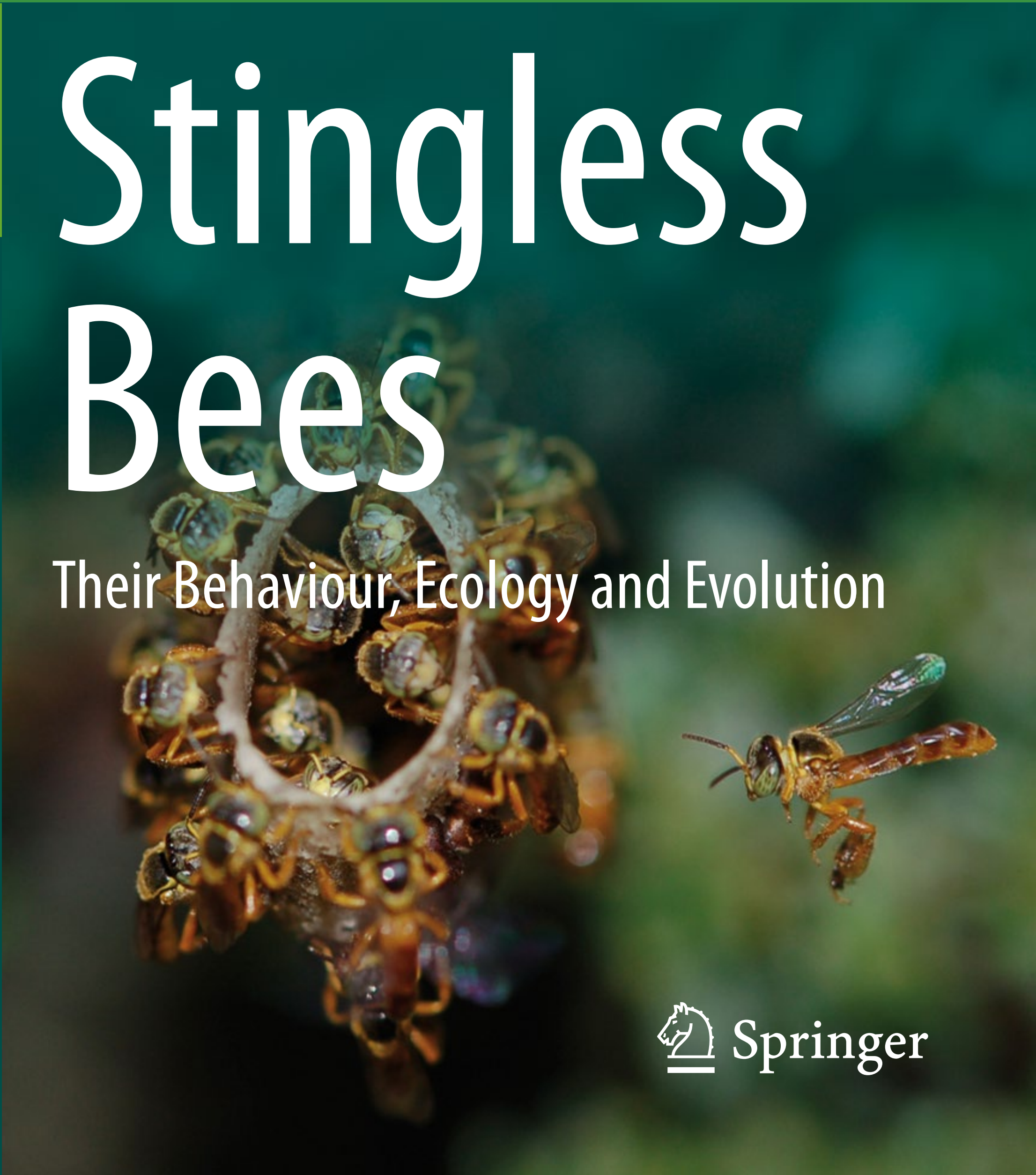
Fascinating Life Sciences

Christoph Grüter



Stingless Bees

Their Behaviour, Ecology and Evolution

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Their Behaviour, Ecology and Evolution

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I would like to dedicate this book to my collaborators, for their support and generosity, to my parents who always encouraged my detours and to Francisca, my partner in crime.

Preface

Interest in bees has been growing in recent years, mainly because it has become clear that bees are having an increasingly hard time in the modern world and that we humans are both responsible for and affected by a decline in the number and diversity of pollinators. But bees are much more than useful pollinators; they are fascinating in so many ways and worth studying in their own right. This is reflected in the thousands of research articles on bees that have been published in the last few decades. Most studies had their focus on temperate honey bees and bumble bees, while tropical bees tend to receive less attention. This also applies to the tropical stingless bees, which is remarkable given that they are the largest, oldest and most diverse group of social bees. Information about stingless bees is often not as easily accessible as it is for honey bees. Numerous important texts on stingless bees are published in non-English languages and some are not published in scientific journals at all. In fact, a wealth of information only exists in the form of unpublished MSc and PhD theses, some of which I have mentioned in this book. One of the main motivations for writing a book about stingless bees was, therefore, to bring these diverse sources of information together and provide a hopefully accessible summary of the behaviour, ecology and evolution of this spectacular group of bees. David Roubik's *Ecology and Natural History of Tropical Bees* (1989) and the accounts of Schwarz (1948), Michener (1974), Sakagami (1982), Wille (1983) and Nogueira-Neto (1997) were particularly important sources of information, and I highly recommend them to readers who would like to know more about tropical bees.

Another reason for writing a book about stingless bees is that I feel that there is a growing momentum in stingless bee research (Hrncir et al. 2016, their Fig. 3) and an increasing interest in stingless beekeeping, or meliponiculture, and stingless bee conservation (see for example Vit et al. 2013, 2018; Heard 2016; Quezada-Euán 2018; Quezada-Euán and Alves 2020). The first stingless bee genomes have recently been published (Kapheim et al. 2015; Freitas et al. 2020), which will add new tools to study stingless bee biology. A renewed appreciation of the importance of stingless bee natural history and ecology will hopefully improve our understanding of the different roles stingless bees play in tropical ecosystems.

One important area that has made much progress in recent years is stingless bee phylogenetics and taxonomy (e.g. Rasmussen and Cameron 2007, 2010). Knowledge of the phylogenetic relationships among stingless bees and other corbiculate bees (Romiguier et al. 2016; Bossert et al. 2017, 2019) is often key to understanding the evolution and expression of phenotypic traits. Having said that, many uncertainties remain regarding species identities and the relationships within some groups stubbornly refuse to be resolved (Chap. 2). The large Neotropical genus *Plebeia*, for instance, remains a phylogenetic puzzle (Rasmussen and Cameron 2010). Readers might take issue with some of the taxonomic names used in this book, and taxonomic disagreements seem unavoidable when dealing with this large and diverse group of bees. It is my hope that we can motivate a new generation of bee taxonomists to undertake long-overdue revisions, e.g. of the genus *Plebeia* and the *Trigonisca* group in the Neotropics and the Afrotropical meliponine fauna—as well as many other groups. A better understanding of their biodiversity will greatly benefit our efforts to study and protect stingless bees.

Readers might also wonder why the honey bee *Apis mellifera* is frequently used as a reference point, rather than the more closely related bumble bees (see Chaps. 1 and 2). Even though stingless bees and honey bees have been on different evolutionary trajectories for longer time periods (Chap. 2), they are often more similar in their lifestyle than bumble bees. As perennial highly eusocial bees, stingless bees and honey bees face similar challenges, but, interestingly, have often found different solutions to these challenges (e.g. nest architecture, Chap. 3; colony migration, Chap. 4; cooperative brood rearing, Chap. 5; division of labour, Chap. 6; colony defence, Chap. 7; or recruitment communication, Chap. 10). Another reason for this bias is that, as a trained honey bee biologist, I often cannot help but examine stingless bees through the lens of a honey bee researcher (and *vice versa*). In fact, I had my first encounter with stingless bees while working with honey bees during my PhD in the laboratory of Walter Farina at the University of Buenos Aires in Argentina. Feisty little guards of the species *Tetragonisca fiebrigi* attacked my honey bees as I tried to train them to a sugar water feeder. This was less than welcome at the time.

I am deeply indebted to Francis Ratnieks for introducing me to the world of stingless bees when I was a postdoc in his laboratory at the University of Sussex, UK. During our trips to Brazil, Francis was a constant source of knowledge, entertainment and inspiration. These trips have led to many wonderful encounters and collaborations. I would like to express my deep gratitude to Cristiano Menezes, Denise Alves and Ayrton Vollet-Neto for sharing their knowledge about stingless bees, for friendship and for improving sections of this book. Cristiano Menezes also generously provided many of the photos shown in this book. Much of my research on stingless bees would not have been possible without the support of Fabio Nascimento, both when I was a postdoctoral researcher in his laboratory at the University of São Paulo and during subsequent visits. Likewise, Sidnei Mateus has been incredibly helpful throughout the years by enthusiastically sharing his knowledge about stingless bees and their habits. Many tricky questions regarding stingless bee phylogeny and taxonomy were navigated with the expert help of Eduardo Almeida, who also provided important feedback for some parts of the book. Lucas von Zuben, Jamille Veiga, Ricardo Oliveira, Luana Santos, Túlio Nunes and Vera Imperatriz-Fonseca generously shared their enthusiasm and expertise during collaborative projects. Jamille Veiga deserves a special mention for putting a lot of work into assembling the data on colony sizes that is summarised in Table 1.3. Michael Hrncir, Friedrich Barth and Robbie I'Anson Price critically read and improved several chapters of this book. I am also very grateful to Robbie I'Anson Price, Tianfei Peng and Simone Glaser for many discussions during trips to Brazil. Many people have kindly allowed me to use their photos and illustrations, as indicated in the figure captions. Nadja Stadelmann has expertly prepared Figs. 1.4 and 1.9.

I would like to thank Tomer Czaczkes, Jelle van Zweden, Sam Jones and Patrícia Nunes-Silva for their company and input while working together on Fazenda Aretuzina in São Simão, the Farm of Paulo Nogueira-Neto. The hospitality of "Dr. Paulo", a pioneer in stingless bee research and a Brazilian environmentalist of historic stature, made much of our research possible. Discussing stingless bees with him was a unique privilege and is a lasting inspiration. Finally, I would like to thank my most important colleague and collaborator, Francisca Segers. Her critical feedback substantially improved several of the chapters of this book.

Our research on stingless bees was possible thanks to the generous support provided by different funding organisations. Research stays at the University of São Paulo in Ribeirão Preto were funded by the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP), the *Swiss National Science Foundation* (SNSF), the *Ethologische Gesellschaft* and the *German Research Foundation* (DFG). Of particular importance was a *Ciência sem fronteiras* ("Science without borders") postdoctoral fellowship financed by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Capes), which allowed me to spend an extended period at the University of São Paulo in the laboratory of Fabio Nascimento. This allowed me to learn more about stingless bees, develop new collaborations and strengthen existing ones. This fellowship provided the foundation for this book.

Bristol, UK Christoph Grüter
June 2020

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Contents

Stingless Bees: An Overview

1

The tropics and subtropics are home to thousands of different types of bees. One bee group that frequently calls an observer's attention are the stingless bees or Meliponini. In the Neotropics, for example, about half of all bees that one is likely to see on flowers belongs to this group (Chap. 9). Stingless bees can be smaller than a fruit fly or as large as the giant honey bee *Apis dorsata*. Like honey bees (Apini), stingless bees live in colonies and produce honey. They are potential pollinators of thousands of plant species and play important roles in human cultures. Like many other animals, stingless bees face new challenges in an increasingly human-modified world, including large-scale habitat loss, the widespread use of agrochemicals, climate change and introduced species, all of which put pressure on stingless bee populations (see Sect. 1.9).

Stingless bees belong to the corbiculate bees (Hymenoptera, Apidae), as do the bumble bees (Bombini), the honey bees (Apini) and the orchid bees (Euglossini). These four groups of bees are characterised by their habit of carrying pollen in a "pollen basket" (or corbicula) on their hind legs (Fig. 1.1). With about 550 described species belonging to dozens of genera, the stingless bees contain more species than the other three groups combined (Fig. 1.2; Chap. 2) (Eardley 2004; Rasmussen and Cameron 2010; Camargo and Pedro 2013; Rasmussen et al. 2017). They can be distinguished from the other corbiculate bees by their lack of a functional sting and a reduced wing venation (Fig. 1.3). The reduction in wing venation was probably the result of the small body size of stingless bee ancestors (Melo 2020). Other typical morphological features of stingless bees are the "penicillum", a tibial tuft of strong and curved bristles at the base of the hind tibia (Fig. 1.1) and the absence of an auricle (pollen press), an expansion of the base of the hind basitarsus (Michener 2007, Fig. 102-2 therein; see also Quezada-Euán 2018 for more details on stingless bee morphology).

The majority of stingless bee species can be found in the Neotropics (77%), followed by the Indo-Malay/Australasian region (16%) and the Afrotropics (7%). It is striking, however, how little we know about most stingless bee species, especially considering their ecological importance for tropical ecosystem and when compared

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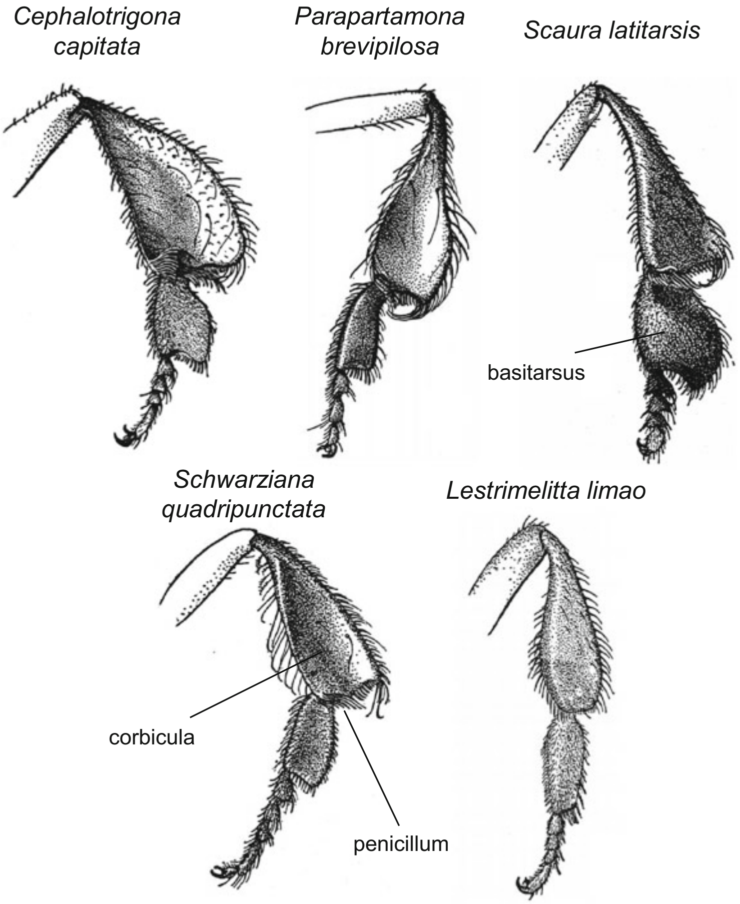

Fig. 1.1 Widened hind tibiae of worker bees with long, thick hairs or setae (pollen basket or corbicula). Note the bladder-like basitarsus in *Scaura latitarsus* (Identity unclear. This species is now considered to be *Scaura amazonica* or *S. aspera* (Nogueira et al. 2019)) (see also *S. longula* in Fig. 8.1) and the reduction of the pollen basket and absence of the penicillum in *Lestrimelitta limao* (original drawings from Schwarz 1948)

to honey bees and bumble bees, the other two groups of eusocial bees. Even though stingless bees represent ~70% of all eusocial bee species, only about 6% of scientific publications on eusocial bees deal with stingless bees[1,2] (see also Hrncir et al. 2016).

Stingless bees are the oldest group of eusocial bees. They appeared in the Late Cretaceous, about 70–87 million years ago and lived alongside dinosaurs for

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[1] About 78% deal with honeybees and about 16% with bumble bees, based on a search in Web of Science in January 2020. Search terms were (1) Apini/honeybees/honey bees, (2) Bombini/bumblebees/bumble bees and (3) Meliponini/stingless bees/stingless bee.

[2] See Quintal and Roubik (2013) for a short history of stingless bee research.

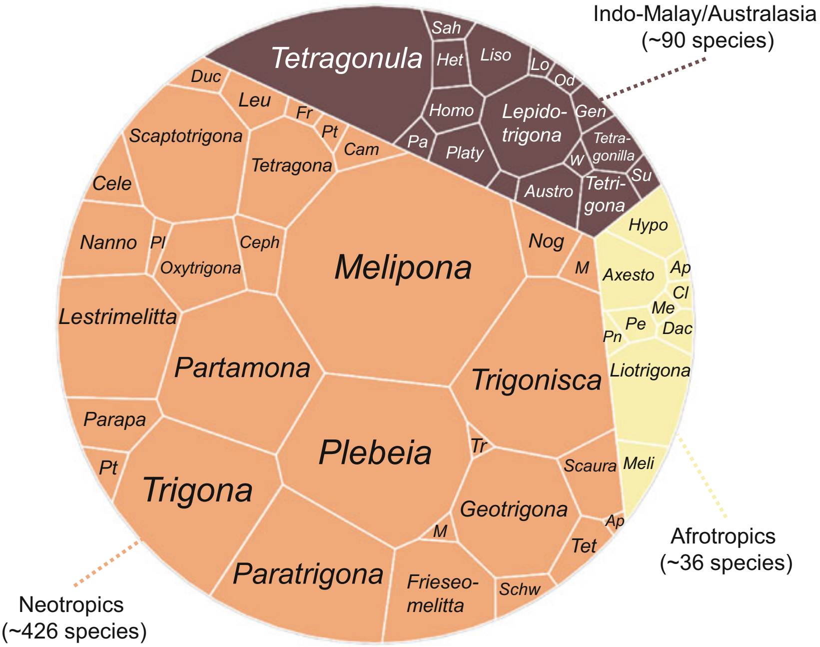

Fig. 1.2 Global diversity of stingless bees. The size of the areas is proportional to the number of species in each genus (see Chap. 2 for detailed information about the number and names of species in each genus)

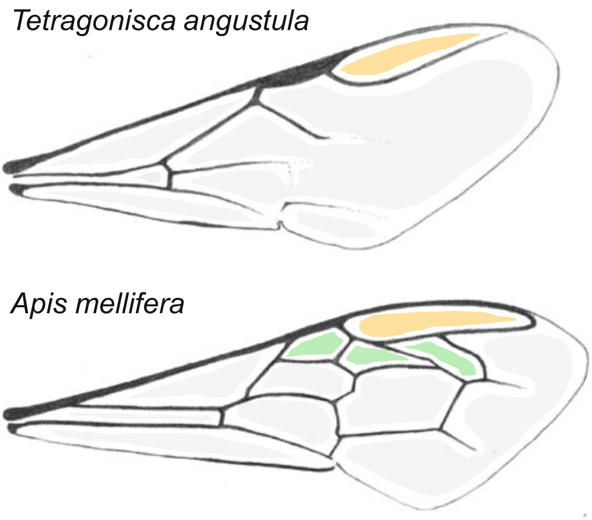

Fig. 1.3 Reduced wing venation in stingless bees (here *Tetragonisca angustula*) compared to honey bees (here *Apis mellifera*). The marginal cell (orange) is often open, the submarginal cells (green in *Apis mellifera*, sm1–sm3 from left to right) are weakly defined or not visible at all in stingless bees. See Michener (2007, Fig. 120-1) for more examples of stingless bee wing venation

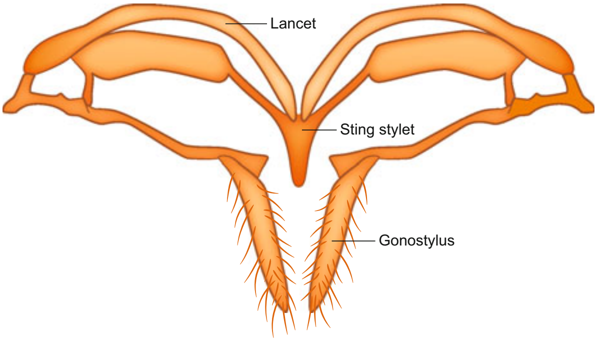

Fig. 1.4 Reduced sting apparatus in *Melipona* (modified from Michener 2007 and Quezada-Euán 2018). The lancet (also called first valvula) and sting stylet (second valvula) are reduced to varying degrees in different stingless bee species (von Ihering 1886; see Michener 2007, Fig. 120-6). In *Apis mellifera*, lancet and sting stylet combine to form a functional sting (illustration by Nadja Stadelmann)

millions of years (Chap. 2). By this time, their ancestors had already lost a functional sting. The name "stingless bee" is slightly misleading, however, as they do have a vestigial leftover of what used to function as a sting (Fig. 1.4) (von Ihering 1886; Schwarz 1948; Kerr and de Lello 1962; Michener 2007). It is somewhat puzzling that the Meliponini, like many ants, lost what would appear to be a formidable weapon. The most likely explanation is that other defence traits were more suited to deal with the enemies that stingless bees commonly faced (Chap. 7) (Kerr and de Lello 1962; Sakagami 1971). The small body size of early stingless bees might have contributed to this change in their defensive portfolio as being stung by a small bee might not have deterred their enemies (Melo 2020). Examples of such alternative defence traits are powerful mandibles, caustic secretions, unpleasant smells or the use of sticky materials to immobilise enemies. The effectiveness of these traits is highlighted by reports of *Lestrimelitta*, *Melipona*, *Oxytrigona* and *Tetragonisca* colonies occasionally attacking and killing honey bee colonies (Nogueira-Neto 1970; Sakagami 1971; Grüter et al. 2016).

Stingless bees share many characteristics with honey bees. They live in perennial colonies (Sect. 1.7), have considerable caste differentiation between queens and workers (Fig. 1.5), they build an elaborate nest that allows them to store large quantities of food and they use complex and interacting communication systems to coordinate their daily activities. As is the case in honey bees, queens cannot start new colonies on their own (Chaps. 4, 5 and 10) (for earlier reviews of stingless bee biology see also Schwarz 1948; Michener 1974; Sakagami 1982; Wille 1983; Roubik 1989; Heard 2016; Quezada-Euán 2018). Stingless bees also differ from honey bees in significant and sometimes astonishing ways (see Sect. 1.5) (Sakagami 1971; Michener 1974), indicating that they evolved a highly eusocial lifestyle independently from honey bees (Chap. 2).

Fig. 1.5 Queen and worker of *Trigonisca mepecheu*, one of the smallest Neotropical stingless bees (adapted from Engel et al. 2019)

1.1 The Queen

The queen is the principal egg layer and differs from the workers in both size and shape (Fig. 1.5). With the exception of *Melipona*, a genus in which queens and workers are reared on the same amount of food and, therefore, are very similar in size, stingless bee queens weigh more than workers (~2–6 times) and they have longer bodies (Figs. 1.5 and 1.6) (Tóth et al. 2004; Grüter et al. 2017; Luna-Lucena et al. 2019). After mating, stingless bee queens become far more physogastric (i.e. they develop a distended abdomen) than honey bee queens and even *Melipona* queens can easily be distinguished from workers by their larger abdomen once they have mated (Fig. 4.1). Compared to workers, queens have relatively smaller heads and eyes (Fig. 1.7) and shorter wings, but often a wider thorax, longer malar spaces and longer antennae (Schwarz 1948; Sakagami 1982).[3]

Stingless bee colonies are usually monogynous, i.e. they have just one egg-laying queen. *Melipona bicolor* is the only currently known exception to the rule as colonies are frequently polygynous (Chap. 5). Depending on the species, queens lay from a dozen (e.g. *Plebeia julianii*) to several hundred (e.g. *Trigona recursa*) eggs each day (Table 5.1). The number of ovarioles per ovary is much lower than in honey bee queens (100–200) and ranges from 4 to 8 in most species (Camargo 1974; Cruz-Landim 2000; Luna-Lucena et al. 2019), but can be as high as 10–15 in some *Trigona* (e.g. *T. spinipes*) and *Lestrimelitta* species (Sakagami 1982; Cruz-Landim 2000). Increases in queen productivity are associated with an elongation of the ovarioles, rather than an increase in their number (Fig. 1.8) (Velthuis 1976;

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[3] For more detailed descriptions of the morphology of different genera, see literature provided in Chap. 2, Sect. 2.4.

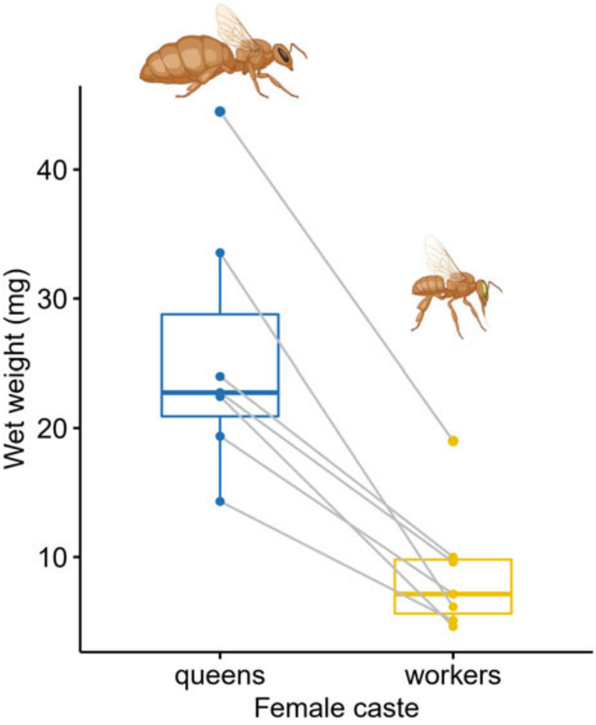

Fig. 1.6 Body weight of queens and workers of seven Neotropical species: *Frieseomelitta varia* (Baptistella et al. 2012), *Nannotrigona testaceicornis* (Ribeiro et al. 2006; Grüter et al. 2017), *Plebeia pugnax*, *P. remota* (Ribeiro et al. 2006), *Scaptotrigona postica* (Velthuis 1976), *Schwarziana quadripunctata* (Ribeiro et al. 2006), *Tetragonisca angustula* (Kerr et al. 1962; Segers et al. 2015) (illustration by Nadja Stadelmann)

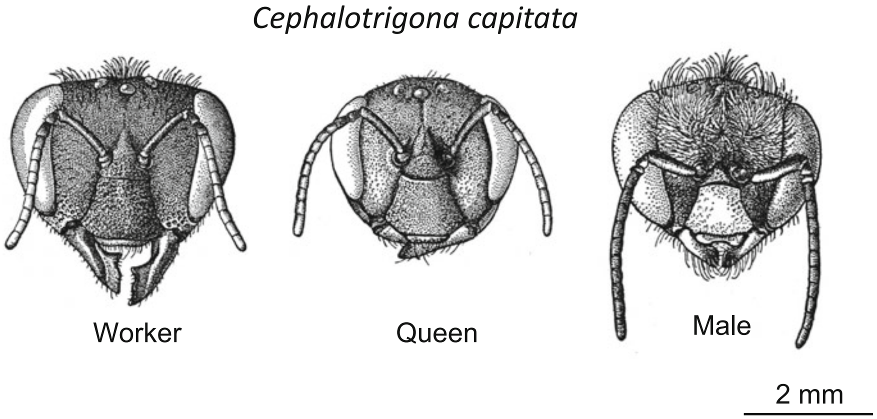

Fig. 1.7 Worker, queen and male head of *Cephalotrigona capitata* (original drawings from Schwarz 1948). Queens have smaller eyes than workers and males. Males have a longer flagellum, but a shorter scape

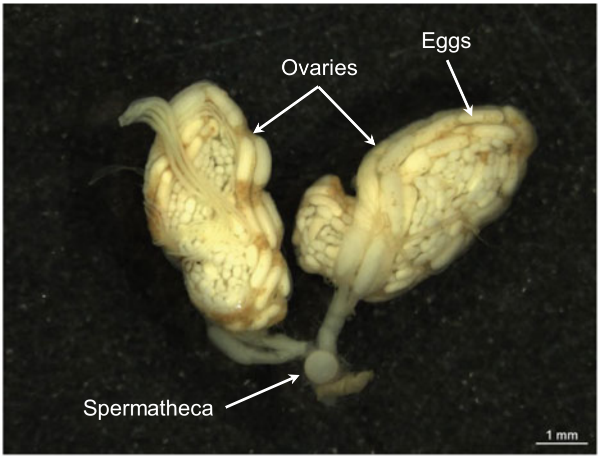

Fig. 1.8 Reproductive tract of a physogastric *Tetragonisca angustula* queen (modified from Santos 2012)

Sakagami 1982). It has been suggested that ovariole number is linked to colony size (Sakagami 1982), but this requires further study. Like workers, queens do not have a functional sting, but vestigial structures seem to be more pronounced than in workers (Schwarz 1948; Sakagami 1982).

The queen does not have pollen baskets and, according to Sakagami (1982), queens of some species do not have wax glands. Wax secretion has, however, been observed in unmated ("virgin") queens of several Neotropical genera (see Chap. 7). Even though we have little data on queen lifespans, the existing information suggests that queens live much longer than workers, usually between 1 and 3 years, but queens can live up to 7 years (Table 1.1) (Kerr et al. 1962; da Silva et al. 1972; Darchen 1977; Imperatriz-Fonseca 1978; Imperatriz-Fonseca and Zucchi 1995; van Veen et al. 2004; Ribeiro et al. 2006).

1.2 The Workers

Most individuals in a stingless bee colony are workers and as the name suggests, they are responsible for performing most of the tasks to maintain the colony. The most important tasks include the building of the nest structures (Chaps. 3 and 4), larval food provisioning (Chap. 5), nest defence (Chap. 7) and foraging (Chaps. 8–10). Worker morphology reflects the need to perform these different tasks (Fig. 1.9). For example, foraging requires them to have relatively large eyes compared to the queen who spends most of her time in the darkness of the nest (Fig. 1.7). Workers of many species have strong mandibles that are employed effectively in defence (e.g. Shackleton et al. 2015). Unlike in honey bees, wax is secreted dorsally, i.e. from wax glands located on top of the abdomen (Fig. 3.7) (Müller 1874).

Table 1.1 Average and maximum lifespan of workers, queens and males

Species	Av. lifespan	Max. lifespan	References
Workers	***Days***		
Austroplebeia australis		240	Halcroft et al. (2013a)
Friesella schrottkyi	30.1		Giannini (1997)
Frieseomelitta languida	33.3		Giannini (1997)
Frieseomelitta varia	42	82	Cardoso (2010)
Meliplebeia beccarii	52.7	73	Njoya and Wittmann (2013)
Melipona beecheii	51	101	Biesmeijer and Tóth (1998)
Melipona bicolor	44	68	Giannini (1997), Bego (1983)
Melipona eburnea	36–43	66	Bustamante (2006)
Melipona fasciculata	42.5	80	Giannini (1997), Gomes et al. (2015)
Melipona favosa	40.5	108	Sommeijer (1984), Roubik (1982)
Melipona fulva	34	84	Roubik (1982)
Melipona lateralis	35–54	73	Bustamante (2006)
Melipona marginata	41.1	70	Mateus et al. (2019)
Melipona seminigra	30	54	Bustamante (2006)
Melipona scutellaris	43.8	75	Santos (2013)
Plebeia droryana	41.7	75	Terada et al. (1975)
Plebeia emerina		63	dos Santos et al. (2010)
Plebeia remota	67.7	96	van Benthem et al. (1995), Grosso and Bego (2002)
Scaptotrigona postica	33.4–39.5	60	Simões and Bego (1979)
Scaptotrigona xanthotricha	94	97	Hebling et al. (1964)
Tetragonisca angustula	24	56	Grosso and Bego (2002), Hammel et al. (2016)
Tetragonula minangkabau	37	60	Inoue et al. (1996)
Trigona pallens	29.4		Cardoso (2010)
Trigona pellucida	41.5	79	Cardoso (2010)
Tetragonula laeviceps	40–50		Inoue et al. (1984)
Males	***Days***		
Melipona eburnea	16	18	Bustamante (2006)
Melipona seminigra	21	23	Bustamante (2006)
Queens	***Years***		
Hypotrigona sp.		>4	Darchen (1977)
Melipona beecheii	>3		van Veen et al. (2004)
Melipona bicolor		7	Imperatriz-Fonseca and Zucchi (1995)
Melipona favosa	>3		Sommeijer et al. (2003)
Melipona quadrifasciata		~3	da Silva et al. (1972)
Paratrigona subnuda	3		Imperatriz-Fonseca (1978)
Plebeia remota	~1.5	>4	Ribeiro et al. (2006)

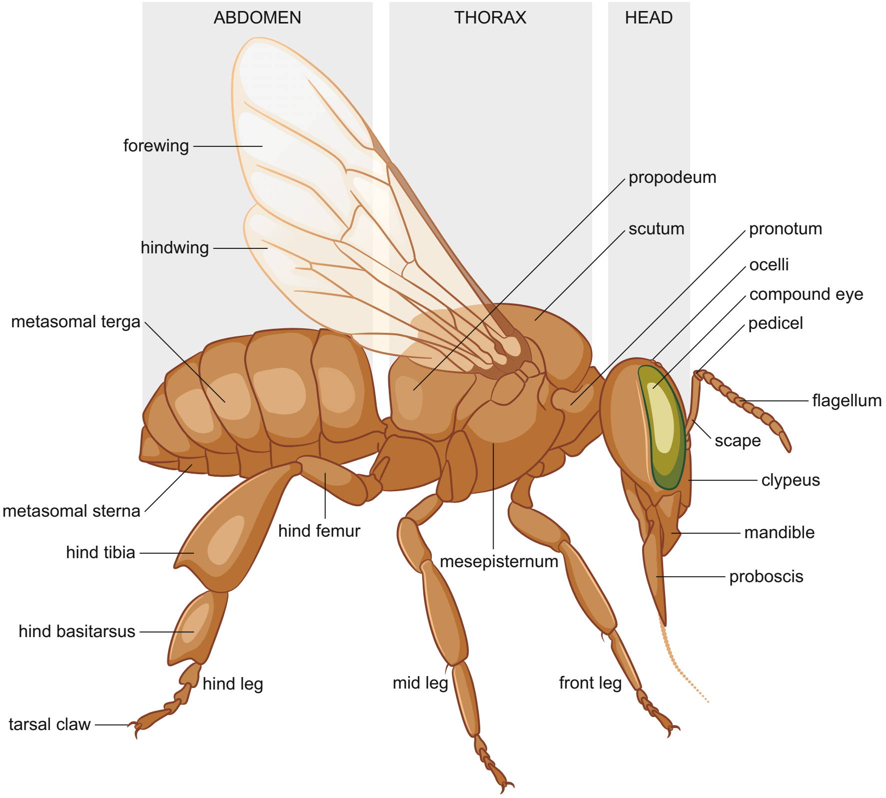

Fig. 1.9 Stingless bee worker morphology (modified from Camargo and Posey 1990, illustration by Nadja Stadelmann)

In contrast to many other groups of social insects, workers of numerous species can have developed ovaries (usually with four ovarioles) even when the queen is present and in some species most males of a colony are the sons of workers (Chap. 5) (Cruz-Landim 2000; Tóth et al. 2004; Grüter 2018; Luna-Lucena et al. 2019). Workers are able to produce males by laying unfertilised eggs. This is possible because males are haploid, which means they have only one set of chromosomes.[4] The workers themselves are diploid and they cannot produce diploid female eggs because they are not able to mate.[5] This system of sex determination is called haplodiploidy and is used by all hymenopterans (ants, bees, wasps and sawflies).

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[4] Stingless bees show considerable variation in the number of chromosomes, ranging from 8 to 18 (present as pairs in workers and queens, $2n = 16$–36) (Tavares et al. 2017; Travenzoli et al. 2019).

[5] There is an unusual kind of honey bee in Africa, the Cape honey bee *Apis mellifera capensis* that has solved this problem. Workers in this sub-species are able to produce other females asexually in a process called parthenogenesis (Goudie and Oldroyd 2014).

Haplodiploidy leads to unusual family and relatedness structures—e.g. males have no fathers and workers are more closely related to their nephews than to their brothers—with far-reaching consequences for colonies (Chaps. 4 and 5).

The pollen basket, located on the hind tibiae, is used to carry pollen, resin, wax and mud, among other substances (Figs. 1.1 and 8.1). The face of the corbicula is widened, concave with long, thick hairs or setae. Its anterior and posterior edges are rimmed with long setae that keep the pollen attached to the leg. There is considerable variation in the shape of the pollen baskets. For instance, the cleptoparasitic *Lestrimelitta* have reduced corbiculae without penicillum, while *Scaura* additionally have a bladder-like hind basitarsus (Figs. 1.1 and 8.1). The function of this structure could be to help foragers collect pollen that has fallen to the ground (see also Chap. 8) (Roubik 2018).

Species differ considerably in size, colour, hairiness and shape. The smallest stingless bee, probably *Liotrigona bitika* from Madagascar (also a contender for smallest bee[6]), is smaller than a fruit fly (Koch 2010), whereas the largest stingless bee, *Melipona fuliginosa* (~125 mg body weight) is the size of a giant honey bee (*Apis dorsata*) (Roubik 1989; Camargo and Pedro 2008). Average worker lifespan can be as short as ~3 weeks (in *Tetragonisca angustula*, Grosso and Bego 2002; Hammel et al. 2016) or as long as ~10 weeks (in *Plebeia remota*, van Benthem et al. 1995) (Table 1.1). The longest worker lifespan has been recorded in *Austroplebeia australis*, who can live up to 270 days during periods of flower scarcity in its Australian habitat (Halcroft et al. 2013a). As foraging activity increases, often with increasing worker age and food source availability, so does mortality (see also Fig. 6.4). For example, *Melipona fasciculata* workers forage at a later age in the rainy season and live more than 50% longer than workers during the dry season (Gomes et al. 2015). In *M. favosa* and *M. fulva*, worker lifespans were about twice as long during the rainy season compared to the dry season when foraging conditions were better (Roubik 1982). The observation that workers live longer in queenless colonies is most likely also explained by a reduction in foraging activity after queen loss (Lopes et al. 2020). In *Plebeia remota*, worker lifespan is linked to the time of year. This species occurs in subtropical areas where brood production is seasonal and van Benthem et al. (1995) observed that winter bees live 25–100% longer than summer bees (see also Terada et al. 1975). In *Apis mellifera*, the difference in lifespan between winter and summer bees is partly linked to the exposure to brood pheromone and vitellogenin titres in the hemolymph and in fat bodies (Bitondi and Simões 1996; Smedal et al. 2009). Vitellogenin is known to affect foraging behaviour, innate immunity and protects against oxidative stress in honey bees (Amdam et al. 2004, 2005; Seehuus et al. 2006). The physiological and molecular factors that affect lifespan in stingless bees remain to be investigated.

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[6] The average worker length of 1.97 mm (Koch 2010) is similar or slightly smaller compared to *Perdita minima* and *Quasihesma clypearis* females (Exley 1980), other candidates for the title of smallest known bee.

Fig. 1.10 *Melipona flavolineata* male. Males have larger eyes than workers and their antennae often form a V-shape (Photo by Cristiano Menezes)

Worker size might also affect lifespan. In *Melipona fasciculata*, larger workers lived longer, especially in the dry season when more plants are flowering and foraging activities are more intense in the Amazon region (Gomes et al. 2015). In *Tetragonisca angustula*, on the other hand, larger workers did not live longer than smaller workers (Hammel et al. 2016).

1.3 The Males

The main function of males (or drones) is to mate with a queen (Chap. 4). They perform little work inside their natal nest (but more than honey bee drones, Chap. 6) and they leave the nest at an age of 2–3 weeks, without ever returning to it (van Veen et al. 1997; Sommeijer et al. 2003). Superficially, males and workers look similar and only an experienced observer can distinguish them without visual help. The similarity stems from the fact that stingless bees build one type of brood cell for both males and workers. Since both types of bee also receive the same amount of larval food they are of similar size and general appearance. Among the more obvious distinguishing external features are the larger eyes in males (Fig. 1.7) and their reduced hind tibiae. However, exceptions exist and males of some Asian stingless bees (e.g. *Tetragonula fuscobalteata*) have hind tibia that are similar to those of workers (but without corbiculae). These males have also been observed to carry pollen and propolis on their hind legs (Chap. 8) (Boongird and Michener 2010). Another difference is the appearance and positioning of the antennae. The antennae of males are often kept in a characteristic V-position (Fig. 1.10). Males have a longer flagellum consisting of 11 flagellomeres (as opposed to 10 in workers and queens), but a shorter scape than workers (Fig. 1.7) (Schwarz 1948; Carvalho et al. 2017; Month-Juris et al. 2020).

There is currently little information available about the lifespan of adult males, but observations indicate that males have shorter lifespans than workers (Table 1.1).

1.4 Stingless Bee Products

1.4.1 Honey

A perennial lifestyle means that colonies need to store honey and pollen for periods with bad foraging conditions. Extreme examples are colonies inhabiting the tropical dry forests of the Caatinga in north-eastern Brazil or the semi-arid zones of Northern Australia. These habitats experience many months of drought every year, which forces colonies into extended periods of lockdown (Halcroft et al. 2013b; Maia-Silva et al. 2015; Hrncir et al. 2019). Honey is produced by the processing of nectars, honeydews and fruit juices by workers and is kept in food pots (Chap. 8) (Fig. 1.11). Because of this way of storing honey, stingless bee honey is often called pot-honey (Vit et al. 2013). Different species produce honeys that vary in colour, texture and flavour, but in general they tend to be more liquid than *Apis mellifera* honey (about 30% water content [~20–42%] vs. 18% water content[7]) (Roubik 1983; Souza et al. 2006; Bijlsma et al. 2006; Dardón et al. 2013; Ferrufino and Vit 2013; Fuenmayor et al. 2013; Biluca et al. 2016; Nordin et al. 2018; Ávila et al. 2018; but see Nweze et al. 2017 who found similar water content in honey samples from honey bees and stingless bees in Nigeria).

The most commonly reported sugars are fructose, glucose, sucrose and maltose. However, a recent analysis by Fletcher et al. (2020) suggests that the disaccharide commonly identified as maltose is, in fact, trehalulose, an isomer of sucrose. This is an intriguing finding because trehalulose has potential health benefits for humans

Fig. 1.11 Interior of a *Duckeola ghilianii* nest, Pará state, Brazil. The honey pots are much larger than the brood cells and are often located in the periphery of the nest. This species does not build protective sheets ("involucrum") around the brood cells (Photo by C. Grüter)

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[7] Some characteristics of stingless bee honey, e.g. the high water content, mean that in some countries pot-honeys do not fulfil the official norms of what can be called "honey" (see the Codex Alimentarius of the FAO/WHO Food Standards Programme). The legal standards are often set with *Apis mellifera* honey in mind (Vit et al. 2004; Alves 2013; Vit 2013).

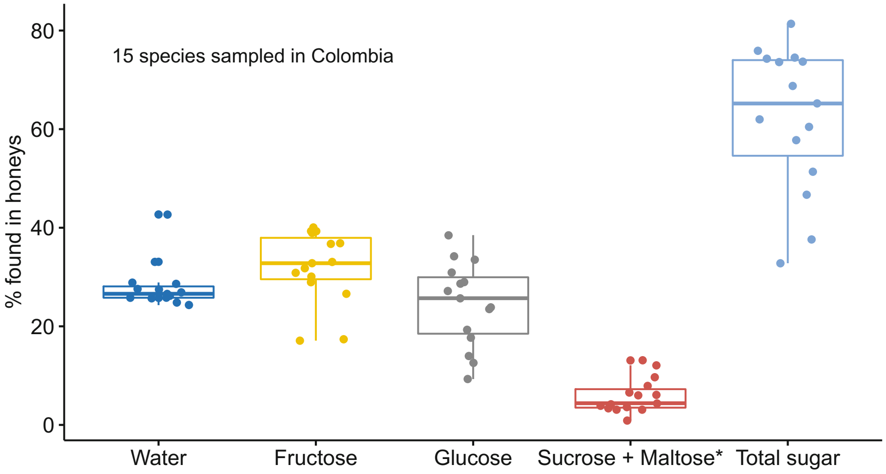

Fig. 1.12 Relative quantities of the main sugars found in stingless bee honeys from Colombia (data from Fuenmayor et al. 2013). *Note that the sugar usually identified as maltose might be trehalulose instead (Fletcher et al. 2020)

(Fletcher et al. 2020). A striking difference can often be found in the maltose/trehalulose content of Neotropical honeys *versus* honeys from Asia and Australia. For example, honeys from 15 species in Colombia contained ~17–40% fructose, ~9–38% glucose and only ~1–13% of sucrose and maltose/trehalulose combined (Fig. 1.12) (Fuenmayor et al. 2013). This is similar to other honeys from Neotropical species (e.g. Biluca et al. 2016). In many Asian and Australian honeys, however, maltose/trehalulose is much more prominent. Honey produced by the Australian *Tetragonula carbonaria* contains ~20% maltose/trehalulose (Oddo et al. 2008), while *Geniotrigona thoracica*, *Heterotrigona itama* and *Lepidotrigona terminata* honey contained ~35%, ~34% and ~53% of maltose/trehalulose, respectively (Nordin et al. 2018; Se et al. 2018). Sucrose, which is the main sugar found in nectars (Chalcoff et al. 2017), is broken down by digestive enzymes and is only found in small quantities in honeys. Total sugar content in stingless bee honey is usually 68–73% (Fig. 1.12) (Roubik 1983; Souza et al. 2006).

Additionally, stingless bee honeys contain various minerals, especially potassium, amino acids, ash (Vit et al. 2013; Biluca et al. 2016, 2019) and flavonoid and phenolic compounds (Vit et al. 2013; Tuksitha et al. 2018).[8] It should be noted that the honey composition, including the sugar content, varies among colonies of the same species and depends on the season, habitat and the collected resources. For example, Fuenmayor et al. (2013) found that *Tetragonisca angustula* honey contained less than 60% sugar in Colombia, whereas Dardón et al. (2013) reported

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[8] These flavonoids and phenolics might be leaking into the honey from the cerumen pots (Heard 2016).

a sugar content of more than 70% in Guatemalan samples (see also Roubik 1983; Souza et al. 2006).

The processing of nectar, honeydew or fruit juice into honey involves (1) the evaporation of water, (2) fermentation due to yeast and bacterial activity and (3) the addition of enzymes and other substances secreted by glands in the heads of workers (Menezes et al. 2013). The fermentation process produces honeys that are more acidic (pH 3–5) than *Apis mellifera* honey (Dardón et al. 2013; Deliza and Vit 2013; Vit 2013; Heard 2016; Nordin et al. 2018; Ávila et al. 2018). Fermentation might in part be responsible for the antioxidant and medicinal properties attributed to stingless bee honeys (see Sect. 1.8) (Rodríguez-Malaver 2013).

Humans extract stingless bee honey by piercing closed honey pots and removing the honey with a syringe or a suction pump (Nogueira-Neto 1997; Souza et al. 2006; Almeida-Muradian 2013; Vit 2013; Heard 2016; Quezada-Euán 2018). The yearly honey production of most species is relatively low compared to *Apis mellifera*, usually between 1 and 3 kg (Cortopassi-Laurino et al. 2006; Kumar et al. 2012; Alves 2013; Ferrufino and Vit 2013; Jaffé et al. 2015; Heard 2016). There are also species, like *Melipona subnitida*, that can produce more than 5 kg of honey per year and managed *Meliponula bocandei* colonies in Angola can produce 10–15 kg (Cortopassi-Laurino et al. 2006). Even more impressive are reports of *Melipona seminigra* or *M. scutellaris* colonies that produced up to 15–25 kg of honey per year (Kerr et al. 1967; see also Alves 2013). Interestingly, yearly honey production seems to depend mainly on body size, rather than colony size (Roubik 1983). Among the most popular honeys are honeys from *Melipona*, *Scaptotrigona* and *Tetragonisca* in the Neotropics and from *Tetragonula* in Australia and Asia (Alves 2013; Heard 2016). Because stingless bees are less productive than honey bees, stingless bee honey tends to be considerably more expensive than *Apis mellifera* honey (Kumar et al. 2012; Alves 2013; Dardón et al. 2013; Ferrufino and Vit 2013; Fuenmayor et al. 2013).

Not all stingless bees produce edible honey. Honey from *Trigonisca mepecheu* (Fig. 1.5) is considered harmful and is not eaten by locals (Engel et al. 2019). Likewise, robber bee (*Lestrimelitta* spp.) honey and pollen are widely described as being toxic (Schwarz 1948; Nogueira-Neto 1997). Von Ihering (1903) reported how eating even small amounts of robber bee honey[9] and pollen led to bouts of cramps and vomiting within about 15 minutes after ingestion, while others have described that consumption of robber bee honey can lead to temporary paralysis (Nogueira-Neto 1997). It should probably not surprise that some honeys (and pollen stores) are better avoided, given that stingless bees have evolved unusual foraging habits, including obligate robbing, the collection of animal faeces and meat from dead animals (Chap. 8). Furthermore, honeys and pollens can be toxic for humans if they originate from certain plant species (e.g. the mass-flowering tree *Luetzelburgia auriculata*, Nogueira-Neto 1997).

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[9] von Ihering (1903, pp. 271–272) does not identify the species, but speculates that the most likely candidate is *Lestrimelitta* (see also Nogueira-Neto 1997, pp. 289 and later for further examples).

1.4.2 Cerumen and Batumen

Stingless bees are industrious builders and they combine various substances, mainly wax and resin to create new building materials (Chap. 3) (Roubik 2006). Unlike honey bees, stingless bees do not usually use pure wax as a building material, but instead mix it with resinous material to create cerumen. Cerumen is used to build most nest structures, such as brood cells, food pots and the involucrum. The involucrum is a protective structure that envelopes the brood (Fig. 3.3). Combining resin and wax creates a firmer building material and the presence of resin is thought to reduce the growth of fungi and bacteria due to its antimicrobial properties (Chap. 3) (e.g. Massaro et al. 2011; Çelemli 2013). To create an even stronger material called batumen, cerumen is combined with more resin, mud, plant material and in some species even animal faeces (Roubik 2006). Layers of batumen cover the walls of the cavity to create a well-protected nesting space.

1.5 Similarities and Differences Between Stingless Bees and Honey Bees

Stingless bees and honey bees share many important features, but they also differ in a number of ways (Table 1.2) (reviewed in Sakagami 1971; Michener 1974). Unlike honey bee queens, stingless bee queens mate with only one male (with exceptions, see Chaps. 4 and 5). Another significant difference is how brood is reared: after oviposition, honey bees feed their larvae progressively until the larvae reach their final size. Then, cells are closed until the adult bee emerges from its cell. In stingless bees, workers first regurgitate larval food into an empty cell (so-called mass provisioning). Afterwards, the queen lays an egg on top of the larval food and workers immediately close the cell (Chap. 5). Laying an egg on larval food is a feature that stingless bees share with many solitary bees (Michener 2007). This way of brood rearing means that adult bees have no direct contact with eggs and the developing larvae and it is likely that this has profound consequences for stingless bee biology. For example, it affects the potential for the transmission of diseases, reduces the scope for worker or queen policing (i.e. the selective killing of worker laid eggs) and prevents workers from removing diploid male larvae, which might explain single mating in stingless bees (Chap. 4).

The general organisation of work in stingless bees and honey bees shows both many parallels and a few noteworthy differences. Workers from both bee groups switch task as they age. Young bees are mostly involved in the brood rearing process and other tasks inside their nest, whereas older bees guard the nest entrance and collect resources (Chap. 6). Building activities play a more important role in the life of stingless bee workers than in honey bee workers. One reason for this is that brood cells are never reused, but built from scratch for each new egg. Furthermore, the protective sheets of cerumen and batumen are constructed and continuously modified to protect the colony (Chap. 3).

Table 1.2 Summary of some of the main differences between stingless bees and honey bees

Trait	Stingless bees	Honey bees (*Apis*)	Chapters
Species diversity	~550 described species	~10 species	2
Building material	Cerumen and batumen: wax mixed with varying amounts resin and other materials	Pure wax for cells, propolis to close openings	3
Brood and food cells	Differ in size, shape and location. Worker and male cells are identical	No difference between worker and food storage cells. Males reared in larger cells	3
Queen leaving the nest when swarming	The virgin queen flies to new nest, mother queen stays in old nest	The mother queen flies to a new nest, virgin queen stays in the old nest	4
Migration	Physogastric queens cannot fly, thus colonies cannot migrate	Mated queens can fly and colonies can migrate to new locations, e.g. when stressed	4
Mating location	Mostly at the newly established nest	At drone aggregations sites	4
Duration of swarming	From days to months	From hours to a few days	4
Mating number	Queens are singly mated	Queens mate with many males	4
Queen determination	Queen destined larvae receive more larval food. Genetic factors are likely to be important in *Melipona*	Queen rearing requires quantitatively and qualitatively different food	5
Brood provisioning	Mass provisioning	Progressive provisioning	5
Brood cells	Open upwards and are built newly for each egg	Open sideways, re-used to rear next generation	5
Worker reproduction	Workers in many species have developed ovaries and produce males	Workers do not have developed ovaries and remain sterile in queenright conditions	5
Waste management	Bees defecate inside the nest and store waste on piles before other bees dump waste outside	Workers defecate outside, dead bees removed by undertakers	6
Defence	Mandibles for biting, resin to immobilise small enemies, soldiers	Mostly by stinging, giant honey bees with collective visual displays	7
Collected resources	Variety of resources next to nectar and pollen	Mostly nectar and pollen	8
Recruitment communication	Variable: from solitary to chemical trails	Waggle dance	10

Another noteworthy difference between stingless bees and honey bees is how they defend their nest. Whilst stingless bee guards do not have a sting, guards of several species show a greater degree of behavioural specialisation, i.e. they guard for extended time periods, and are often larger than other workers (Chap. 7) (Grüter et al. 2011, 2017; Wittwer and Elgar 2018; Baudier et al. 2019).

Honey bees use the waggle dance to communicate the location and odour of profitable food sources to their nestmates (von Frisch 1967; Couvillon 2012; I'Anson Price and Grüter 2015). Stingless bees also perform striking movement patterns inside their nest and it has been suggested that their spinning and zig-zagging might encode information about the location of food sources, but convincing evidence for this is lacking (Chap. 10) (Nieh 2004; Hrncir 2009). Pheromone trails, on the other hand, are used by some species to recruit accurately to profitable food sources (Nieh 2004; Jarau 2009; Leonhardt 2017).

1.6 Colony Size

Colony size is a fundamental social trait that interacts with many key aspects of social life (Bourke 1999; Dornhaus et al. 2012). For instance, colony size is linked to division of labour (Oster and Wilson 1978), colony productivity (Karsai and Wenzel 1998), reproductive conflicts (Bourke 1999), the benefits and method of recruitment (see also Chap. 10) (Beckers et al. 1989) and competitive interactions (Hölldobler 1976; Lichtenberg et al. 2010; Hrncir and Maia-Silva 2013). Colony size estimates (the number of adult bees) vary greatly among stingless bee species and range from less than 100, e.g. in *Melipona phenax*, to more than 100,000 in *Trigona amazonensis* (Table 1.3). The average colony size of the 104 species included in Table 1.3 is ~5200, with a median colony size of 1500 (Fig. 1.13). Most species that build brood cells arranged in clusters (see Chap. 3) have colonies that are smaller than 1000 workers, whereas a majority of species with brood combs have a colony size that is larger than 1000 (Fig. 1.13).

Very large colonies are often found in the Neotropical genera *Tetragona* and *Trigona*. Many *Trigona* species build external nests, which are not constrained by the size of a nesting cavity. *Trigona corvina*, for example, builds external nests that can weigh more than 100 kg (Roubik and Patiño 2009). Colony size estimates of 180,000–200,000 bees in *Trigona amazonensis* and *T. spinipes* have been questioned by some researchers (Wille and Michener 1973; Roubik 1989) and the estimates in Table 1.3 should be considered preliminary for most species. Estimates are frequently based on only one or two colonies and the methods to estimate colony sizes are often imprecise, such as estimating the perceived number of flying bees combined with counts of brood cells. Some studies provide no information at all about how colony sizes were assessed. For example, in their influential paper published in 1958, Lindauer and Kerr stated that *Trigona spinipes* (there as "*T. ruficrus*") colonies contain 100,000–150,000 bees before changing this to 5000–180,000 bees in their 1960 paper,[10] without mentioning the methods used to estimate colony size in either publication.

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[10] The 1960 paper is largely a translation of their 1958 paper, which was in German.

Table 1.3 Colony size estimates and brood cell arrangement

Region	Species	Colony size range	Av. colony size	Brood cell type	References
Afrotropical	*Apotrigona nebulata*	195–2000	1700	Combs	Darchen (1969), Kajobe (2007)
	Hypotrigona araujoi	2000–2500	2250	Combs	Portugal-Araojo and Kerr (1959), as “landula”
	Hypotrigona gribodoi	100–750	450	Clusters	Bassindale (1955)
	Meliponula bocandei	1000–1300	1170	Clusters	Kajobe (2007), Njoya et al. (2018)
	Plebeina armata (=hildebrandti)		3091	Combs/spirals	Namu and Wittmann (2016)
Indo-Malay/Australasia	*Austroplebeia australis*		2000	Clusters	Hammond and Keller (2004)
	Austroplebeia cassiae		2000	Clusters	Hammond and Keller (2004)
	Lepidotrigona hoozana		10,000	Combs	Santos et al. (2014)
	Lepidotrigona ventralis	258–12,167	4221	Combs	Chinh et al. (2005)
	Lisotrigona carpenteri	50–375	144	Clusters	Chinh et al. (2005)
	Tetragonula carbonaria	2500–11,000	6750	Spirals	Tóth et al. (2004), Halcroft et al. (2013a)
	Tetragonula hockingsi	3000–10,000	7000	Semi-combs	Tóth et al. (2004)
	Tetragonula iridipennis		2500	Clusters/semi-combs	Schwarz (1939)
	Tetragonula laeviceps	487–2800	747	Clusters	Sakagami et al. (1983), Chinh et al. (2005)
	Tetragonula mellipes	1000–5000	2000	Clusters	Tóth et al. (2004)
	Tetragonula minangkabau	300–2600	750	Clusters	Inoue et al. (1996), Tóth et al. (2004)
Neotropical	*Aparatrigona isopteraphila*	80–350	185	Combs/spirals	Roubik (1983)
	Cephalotrigona capitata	1000–1500	1250	Combs	Michener (1974)
	Cephalotrigona zexmeniae	400–1000	700	Combs	Roubik (1983)
	Duckeola ghilianii		10,000	Semi-combs	Tóth et al. (2004)

(continued)

Table 1.3 (continued)

Region	Species	Colony size range	Av. colony size	Brood cell type	References
	Friesella schrottkyi	300–2500	1400	Semi-combs	Sakagami et al. (1973), Michener (1974)
	Frieseomelitta flavicornis		375	Clusters	Roubik (1979), there as *T. savannensis*
	Frieseomelitta longipes	1051–4393	2635	Clusters	Leão (2019)
	Frieseomelitta nigra	400–1500	950	Clusters	Tóth et al. (2004)
	Frieseomelitta paupera	500–700	600	Clusters	Sommeijer et al. (1984)
	Frieseomelitta silvestrii	400–600	500	Clusters	Michener (1974)
	Frieseomelitta varia	800–1600	1200	Clusters	Tóth et al. (2004)
	Geotrigona leucogastra	200–450	325	Combs	Roubik (1983)
	Geotrigona mombuca	2000–3000	2500	Combs	Tóth et al. (2004)
	Geotrigona subterranea	2726–11,074	7485	Combs	Barbosa et al. (2013)
	Lestrimelitta danuncia		900	Combs	Roubik (1983)
	Lestrimelitta guayanensis		2890	Combs	Roubik (1979)
	Lestrimelitta limao	2000–7000	4500	Combs	Grüter et al. (2016)
	Lestrimelitta niitkib	3000–5000	4000	Combs	Quezada-Euán and González-Acereto (2002)
	Melipona beecheii	300–2000	800	Combs	van Veen et al. (2004)
	Melipona bicolor	150–800	425	Combs	Tóth et al. (2004)
	Melipona carrikeri		210	Combs	Wille and Michener (1973)
	Melipona costaricensis		2000	Combs	Wille and Michener (1973)
	Melipona crinita	180–450	315	Combs	Roubik (1983)
	Melipona fasciata	200–2500	1000	Combs	Roubik (1983), Tóth et al. (2004)
	Melipona fasciculata	300–1000	600	Combs	Gomes et al. (2015), Leão (2019)
	Melipona favosa	60–700	400	Combs	Sommeijer (1984), Roubik (1982)

(continued)

Table 1.3 (continued)

Region	Species	Colony size range	Av. colony size	Brood cell type	References
	Melipona flavolineata	770–3000	1500	Combs	Grüter et al. (2016), Leão (2019)
	Melipona fuliginosa	250–600	383	Combs	Roubik (1983)
	Melipona fulva	300–500	400	Combs	Roubik (1982)
	Melipona marginata	160–2500	1330	Combs	Tóth et al. (2004)
	Melipona micheneri	50–120	85	Combs	Roubik (1983)
	Melipona phenax	60–120	90	Combs	Roubik (1983)
	Melipona quadrifasciata	300–1500	900	Combs	Michener (1974), Tóth et al. (2004)
	Melipona rufiventris	500–700	600	Combs	Nieh et al. (2005)
	Melipona scutellaris	1000–2000	1500	Combs	Tóth et al. (2004)
	Melipona seminigra	1000–3000	2000	Combs	Grüter et al. (2016)
	Melipona trinitatis	1000–2000	1500	Combs	Bijlsma et al. (2006)
	Melipona triplaridis	70–550	340	Combs	Roubik (1983)
	Nannotrigona mellaria		1000	Combs	Roubik (1983)
	Nannotrigona perilampoides	700–1200	950	Combs/spirals	Quezada-Euán and González-Acereto (2002)
	Nannotrigona testaceicornis	2000–3000	2500	Combs/spirals	Michener (1974)
	Nogueirapis mirandula	2281–4076	3180	Combs	Wille (1966)
	Oxytrigona mellicolor		5442	Combs	Roubik (1983)
	Oxytrigona obscura		1900	Combs	Roubik (1983)
	Paratrigona ornaticeps	1100–2400	1710	Combs	Roubik (1983)
	Paratrigona opaca		250	Combs	Roubik (1983)
	Partamona aff. *cupira*	280–6457	2084	Combs	Wille and Michener (1973), Roubik (1983)
	Plebeia droryana	1070–3000	2400	Combs	Tóth et al. (2004), Roldão-Sbordoni et al. (2018)

(continued)

Table 1.3 (continued)

Region	Species	Colony size range	Av. colony size	Brood cell type	References
	Plebeia minima	200–900	350	Clusters	Leão (2019)
	Plebeia franki	70–250	113	Clusters	Roubik (1983)
	Plebeia frontalis	100–1900	1000	Combs	Wille and Michener (1973), Roubik (1983)
	Plebeia jatiformis	650–950	800	Combs	Roubik (1983)
	Plebeia julianii		300	Semi-combs	Drumond et al. (1998)
	Plebeia mosquito		1175	Combs	von Ihering (1903), Wille and Michener (1973)
	Plebeia nigriceps	100–200	150	Combs	Witter et al. (2007)
	Plebeia remota	800–5000	2900	Combs	van Benthem et al. (1995), Tóth et al. (2004)
	Plebeia saiqui		7000	Combs	Witter et al. (2007)
	Plebeia tica		612	Clusters	Wille (1969)
	Plebeia tobagoensis	40–1500	1200		Bijlsma et al. (2006), Hofstede and Sommeijer (2006)
	Scaptotrigona postica	6000–10,000	7400	Combs	Leão (2019)
	Scaptotrigona barrocoloradensis	3000–7827	5400	Combs	Roubik (1983), Tóth et al. (2004)
	Scaptotrigona luteipennis	3000–10,000	5600	Combs	Roubik (1983), as *S. pachysoma*
	Scaptotrigona panamensis		5201	Combs	Roubik (1983)
	Scaptotrigona pectoralis	2000–5200	3600	Combs	Roubik (1983), Quezada-Euán (2018)
	Scaptotrigona tubiba		1764	Combs	Roubik (1979)
	Scaura latitarsis	387–450	400	Combs/spirals	Wille and Michener (1973), Roubik (1983)
	Schwarziana quadripunctata	500–2500	1500	Combs	Tóth et al. (2003)
	Tetragona beebei	1500–2000	1750	Spirals	Roubik (1983), as *T. dorsalis beebei*
	Tetragona clavipes	5383–29,000	7343	Combs/spirals	Roubik (1979), Duarte et al. (2016)
	Tetragona dorsalis	1500–75,000	27,774	Combs/spirals	Roubik (1983), Tóth et al. (2004)

(continued)

Table 1.3 (continued)

Region	Species	Colony size range	Av. colony size	Brood cell type	References
	Tetragonisca angustula	2000–8000	5000	Combs	Tóth et al. (2004)
	Tetragonisca buchwaldi	1326–2979	2028	Combs	Wille (1966), Wille and Michener (1973)
	Tetragonisca weyrauchi	2000–3000	2500	Combs	Cortopassi-Laurino and Nogueira-Neto (2003)
	Trichotrigona camargoiana		200	Clusters	Pedro and Cordeiro (2015)
	Trichotrigona extranea	43–163	107	Clusters	Camargo and Pedro (2007)
	Trigona amazonensis	100,000–200,000	150,000	Combs	Roubik (1989, pp. 109), Inoue et al. (1996)
	Trigona branneri	43,758–59,820	51789	Combs	Roubik (1979)
	Trigona cilipes	400–3204	1800	Combs	Roubik (1979, 1983)
	Trigona corvina	7000–25,000	16,000	Combs	Michener (1974), Roubik (1983)
	Trigona ferricauda		1000	Combs	Roubik (1983)
	Trigona fulviventris	2000–10,000	5750	Combs	Roubik (1983)
	Trigona fuscipennis	5000–10,000	7500	Combs	Roubik (1983)
	Trigona hyalinata	Up to 40,000		Combs	Nieh et al. (2003)
	Trigona hypogea	1200–5000	1750	Combs/spirals	Roubik (1983), Camargo and Roubik (1991)
	Trigona necrophaga	1000–3000	2000	Combs/spirals	Camargo and Roubik (1991)
	Trigona nigerrima	700–1800	1398	Combs	Roubik (1983)
	Trigona pallens	658–8170	4400	Combs/semi-combs	Roubik (1979)
	Trigona silvestriana		3000	Combs	Roubik (1983)
	Trigona spinipes	5000–180,000	92500	Combs	Michener (1974)
	Trigona williana		2492	Semi-combs	Roubik (1979)

(continued)

Table 1.3 (continued)

Region	Species	Colony size range	Av. colony size	Brood cell type	References
	Trigonisca atomaria	160–500	330	Clusters	Wille and Michener (1973), Roubik (1983)
	Trigonisca buyssoni	136–600	368	Clusters	Wille and Michener (1973), Roubik (1983)

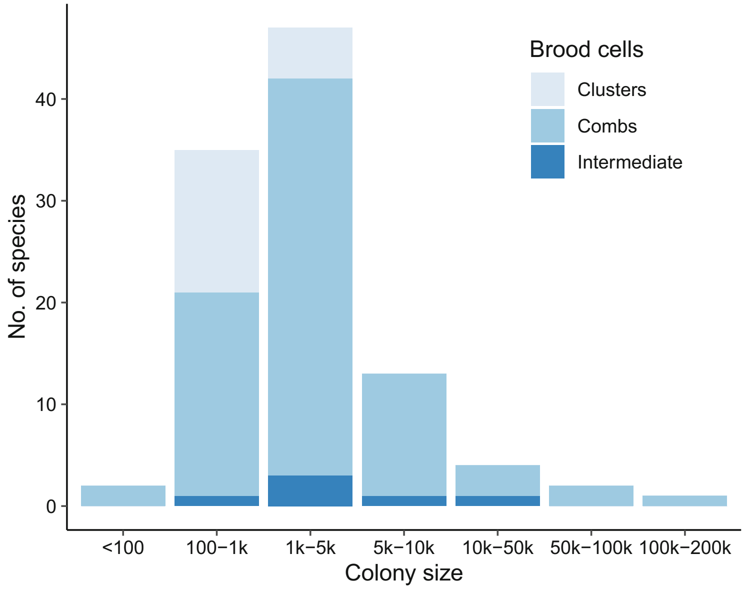

Fig. 1.13 Colony sizes of 104 stingless bee species (Table 1.3). Shown are colony sizes (k = thousand) and the type of brood cell arrangement (see Chap. 3). The "combs" category includes species that build spirals. "Intermediate" refers to semi-combs

One way to obtain accurate estimates would be to close colonies when all foragers are inside, kill or anesthetise all bees and count or weigh them (see e.g. Roldão-Sbordoni et al. 2018). However, this is not usually feasible, especially when conducting field research. Therefore, researchers have attempted to estimate colony size based on traits that are easier to measure, such as the number of brood cells (von Ihering 1903; Roubik 1979). Roubik (1979) suggested the formula $B_c = \frac{1}{4}\,\mathrm{Br} + \frac{1}{2}\,B_e$, where B_c is the estimate of the total number of adult bees, Br is the total brood count and B_e is the field estimate of the total adult bees. The field estimate refers to the number of bees in flight near the opened nest and on the combs. Von Ihering (1930, cited in Duarte et al. 2016) used the formula $x + x/2$, where x is the number of

Fig. 1.14 A *Trigona hyalinata* nest on the wall of "Bloco 7" of the University of São Paulo, Ribeirão Preto, that was 18 years old at the time of writing (established in 2002, photo taken in 2016 by C. Grüter). Any change in ownership would most likely have been noticed by the numerous entomologists that walk past this nest on a daily basis

brood cells, to calculate the total population (including all brood). This formula means that the number of adult bees is estimated to be ~50% of the number of brood cells. Adult bee population estimates in Roubik (1983), on the other hand, represented about 1/3 of the number of brood cells. Roldão-Sbordoni et al. (2018) counted both brood and adult population and found that adult population (i.e. colony size as used here) was ~40% of the brood population. Any rule of thumb that is based on the number of brood cells should be used with caution because the ratio of adult to immature bees is likely to vary with season and species (see Chap. 5). For some biological questions, it is enough to assess the relative colony sizes ("colony A is larger than colony B"). In these situations, counting brood cells or comparing foraging traffic can be straightforward solutions.

1.7 Colony Longevity

Stingless bee colonies can, in theory, exist continuously for very long time periods because aging or dead queens can be replaced by new queens (Chap. 4). Possibly in anticipation of the risk of queen loss, some colonies even keep "spare" unmated queens in "imprisonment" chambers (Chap. 4). Indeed, various examples of colonies living in the same cavity for more than 10 years have been reported (e.g. Schwarz 1948). Kerr et al. (1967) studied a *Frieseomelitta flavicornis* colony that was 12 years old when the study was performed and Starr and Sakagami (1987) describe a *Tetragonula sapiens* nest of at least 10 years of age. A *Trigona hyalinata* colony on the campus of the University of São Paulo in Ribeirão Preto continuously existed for 18 years at the time of writing this book (Fig. 1.14) and reports of *T. hyalinata* nests reaching 20–30 years are not uncommon (Sidnei Mateus, personal communication). Murillo (1984, cited in Roubik 1989, p. 198) examined 35 managed colonies of

Melipona beecheii in southern Mexico and reported an average colony age of 10–19 years. Astonishingly, a managed colony of *M. beecheii* has reportedly reached an age of at least 61 years (Slaa 2006) and Schwarz (1948) mentioned a managed colony of the same species that, according to personal communication, was more than a century old. It should be noted, however, that it can be difficult to distinguish continuous occupation by the same colony from successive occupation by different colonies from the same species. Michener (1946), for example, observed a nest site that was occupied by three different *Tetragonisca angustula* colonies in a 14-month observation period. In the case of managed colonies or colonies that are regularly checked we can be more confident of a continuous history of residence.

Estimation of median life expectancy of colonies requires frequent and continuous monitoring. Slaa (2006) performed such a study to quantify the yearly colony mortality rate of stingless bee species in Costa Rica during a 4-year period. She estimated that the annual survivorship probability of the different species was as high as 0.96. Based on this, she inferred an average colony lifespan of ~23 years. This was considerably longer than Africanised honey bees living in the same habitats (Slaa 2006). Eltz et al. (2002) and Kajobe and Roubik (2006) found yearly colony survival probabilities of ~85–88% in Ugandan and Malaysian species, suggesting an average colony longevity of ~4–7 years. These estimates in combination with the low propensity to swarm (Chap. 4) suggest that stingless bees follow a life-history strategy of high colony survival and relatively low colony reproduction (Slaa 2006).

1.8 Importance for Humans

Humans have kept stingless bees and used their products since Millenia (Crane 1992, 1999; Jones 2013; Źrałka et al. 2014, 2018; Quezada-Euán 2018; Quezada-Euán and Alves 2020). The Mayans, in particular, incorporated meliponiculture in their social, economic and religious activities (Cortopassi-Laurino et al. 2006; Rosales 2013; Quezada-Euán 2018; Źrałka et al. 2018; Quezada-Euán and Alves 2020). Honey and cerumen, for example, were used for trading with the Aztecs (Nogueira-Neto 1951; Quezada-Euán 2018) and as a way to pay taxes to Spanish conquistadores in the 16th century (Jones 2013; Quezada-Euán 2018). Stingless bees continue to be important in the traditions and cultures of many native communities, such as the Kayapó in Brazil (Camargo and Posey 1990; Quezada-Euán et al. 2018), the Aché in Paraguay (sometimes called "civilisation of honey") (Nogueira-Neto 1951), the Abayanda pygmies in Uganda (Byarugaba 2004), rural communities in Madagascar (Randrianandrasana and Berenbaum 2015), indigenous communities in the Himalayas (Bhatta et al. 2020) and the Yolngu Aboriginal communities in Australia (Fijn and Baynes-Rock 2018).[11]

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[11] The first report in the European literature on stingless bees and how they were used by humans appeared in "Warhaftig Historia" published 1557. In it, the German mercenary Hans Staden described how the Brazilian Tupinambá tribe that held him captive for many months regularly collected stingless bee honey for consumption (Engels 2009, 2013).

Meliponiculture continues to provide income to people in many communities in the tropics (Cortopassi-Laurino et al. 2006; Kwapong et al. 2010; Alves 2013; Tornyie and Kwapong 2015; Quezada-Euán et al. 2018). Dozens of different species are kept for honey or cerumen production (Crane 1992; Cortopassi-Laurino et al. 2006). Among the most popular species are *Tetragonula carbonaria* in Australia (Heard 2016; Chapman et al. 2018) and *Melipona beecheii*, *M. eburnea*, *M. quadrifasciata*, *M. scutellaris* and *Tetragonisca angustula* in the Neotropics (Jaffé et al. 2015; Quezada-Euán 2018; Quezada-Euán and Alves 2020). More recently, stingless bees have been employed to improve the pollination of crops (Chap. 9) (Heard 1999; Slaa et al. 2006; Giannini et al. 2015).

1.8.1 Meliponiculture

In Mesoamerica, the Mayans have practiced large-scale intensive meliponiculture[12] since pre-Columbian times (Fig. 1.15) (Źrałka et al. 2014, 2018; Quezada-Euán 2018). The clay hive shown in Fig. 1.15a has been dated to the Protoclassic phase of the Maya culture (ca. 100 BC–300 AD) and is the oldest known Mayan beehive (Źrałka et al. 2014, 2018). This and other artefacts suggest that meliponiculture, mainly with *Melipona beecheii*, has been practiced in Mesoamerica continuously for the last ~1700–2000 years (Crane 1992; Źrałka et al. 2014, 2018; Quezada-Euán 2018; Quezada-Euán and Alves 2020).

The main products of meliponiculture are honey, resin and cerumen (Nogueira-Neto 1997; Cortopassi-Laurino et al. 2006; Kwapong et al. 2010; Jaffé et al. 2015; Heard 2016; Quezada-Euán 2018; Quezada-Euán et al. 2018), but many people keep stingless bees simply for pleasure and relaxation (Carvalho et al. 2018). Colonies are collected from the wild and transferred to wooden boxes, logs or clay pots (Fig. 1.16). In the last decades, different types of rational hives have been developed, most notably by Paulo Nogueira-Neto in Brazil (Nogueira-Neto 1997). Experienced beekeepers can establish new colonies by dividing existing ones (Quezada-Euán 2018; Źrałka et al. 2018), a practice that was already used by the ancient Mayans (Quezada-Euán 2018; Źrałka et al. 2018).

Keeping stingless bees in artificial housing is relatively widespread in the Americas, particularly in Brazil and Mexico (Jaffé et al. 2015; see Quezada-Euán 2018 for a comprehensive description of meliponiculture in Mexico), but is less widespread in Africa where *Apis mellifera* is traditionally preferred and the

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[12] Tomás López Medel, who was a government official in Yucatán in the 1550s wrote about beekeeping and honey production by the Mayans in his treatise De los tres elementos: “There is no part of the Indies, nor do I believe Spain, where as much honey and wax are produced as in the province of Yucatán, and therefore the Indians and natives of those provinces [. . .] are not content with honey and wax brought from the mountains and common places, but also have many hives in their houses and other places.” (from Źrałka et al. 2018).

Fig. 1.15 Mayan artefacts showing the importance of stingless bees in culture and religion. (**a**) Clay hive from the Protoclassical period, discovered at the Nakum site in Guatemala (Źrałka et al. 2014, 2018). It is not clear whether the hive was used to keep bees or as a representation of a bee hive. (**b**) Deity with a beehive as a necklace (from Źrałka et al. 2014). (**c**) Representations of the god "Mulzencab" or "Ah-Muzen-Cab" in the Madrid codex, p. 105. The Madrid codex or Tro-Cortesianus codex is one of only three surviving Mayan books and is dated to ~900–1500 AD

extraction of honey and cerumen from wild stingless bee nests often destroys colonies (Cortopassi-Laurino et al. 2006). Meliponiculture is practiced, for example, in Angola and Tanzania and interest in managing stingless bees is increasing in other African countries as well (Cortopassi-Laurino et al. 2006; see Kwapong et al. 2010 for an introduction to meliponiculture in Ghana). Meliponiculture is also gaining popularity in Australia where colonies are kept for the production of honey, crop pollination (e.g. of *Macadamia*) or conservation (Heard 1999, 2016). Managing stingless bee colonies is not common in tropical Asia where honey can be obtained in larger quantities from native honey bees, e.g. *Apis cerana* or *A. dorsata* (Cortopassi-Laurino et al. 2006; Kumar et al. 2012). However, the Kani communities in southern India have developed bamboo logs to keep *Tetragonula iridipennis* colonies (Fig. 1.16d) (Kumar et al. 2012) and meliponiculture is being developed in other parts of Asia as well, such as southwest China (Pan et al. 2020).

1.8.2 Medicinal Use of Stingless Bee Products

The medicinal use of stingless bee honey and cerumen is often the main reason why stingless bee products are harvested (Cortopassi-Laurino et al. 2006; Kumar et al. 2012; Choudhari et al. 2012; Chanchao 2013; Rodríguez-Malaver 2013; Zamora et al. 2013; Carvalho et al. 2018; Quezada-Euán et al. 2018; Bhatta et al. 2020). Numerous beneficial effects have been attributed to stingless bee honey and cerumen or some of their compounds (for review see e.g. Vit et al. 2004; Rosales 2013; Rao

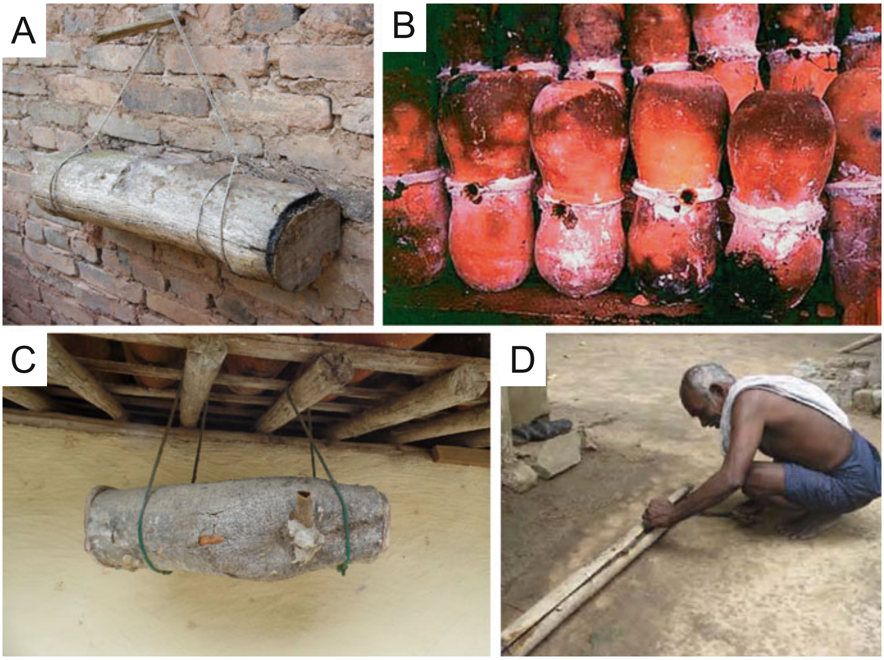


Fig. 1.16 Different types of log and clay hives. (**a**) Log hive containing a *Tetragonula* colony from Xishuangbanna, Yunnan province in China (photo by Bingfeng Zhou). (**b**) Clay pots of *Scaptotrigona pectoralis* from Mexico (from Vit et al. 2004). (**c**) Log hive of *Scaptotrigona* sp. at Sítio Xixá, state of Pernambuco, Northeast Brazil (from Carvalho et al. 2018). (**d**) Bamboo logs used by the Kani people in southern India to keep stingless bees (Kumar et al. 2012)

et al. 2016; Amin et al. 2018). Nepali communities in India use the honey of *Lepidotrigona arcifera* for various health problems, ranging from diarrhoea to asthma (Biswa et al. 2017). Abayanda pygmies in Uganda use stingless bee honey against constipation (Byarugaba 2004). Communities in northern Argentina treat a wide range of illnesses related to the digestive, respiratory and reproductive system with *Plebeia* honey (Flores et al. 2018). In Bolivia, Guatemala, Mexico and Venezuela, the honey of *Tetragonisca angustula* is particularly often used to treat eye problems, among many other ailments (Vit et al. 2004; Dardón et al. 2013; Ferrufino and Vit 2013). More research is needed to assess the effectiveness of stingless bee products as treatments for different illnesses.

1.8.3 Spiritual and Religious Importance of Stingless Bees

In some native communities, stingless bees are considered to be sacred and bees often play important roles in their cosmology and mythology (Carvalho et al. 2018; Quezada-Euán 2018; Quezada-Euán et al. 2018). Especially *Melipona beecheii* (called Xunan kab or "lady bee" in Mayan) has a long history of cultural and religious importance in the Mayan culture and its honey considered sacred (Cortopassi-Laurino et al. 2006; Quezada-Euán 2018; Źrałka et al. 2018). One of the Mayan gods of honey and bees, "Mulzencab" or "Ah-Muzen-kab", is repeatedly depicted in the *Madrid Codex* as what seems to be a bee in defensive position seen from the front (Fig. 1.15c). The Kayapó in Brazil use cerumen and honey in their religious ceremonies (Cortopassi-Laurino et al. 2006; Quezada-Euán et al. 2018) and communities in north-western Argentina use honey as offerings to Pachamama, the "mother of earth" and goddess of fertility in cultures of Andean communities (Flores et al. 2018). In some areas in Madagascar, on the other hand, the consumption of stingless bee honey is considered a taboo and some American communities discard pollen as "filth" (Randrianandrasana and Berenbaum 2015; Quezada-Euán et al. 2018).

1.8.4 Other Uses of Stingless Bee Products

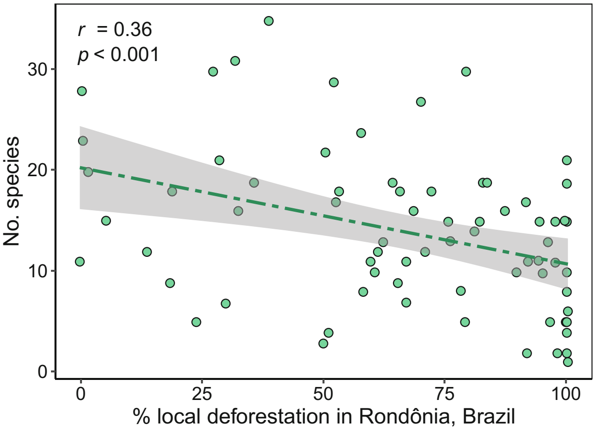
Cerumen and wax are versatile building materials, either in their natural state or after being cooked (Stearman et al. 2008) and are used for the production of everyday objects or foods. The Mayans used cerumen to create the moulds for their gold ornaments and figurines in a process known as "lost wax casting" (Schwarz 1945; Crane 1992; Jones 2013). Aboriginal artists in Australia used cerumen to protect their rock paintings from damage by rain (Schwarz 1945). Cerumen and resin are also used as an adhesive or sealing material in the production of weapons, e.g. arrows and blowguns, or when making canoes by the Yuquí in Bolivia (Stearman et al. 2008) or the Kitchwa in Ecuador (see Schwarz 1945 and Quezada-Euán et al. 2018 for more examples).

In the Andes region, stingless bee honey is occasionally added to alcoholic drinks that serve as an aphrodisiac, as well as to *chicha*, a drink of cultural importance to communities in the Andean region (Flores et al. 2018). Pollen is sometimes used as a supplement in food and alcoholic drinks (Flores et al. 2018). Finally, the bee larvae and pupae themselves are consumed by humans in some communities, e.g. in Mexico (Ramos-Elorduy 2002), Brazil, Ecuador, Paraguay or Northern Australia (Fijn and Baynes-Rock 2018; Quezada-Euán et al. 2018).

1.9 Emerging Challenges in the Anthropocene

Like many other insects, stingless bees face new challenges, such as agrochemicals, new pathogens or climate change (Potts et al. 2010, 2016; Ramírez et al. 2013; Lima et al. 2016; Tsvetkov et al. 2017; Giannini et al. 2017, 2020; Sánchez-Bayo and Wyckhuys 2019; Guimarães-Cestaro et al. 2020). A key challenge is the rapid anthropogenic alteration of the environment, mainly the conversion of natural and semi-natural land into urban or intensely farmed land (e.g. Winfree et al. 2009; Potts et al. 2010, 2016). In 2019 alone, 38,000 km^2 of tropical primary forest were lost due

Fig. 1.17 Number of stingless bee species sampled (as bees were collecting resources) at different locations in Rondônia, Brazil. The *x*-axis shows the percentage of deforested area within 500 m of the sampling site. Grey area shows the 95% confidence interval (modified from Brown and Oliveira 2014)



to deforestation, an area approximately the size of the Netherlands.[13] As a result, many human-modified landscapes no longer provide enough food or nesting space to maintain stingless bees (e.g. Brown and Albrecht 2001; Cairns et al. 2005; Brosi et al. 2008; Ramírez et al. 2013; Kaluza et al. 2018). Stingless bees might be particularly vulnerable to deforestation because a majority of species live in tree cavities (Chap. 3) and because mass-flowering trees are a critical food source for many of them (Chap. 8). A better understanding of how deforestation affects stingless bee populations is urgently needed given the pollination services they provide to natural and cultivated plants. One study assessing the effects of deforestation on stingless bee diversity found that the number of stingless bee species in a habitat in western Brazil decreased as the degree of local deforestation increased (Fig. 1.17) (Brown and Oliveira 2014). It is also noteworthy, however, that some species show a surprising ability to adapt to highly modified environments (Chap. 3).

1.9.1 Pesticides

There is accumulating evidence that pesticides have contributed to bee decline in temperate areas (e.g. Henry et al. 2012; Gill et al. 2012; Goulson et al. 2015; Siviter et al. 2018a). Neonicotenoids (e.g. imidacloprid, thiamethoxam) and phenylpyrazols (e.g. fipronil), for example, are neurotoxins that impair the functioning of the central nervous system of bees (e.g. Goulson et al. 2015; Samuelson et al. 2016; Siviter et al. 2018b). Pesticides acquired in low doses might not kill individuals and colonies immediately, but they can have so-called sub-lethal effects by impairing learning and memory, navigation and reproduction (e.g. Henry et al. 2012; Gill et al. 2012; Balbuena et al. 2015; Samuelson et al. 2016; Siviter et al. 2018a). Given these negative effects of pesticides on honey bees and bumble bees and an increased use of

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[13] Data from https://www.globalforestwatch.org/

pesticides in countries like Brazil (Santos et al. 2018), concerns about negative effects on stingless bees are warranted and a growing number of studies have assessed how different species respond to pesticides and other agrochemicals that are commonly used in the tropics (reviewed in Lima et al. 2016). Worryingly, studies assessing the toxicity (e.g. measured as the dose that leads to a 50% mortality, the LD_{50}) of different pesticides show that pesticides such as fipronil, imidacloprid or thiamethoxam are even more toxic to stingless bees than to honey bees (Valdovinos-Núñez et al. 2009; Jacob et al. 2013, 2019a; Sarto et al. 2014; Costa et al. 2015; Moreira et al. 2018, but see Jacob et al. 2019b). The reported effects of pesticides on stingless bees are numerous and varied (Lima et al. 2016). For example, pesticides present in brood food increase larval mortality, impair brain development, reduce adult activity and cause larvae in queen cells to develop into workers (Tomé et al. 2012; Barbosa et al. 2015; Santos et al. 2016; Seide et al. 2018). Adult workers ingesting sub-lethal doses of commonly used pesticides show reduced locomotion, trophallaxis and antennation among nestmates, indicating that pesticides potentially impair the social functioning of colonies (Boff et al. 2018; Araujo et al. 2019; Jacob et al. 2019a, b). Most studies to date have been performed under laboratory conditions and we currently know relatively little about whether the applied doses are similar to those experienced by adult stingless bees and larvae in more natural conditions. Gómez-Escobar et al. (2018), however, tested the effects of a pesticide (Spinosad based) that was repeatedly applied in a mango orchard according to manufacturer recommendations. Their data indicate that colonies of *Scaptotrigona mexicana* placed in the orchard had reduced colony growth compared to colonies in a control orchard. Again, the pesticide appears to be more harmful to stingless bees than to honey bees (Gómez-Escobar et al. 2018). More research is needed that assesses the impact of widely used pesticides under field conditions. This would lead to a better understanding of the potential population level impacts of pesticide use on stingless bees.

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Evolution and Diversity of Stingless Bees

2

Stingless bees (Meliponini) belong to the corbiculate bees, a monophyletic group of bees characterised, among other things, by their particular pollen-carrying structures ("pollen baskets" or corbiculae) that form a part of the hind legs (Chap. 1; Fig. 1.1) (Michener 2007). The evolution of the concave pollen baskets allowed corbiculate bees to transport large amounts of pollen and especially resin in an efficient way (Martins et al. 2014).[1] Three other tribes belong to the corbiculate bees, the highly eusocial honey bees (Apini), the "primitively eusocial" bumble bees (Bombini), and the mostly solitary orchid bees (Euglossini) (Grimaldi and Engel 2005; Michener 2007). The term *higher eusocial* (sometimes also *advanced eusocial* or *superorganismal*) is often used to refer to species with morphologically distinct queen and worker castes (Michener 1974, 2007; Danforth et al. 2013; Boomsma and Gawne 2018). Occasionally, a perennial colony lifestyle (Michener 2007) or extensive food exchange among the adults (Michener 1974) is mentioned as defining features of highly eusocial colonies. In *primitively eusocial* colonies, queens and workers appear morphologically similar and can be distinguished mainly by their size (Michener 1974, 2007; Danforth et al. 2013).[2] Stingless bees can be distinguished from the other corbiculate bees by a number of morphological traits, including a reduced forewing venation, the penicillum, the absence of an auricle (pollen press), the presence of a jugal lobe in the hind wing, and the reduced sting (Figs. 1.1 and 1.3) (see Michener 2007 for details).

The relationships among the four corbiculate bee tribes have been a matter of much debate. Two principal and related questions are (1) whether stingless bees are more closely related to honey bees or to bumble bees and (2) whether they have evolved higher eusociality independently from honey bees versus a single origin of

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[1] It is hypothesized that the evolution of a corbicula is related to an increased collection and use of resin for nesting (Melo 2020).

[2] Closer examination reveals many other differences, e.g. in behaviour and physiology. See Boomsma and Gawne (2018) for a recent, thorough discussion of the terminology.

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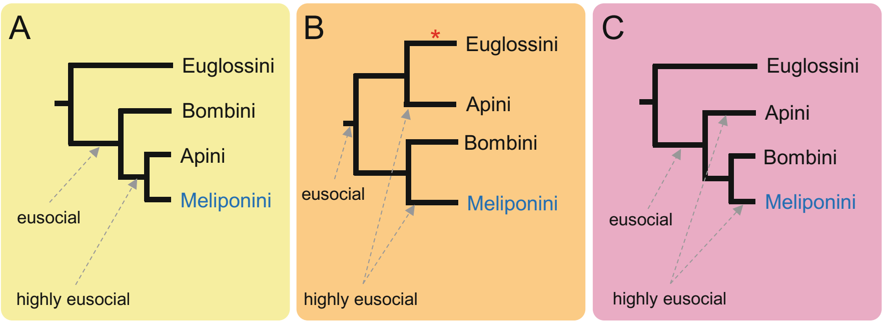

Fig. 2.1 Three possible phylogenetic trees for the four tribes of extant corbiculate bees. (**a**) was suggested by Michener (1944) and Noll (2002). The red star in (**b**) indicates that eusociality was lost in the Euglossini (Cardinal and Danforth 2011)

higher eusociality in the common ancestor of stingless bees and honey bees. Darwin was among the first to speculate about the relationships among the three eusocial groups of corbiculate bees in the *Origin of Species* (Darwin 1859, Chap. 7). Based on the architecture of the brood combs of the stingless bee *Melipona beecheii* he argued that this species is intermediate between bumble bees and honey bees, but more closely related to bumble bees. In the last decades, numerous studies have explored this hypothesis and addressed the relationships among the different corbiculate bee groups. A key challenge has been that phenotypic and molecular data often lead to different conclusions (e.g. Danforth et al. 2013; Almeida and Porto 2014; Porto et al. 2016). Studies relying mainly on phenotypic (morphological and/or behavioural) data have suggested that Meliponini and Apini are sister groups and that higher eusociality evolved once in their common ancestor (Fig. 2.1a) (Schultz et al. 2001; Noll 2002; but see Winston and Michener 1977), whereas molecular studies predominantly suggested Meliponini and Bombini as sister groups and two independent origins of higher eusociality in Apini and Meliponini (Fig. 2.1b, c) (Lockhart and Cameron 2001; Cameron and Mardulyn 2001; Rasmussen and Cameron 2007; Kawakita et al. 2008; Cardinal and Danforth 2011, 2013; Martins et al. 2014; Martins and Melo 2016; Romiguier et al. 2016; Bossert et al. 2017, 2019). In a comprehensive analysis, Payne (2014) combined morphological, behavioural and molecular data to resolve the phylogenetic relationships among the corbiculate bees and found strong support for the second scenario. More recent analyses provide further support for a dual origin of higher eusociality in corbiculate bees and a sister group relationship between stingless bees and bumble bees. According to the currently best supported scenario (Fig. 2.1c), eusociality evolved once in the common ancestor of the Apini, the Bombini and the Meliponini (Romiguier et al. 2016; Bossert et al. 2017, 2019). This suggests that the ancestors of present-day honey bees and stingless bees independently evolved a highly eusocial lifestyle, i.e. distinct morphological female castes, perennial colonies and extensive food sharing (but see Melo 2020). Two separate origins of higher eusociality would help to explain why honey bees and stingless bees have found markedly different solutions to the problem of colony reproduction (Chap. 4), brood rearing (Chap. 5), colony defence (Chap. 7) or foraging communication (Chap. 10) (see also Table 1.2).

2.1 Present-Day Diversity and Distribution

2.1.1 How Many Species Are There?

About 550 species belonging to ~58 genera have been described (Table 2.1 lists 552 species; Figs. 1.2 and 2.2), which makes the Meliponini the largest and most diverse group of corbiculate bees. In comparison, there are ~11 species of honey bees, ~250 species of bumble bees and ~200–250 species of orchid bees (Michener 2007; Danforth et al. 2013; Ascher and Pickering 2018). Furthermore, many undescribed stingless bee species exist (e.g. Eardley 2004; Michener 2007; Rasmussen and Camargo 2008; Freitas et al. 2009; Rasmussen and Cameron 2010; Pedro 2014; Hurtado-Burillo et al. 2017; Ndungu et al. 2017; Roubik 2018). For example, the tiny Neotropical *Plebeia minima* represents a species group that is likely to consist of several species (Drumond et al. 2000). Roubik (2018), in a survey of a relatively small parcel of the hyperdiverse Yasuní National Park in the Ecuadorian Amazon, reported 16 undescribed species of *Trigonisca* alone. Overall, 43 of the 100 species recorded in Yasuní seemed to be new to science (Roubik 2018). When surveying the stingless bees of Brazil, Pedro (2014) reported 244 described and 89 undescribed species in the Entomological Collection “Prof. J.M.F. Camargo” (University of São Paulo, Ribeirão Preto) that await careful taxonomic revision. Thus, the Neotropics alone are probably home to more than 500 species.

Table 2.1 provides a list of genera that relies mainly on the names proposed by Moure (1961), updated by Rasmussen and Cameron (2007, 2010). Others have proposed a different “lumping” system to avoid overabundance of genera (see Wille and Michener 1973; Wille 1979; Michener 1990, 2007). I follow Sakagami’s (1982) reasoning that Moure’s taxa represent natural groups that show significant behavioural, ecological and genetic differences (see also Rasmussen and Cameron 2010; Roubik 2018). Sakagami (1982) also points out that the two classification systems often do not contradict each other greatly and, thus, show substantial correspondence.[3] (Rasmussen (2008) and Rasmussen et al. (2017) provide a helpful comparison of the different genus names.)

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[3] Note that many species have changed their scientific name, some more than once. Discrepancies between the names used in this book and those used in the cited literature are best resolved by consulting Camargo and Pedro (2013), Rasmussen and Cameron (2007, 2010), Rasmussen et al. (2017) and Eardley (2004).

Table 2.1 Stingless bee genera and the number of species in them. See the genus and species list (Sect. 2.4) for references

Region	Genus	No. of species
Neotropics	*Aparatrigona* Moure, 1951	2
	Camargoia Moure, 1989	3
	Celetrigona Moure, 1950	4
	Cephalotrigona Schwarz, 1940	5
	Duckeola Moure, 1944	2
	Friesella Moure, 1946	1
	Frieseomelitta Ihering, 1912	16
	Geotrigona Moure, 1943	22
	Lestrimelitta Friese, 1903	23
	Leurotrigona Moure, 1950	4
	Melipona Illiger, 1806	74
	Meliwillea Roubik, Lobo and Camargo, 1997	1
	Mourella Schwarz, 1946	1
	Nannotrigona Cockerell, 1922	10
	Nogueirapis Moure, 1953	4
	Oxytrigona Cockerell, 1917	11
	Parapartamona Schwarz, 1948	7
	Paratrigona Schwarz, 1938	32
	Paratrigonoides Camargo and Roubik, 2005	1
	Partamona Schwarz, 1938	32
	Plebeia Schwarz, 1938	42
	Plectoplebeia Melo, 2016	1
	Ptilotrigona Moure, 1951	3
	Scaptotrigona Moure, 1942	23
	Scaura Schwarz, 1938	8
	*Scaura (*formerly *Schwarzula* Moure, 1946)	2
	Schwarziana Moure, 1943	4
	Tetragona Lepeletier and Audinet-Serville, 1828	13
	Tetragonisca Moure, 1946	4
	Trichotrigona Camargo and Moure, 1983	2
	Trigona Jurine, 1807	32
	Trigonisca (formerly *Dolichotrigona* Moure, 1950)	10
	Trigonisca Moure, 1950	27
	31 genera	*426*
Afrotropical	*Apotrigona* Moure, 1961	1
	Axestotrigona Moure, 1961	7
	Cleptotrigona Moure, 1961	1
	Dactylurina Cockerell, 1934	2
	Hypotrigona Cockerell, 1934	5
	Liotrigona Moure, 1961	12
	Meliplebeia Moure, 1961	4
	Meliponula Cockerell, 1934	1

(continued)

Table 2.1 (continued)

Region	Genus	No. of species
	Plebeiella Moure, 1961	2
	Plebeina Moure, 1961	1
	10 genera	*36*
Indo-Malay/ Australasia	*Austroplebeia* Moure, 1961	5
	Geniotrigona Moure, 1961	2
	Heterotrigona Schwarz, 1939	3
	Homotrigona Moure, 1961	4
	Lepidotrigona Schwarz, 1939	13
	Lisotrigona Moure, 1961	6
	Lophotrigona Moure, 1961	1
	Odontotrigona Moure, 1961	1
	Papuatrigona Michener and Sakagami, 1990	1
	Pariotrigona Moure, 1961	1
	Platytrigona Moure, 1961	5
	Sahulotrigona Engel and Rasmussen, 2017	2
	Sundatrigona Inoue and Sakagami, 1993	2
	Tetragonilla Moure, 1961	4
	Tetragonula Moure, 1961	34
	Tetrigona Moure, 1961	5
	Wallacetrigona Engel and Rasmussen, 2017	1
	17 genera	*90*
Total	58 genera	552

2.1.2 Where Can Stingless Bees Be Found?

Stingless bees live in the tropical and subtropical regions of Africa, Asia, Australia and the Americas (Fig. 2.3). They are most abundant and species-rich in the Neotropics (~426 species), where they occur from Cuba and Mexico (State of Sinaloa) in the north to Argentina in the south (Fig. 2.3). The species with the southernmost distributions in Argentina (*Plebeia* spp.) can be found in the provinces of San Luis, Santa Fe and Buenos Aires, including the city of Buenos Aires (Roig-Alsina et al. 2013; Mazzeo and Torretta 2015; Roig-Alsina and Alvarez 2017). In Africa (~36 species, including seven species endemic to Madagascar[4]), stingless bees are most diverse in equatorial regions (Eardley 2004; Eardley and Kwapong 2013; Anguilet et al. 2015). To the north, the Sahara Desert is a natural barrier. To the south, stingless bees reach as far as South Africa and southern Madagascar. Most

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[4] The number of African species varies greatly depending on the source (e.g. 32 in Kerr and Maule 1964; 19 in Eardley 2004; 21 in Anguilet et al. 2015; 24 in Kajobe and Roubik 2018) and Eardley and Kwapong (2013) highlight that diversity might be higher than is currently known. Based on these and other sources (e.g. Michener 2007; Rasmussen 2008), I have included 36 species (see Sect. 2.4.2), but taxonomic revisions of African genera are needed (Eardley and Kwapong 2013).

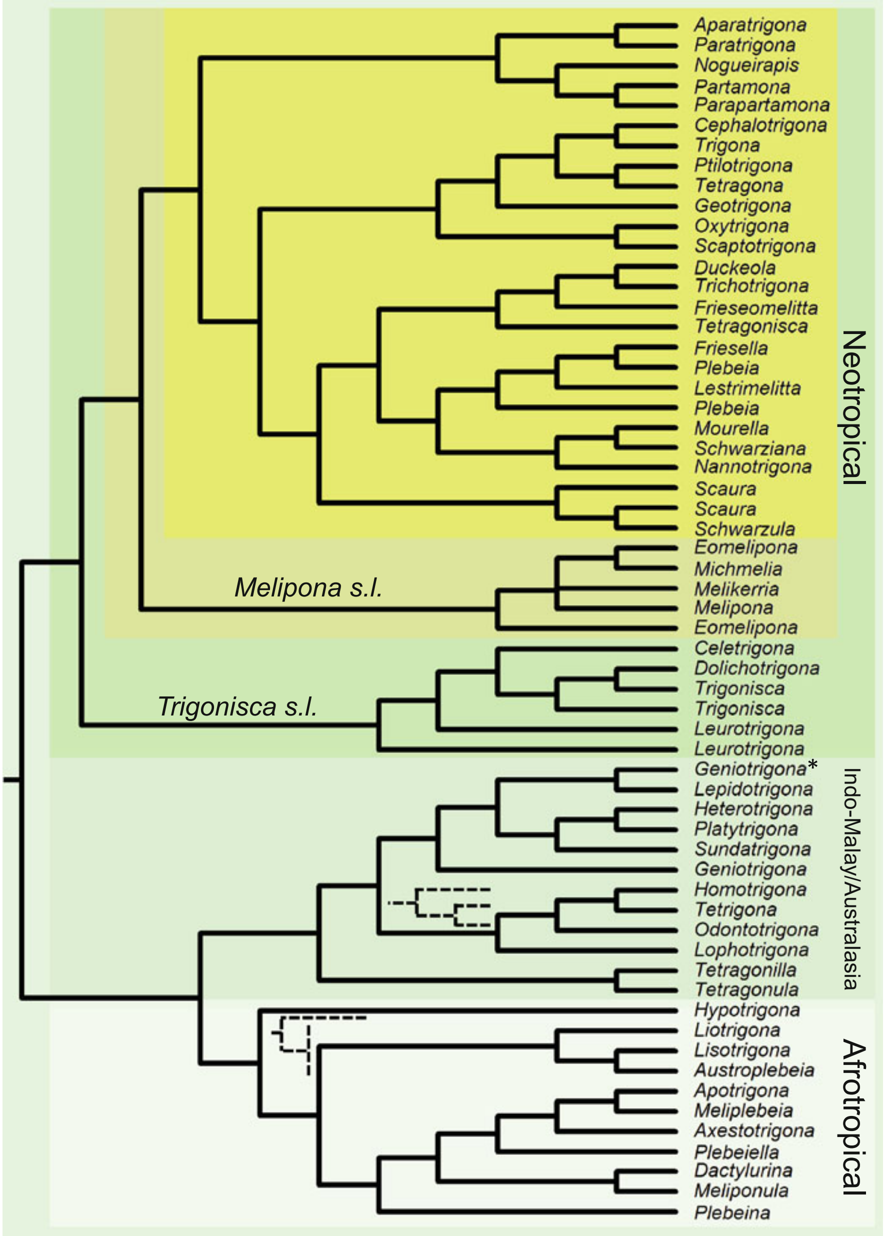

Fig. 2.2 Stingless bee phylogeny at genus level including most genera (modified from Rasmussen and Cameron 2010). Alternative resolutions are shown as dotted lines; s.l. = sensu *lato* (in the broad sense). Based on this tree, Rasmussen and Cameron (2010) suggested to synonymise *Schwarzula* under *Scaura* and *Dolichotrigona* under *Trigonisca*. *Geniotrigona incisa* (the *Geniotrigona** closely related to *Lepidotrigona*) has since been placed in the new genus *Wallacetrigona* as *W. incisa* (Rasmussen et al. 2017) (see Table 2.1). Note that *Leurotrigona* and *Plebeia* are not monophyletic

Fig. 2.3 Present-day distribution of stingless bees (blue areas) (based on Kerr and Maule 1964; Sakagami 1982; Camargo et al. 1988; Pauly et al. 2001; Camargo and Pedro 2003; Eardley 2004; Rasmussen 2008, 2013; Vit et al. 2013; Dollin et al. 2015). See text for more details

African species live in tropical forests or in both tropical forests and savannahs. Two species have been recorded from desert areas (*Hypotrigona penna* and *Liotrigona* sp.) (Eardley and Kwapong 2013). In Asia and Australia (~90 species), stingless bee distribution stretches from India (and possibly southeast Pakistan) in the west (Rasmussen 2013) to the Solomon Islands in the east and from Nepal, China (Yunnan, Hainan) and Taiwan in the north to Australia in the south (Rasmussen 2008, 2013; Pan et al. 2020). The southernmost distribution of *Tetragonula carbonaria* in Australia (New South Wales) includes areas with temperate climate (Halcroft et al. 2013). Phylogenetic analyses confirm a principal division into three different groups that diverged at an early stage in the evolutionary history of Meliponini: the Afrotropical, the Indo-Malay/Australasia and the Neotropical lineages (Fig. 2.2) (Rasmussen and Cameron 2010).

While the highest diversity is often found in lowland rain forests, particularly in the Amazon basin (Biesmeijer and Slaa 2006; Melo 2020), stingless bees can inhabit places at elevations of up to 2500 m in Taiwan (*Lepidotrigona hoozana*), 3400 m in the Colombian, Ecuadorian and Peruvian Andes (e.g. *Parapartamona* spp.) and 2500 m in Sumatra, Borneo or New Guinea (Roubik 1989; Gonzalez and Smith-Pardo 2003; Sung et al. 2011). In order to survive at these elevations, colonies need to be able to maintain nest temperatures to some degree and they may stop brood production during the coldest periods of the year (see Chaps. 3 and 5).

2.2 The Origin of Stingless Bees

The fossil record of stingless bees is better than for most other bee groups and twelve extinct species of stingless bees are currently known (Engel and Michener 2013). Fossils are found in amber (fossilised tree resin) and copal (young amber) where bees are often preserved in excellent condition (Fig. 2.4). This comparatively good fossil record might be linked to the fact that stingless bees collect large amounts of

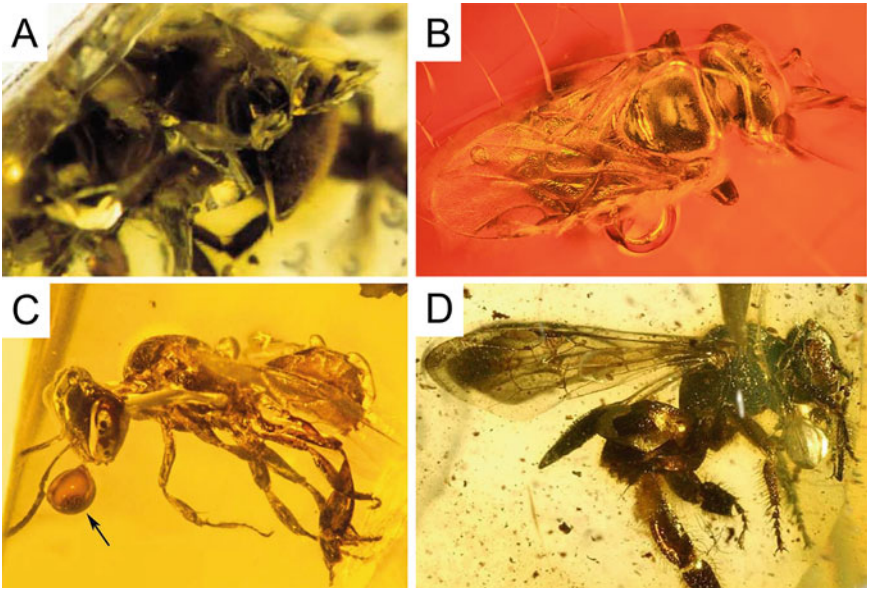

Fig. 2.4 (**a**) *Cretotrigona prisca* worker bee, the oldest known fossil stingless bee, dating from ~65 to 70 Mya (from Michener and Grimaldi 1988, Engel 2000a). (**b**) *Exebotrigona velteni*, a specimen found in Baltic amber from the Eocene (from Engel and Michener 2013, with permission from Michael S. Engel©, 2020). (**c**) *Proplebeia dominicana*, the most common fossil stingless bee with an Orchid pollinarium (arrow) (*Globosites apicola*) attached to its head (from Poinar 2016, www.schweizerbart.de/journals/njgpa). (**d**) *Melissites trigona* Engel (Melikertini) in mid-Eocene Baltic amber (from Engel 2011)

tree resin for nest building and defence and foragers are, thus, more likely to end up trapped in resin. Despite a comparatively good fossil record, we still have a relatively poor understanding of the evolutionary history of stingless bees or how they came to inhabit so many different places around the world.

The oldest fossil stingless bee is *Cretotrigona prisca*, a small worker bee (~5 mm body length) found in New Jersey amber in North America (Fig. 2.4a) (Michener and Grimaldi 1988a, b). The bee is assumed to have lived during the late Cretaceous (Maastrichtian), c. 65–70 Mya (million years ago; Engel 2000a). *Cretotrigona prisca* is also the oldest definitive fossil of an apid bee and the oldest fossil of a eusocial bee (Engel 2000a). It can be recognised as a worker bee by its small metasoma (abdomen) (Fig. 1.8), which is reduced in workers due to the reduction of the ovaries. This feature suggests that *C. prisca* was a highly eusocial bee (Michener and Grimaldi 1988a, b; Engel 2000a). Three fossil species (*Exebotrigona velteni*,[5] *Liotrigonopsis rozeni* and *Kelneriapis eozenica*) were found in Baltic amber

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[5] This species was originally considered to be from Fushun amber in China (Engel and Michener 2013), but has since been re-analysed and is now considered to be from Baltic amber from the Lutetian age (~48–41 Mya) (M.S. Engel, personal communication, July 2020).

from the middle Eocene (~45 Mya) (Engel 2001a; Engel and Michener 2013). The most common fossil stingless bee, *Problebeia dominicana*, is frequently found in Dominican and Mexican amber dating from the Early Miocene (20–15 Mya) (Camargo et al. 2000). Another species from Early Miocene Mexican amber is *Nogueirapis silacea*, an extinct species that belongs to a genus that currently contains four still existing species (Table 2.1).

What does the age of these fossils and our current understanding of present-day phylogenetic relationships tell us about the age of stingless bees as a group? *Cretotrigona prisca* appears to be a eusocial bee with remarkable similarity to some extant stingless bees. Thus, 65–70 million years provides a lower boundary for the emergence of stingless bees. On the other hand, they must be much younger than 120 million years, the time when flowering plants diversified and bees as a group appeared (Cardinal and Danforth 2013; Danforth et al. 2013). Two recent studies that explored the evolution of bees suggest that corbiculate bees appeared about 84–87 Mya (Cardinal and Danforth 2013; Martins et al. 2014). This suggests that stingless bees evolved in the Late Cretaceous, about 70–87 Mya (see also Rasmussen and Cameron 2010; Cardinal and Danforth 2011).

2.3 Biogeography

Present-day stingless bees inhabit most tropical terrestrial habitats, but where their journey began and which routes they took to reach the different regions of the world remain fascinating questions. For example, how did they colonise isolated landmasses like Guadeloupe, Madagascar, Australia or the Solomon Islands? The intricate geological history, the movement of continental plates, dramatic changes in climate and sea levels during the last 100 million years have led to a complex history of vicariance (i.e. the formation of physical and biotic barriers that split ancestral populations and led to speciation) and dispersal events (Camargo and Pedro 1992; Rasmussen and Cameron 2010). Water barriers, even if narrow, represent a substantial problem for stingless bees. Their mode of swarming by progressive colony establishment through the transport of material from the mother nest to the new nest means that swarming distances are short, usually less than 300 meters (see Chap. 4). This makes it unlikely that stingless bee swarms can travel significant distances across water (Kerr and Maule 1964).[6] The following observations illustrate this feature of stingless bee biology. Francisco et al. (2016, 2017) studied the population genetic structure of the common Neotropical stingless bee *Tetragonisca angustula* and the bumble bee *Bombus morio* on small islands in front of the Brazilian coast. Many of these islands are separated by less than 5 km from the mainland. Despite this relative proximity to the mainland, *T. angustula* was absent from several of the islands and colonies where most likely introduced by humans on

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[6] Honey bee swarms can travel much further (Seeley 2010) and bumble bee colonies are founded by single queens, for which water bodies are easier to cross.

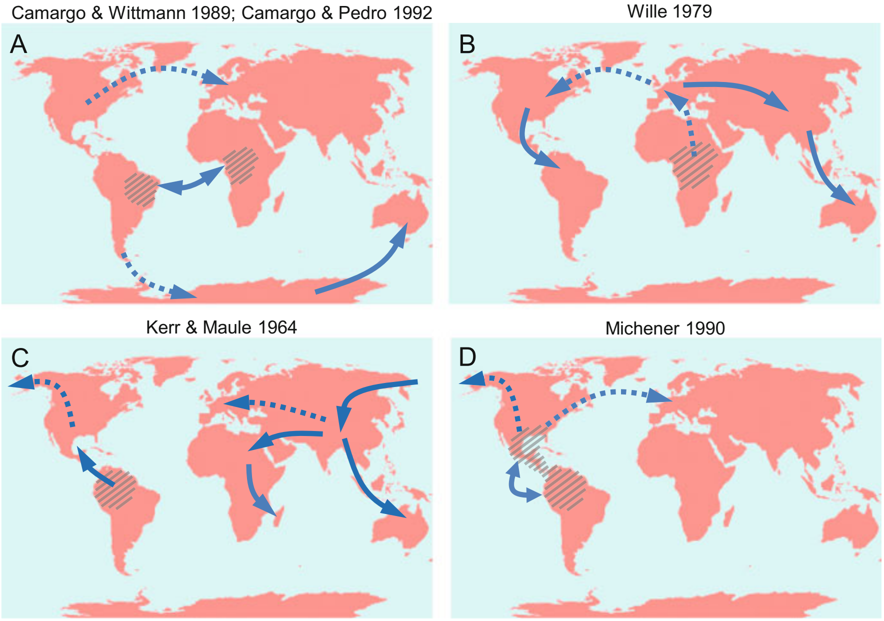

Fig. 2.5 Different biogeographic scenarios to explain the present-day distribution of stingless bees. The striped areas indicate the hypothesised region of origin. Arrows with an unbroken line indicate migrations to locations where stingless bees still exist. Arrows with a broken line indicate migrations to locations where stingless bees do not exist at present. See text for descriptions

others (Francisco et al. 2017). The genetic structure of colonies suggested that queens (and therefore swarms) are unable to cross water barriers larger than a few hundred meters. Bumble bees, on the other hand, were present on most islands and the genetic structure of the populations in combination with observations of queens flying over water suggests that water barriers of several kilometre width do not represent a major obstacle for *Bombus morio* (Francisco et al. 2016).

2.3.1 Biogeographic Scenarios

Several scenarios have been proposed to explain the current pantropical distribution of stingless bees. They roughly fall into two categories (Fig. 2.5): those suggesting that stingless bees originated in tropical Africa (Wille 1979; Michener 2007; Rasmussen and Cameron 2010) and those suggesting tropical America as the place of origin (Kerr and Maule 1964; Michener 1990; Kajobe and Roubik 2018; Melo 2020). Both views try to explain the genetic dissimilarity between Neotropical and Afrotropical species (Fig. 2.2) (Rasmussen and Cameron 2010; Bossert et al. 2019). The history of stingless bees involved a considerable range expansion during periods in the Paleogene when the average temperature of Earth was higher.

An alternative to an African vs. American origin was proposed by Camargo and Wittmann (1989) (Fig. 2.5a) (see also Michener 1979; Camargo and Pedro 1992). They suggested that stingless bees originated in Gondwana about 130–100 Mya, at a time when Africa and South America were still connected. In this scenario, a later migration to Australia could have occurred via Antarctica, or via a northern route to Eurasia followed by a migration to Southeast Asia and Oceania. There is indeed evidence for biological dispersal events between South America and Antarctica to New Zealand and Australia via the South Sandwich Islands and South Georgia, which would have formed a continuous land bridge during some periods of the Late Cretaceous (Morley 2003). At the time when this hypothesis was formulated, *Cretotrigona prisca* was still considered to be much older (Michener and Grimaldi 1988b). Given the re-evaluation of the age of *C. prisca* by Engel (2000a, b) and recent progress in stingless bee and corbiculate bee phylogenetic relationships (Rasmussen and Cameron 2007, 2010; Cardinal and Danforth 2013; Martins et al. 2014) a Gondwanan origin seems unlikely. Instead, it is more likely that corbiculate bees evolved at a time when Africa and South America were already separated by a growing South Atlantic (direct land connections between Africa and South America were lost around 100 mya, Morley 2003).

Out-of-Africa

An African origin was suggested by Wille (1979) (Fig. 2.5b) and Rasmussen and Cameron (2010). Wille (1979) considered African species to be more "primitive" than Neotropical species (but see Michener (1990) for arguments against this interpretation of the traits studied by Wille). From Africa, he suggested, they migrated first to Europe during the Eocene and then to North America, presumably via Greenland and to Asia (Wille 1979). This could explain why stingless bee fossils were found in European Baltic amber from the middle Eocene (~45 Mya). The timing of these events, however, is contradicted by the presence of *Cretotrigona prisca* in Late Cretaceous New Jersey amber.

Rasmussen and Cameron (2010) also proposed an African origin of stingless bees and argue that island chains between Africa and South America might have allowed stingless bee populations to stay connected until the separation of New World and Old World species around 70 Mya. Island chains between these two landmasses can explain the simultaneous appearance of plants on both sides of the South Atlantic until the Cretaceous–Tertiary boundary (Rasmussen and Cameron 2010). However, plants were probably able to cross these water barriers by wind, water and avian vectors (Morley 2003) and, as discussed above, it is unlikely that stingless bee colonies would have been able to cross the South Atlantic as easily as plants. An alternative mode of expansion was suggested by Kerr and Maule (1964), who proposed that floating islands or floating natural rafts could have served as a means of dispersal. This idea was inspired by the observation that in the Amazon River, especially after heavy rains, floating islands with a large number of animals and floating trees can be seen on the river. In the same way stingless bee colonies could have crossed a wide water barrier. Colonies of stingless bees can stay alive for 20–40 days when being transported (Kerr and Maule 1964). Kerr and Maule (1964) themselves, however, were not convinced by their idea because of the absence of stingless bees on many Caribbean islands. Michener (1979) agreed with their objection and pointed out that despite a great species diversity of stingless bees in Central and South America, only two species exist on some of the Antillean islands. One of them, *Melipona beecheii*, was probably brought to Cuba and Jamaica by humans as it is very popular for beekeeping since Mayan times (Chap. 1) (Michener 1979; Camargo et al. 1988; Genaro 2008). The other species, *Melipona variegatipes*, is known only from three islands that are part of the Lesser Antilles (Guadeloupe, Domenica and Monserrat) (Camargo et al. 1988; Camargo and Pedro 2013). Other and more common species from Central and South America might be expected to inhabit the Antilles if the colonisation of the Antilles had occurred by rafting. Thus, neither Kerr and Maule (1964) nor Michener (1979) considered rafting to be a plausible dispersal method used by stingless bee colonies to cross large water barriers. How and from where *M. variegatipes* reached the Lesser Antilles remains a fascinating question (this species separated from its Central and South American relatives, e.g. *M. beecheii*, *M. fasciculata* and *M. grandis*, about 20–30 Mya, see Rasmussen and Cameron 2010). The fact that *M. variegatipes* does not occur on the Greater Antilles (Hispaniola, Cuba, Jamaica, Puerto Rico, etc.) and morphological similarities with South American *Melipona* species suggest a colonisation from South America in combination with local extinctions on some of the smaller islands of the Lesser Antilles (Camargo et al. 1988). Both a land bridge and dispersal over water from South America remain possible (e.g. Iturralde-Vinent and MacPhee 1999; Hedges 2006; Ali 2012).

There are further reasons not to dismiss floating islands and rafts as a way of dispersal from Africa to South America. There is phylogenetic evidence that primates came from Africa to South America across the Atlantic during the middle Eocene (Antoine et al. 2012; Bond et al. 2015) and floating island rafting is considered a plausible explanation (Houle 1998; Bond et al. 2015). Houle (1998) argues that with the right wind conditions it would have taken a floating island about 1 week to cross the Atlantic barrier 50 Mya. It would have required even less time 70 Mya and paleowind directions appear to have been favourable to a westerly dispersal across the South Atlantic (Houle 1998). Stingless bee colonies with some honey and pollen reserves could survive this time on water. A single floating tree containing multiple nests of the same species could potentially lead to the successful migration from Africa to South America (see also Michener 1979). However, in the absence of clear phylogenetic evidence for a dispersal event across the South Atlantic (as is the case for Primates) the floating island hypothesis remains somewhat unsatisfactory. Furthermore, the current lack of fossils supporting an African origin and the low diversity of stingless bees in Africa continue to cast doubt on an African origin.

Out-of-America

A Neotropical origin was proposed by Kerr and Maule (1964) (Fig. 2.5c) and seems plausible because the Neotropics are home to the largest number and the greatest diversity of living species (Rasmussen and Cameron 2010; Table 2.1). Additionally, and contrary to Wille (1979), Kerr and Maule (1964) argued that some Neotropical species are more specialised and have more "primitive" traits (i.e. these traits are assumed to be phenotypically similar to those expressed by ancestors), which, according to the authors, supports an evolutionary origin in South America. Stingless bees would then have expanded first to North America and, subsequently, to Asia via the Bering Strait during the Eocene. From there they could have reached Africa, Australia and other islands in Asia during the Oligocene (Kerr and Maule 1964). As is the case with most other scenarios, the plausibility of this hypothesis relies strongly on land bridges connecting now distant landmasses at some point during the last 80 million years.

Based on his phylogenetic analysis, Michener (1990) (Fig. 2.5d) also assumed that stingless bees evolved in the Neotropics at a time when large parts of North America still had a tropical climate. This hypothesis includes the possibility that stingless bees evolved in present-day North America. From North America, stingless bees would have migrated to Asia via the Bering Strait and to Europe via Greenland. Michener (1990) did not provide details about the potential timeline of these different migrations and Rasmussen and Cameron (2010) have argued that an American origin is unlikely because South America and North America were separated for most of the past 100 million years. A connection between South and North America is required for both Kerr and Maule's (1964) and Michener's (1990) scenario.

Despite these obstacles, an American birthplace remains likely, not least because we have physical evidence that stingless bees (*Cretotrigona prisca*) were present in North America early in their evolution, c. 65–70 Mya (see also Kajobe and Roubik 2018). As mentioned above, an American origin was deemed implausible partly because South America has been isolated from North America during long periods of the last 100 million years. However, the details of land connections during early periods of the separation of continents are often poorly understood (Morley 2003) and the tectonics of the Caribbean region are complex and controversial (Pindell et al. 1988; Morley 2003; Ali 2012). There is evidence for intercontinental land bridges between the Americas (present-day Yucatan and Colombia), e.g. during the Late Cretaceous and Early Paleocene (~65–80 Mya), possibly formed by the Aves Ridge and the Greater Antilles (Iturralde-Vinent and MacPhee 1999; Morley 2003). This could have allowed stingless bees to migrate in both directions and enabled them to be present throughout tropical America shortly after their evolution (as suggested by Michener 1990). Evidence for this dispersal route comes from fossils of large-bodied land vertebrates, including hadrosaurian and ceratopian dinosaurs during the Campanian (83–72 Mya), but also from smaller vertebrates like snakes and lizards (Morley 2003). Furthermore, phylogenetic analyses suggest that the Centridini bees, which are the closest relatives of the corbiculate bees (see above), were also using this connection between South and North America around 75–85 Mya (Martins and Melo 2016). The exchange of animals further increased towards the Cretaceous–Paleogene boundary (Morley 2003). The North–South connection then broke up during the Middle Eocene (~49–39 Mya), which meant that stingless bee populations in North and South America became temporarily separated.

The close relationship between corbiculate bees and the Centridini bees (see above) (Danforth et al. 2013; Martins et al. 2014) further supports a Neotropical birthplace (Melo 2020). The Centridini are thought to have evolved in tropical South America and diverged from the corbiculate bees around 86 Mya in the Late Cretaceous (Martins and Melo 2016). Consequently, the corbiculate bees are thought to have evolved and differentiated in the Americas as well (Martins et al. 2014; Martins and Melo 2016). This places the corbiculate ancestors of stingless bees in the Americas not too long before stingless bees appeared.

From North America, two other migration routes would have been possible: the Beringia route, connecting East Asia and North America and the Thulean route, connecting North America and Europe via Greenland (Brikiatis 2014). Land connections across Beringia existed during warm periods in the Late Cretaceous (~65 Mya), the Late Paleocene (~58 Mya) and during the Early Eocene (Morley 2003; Brikiatis 2014). The Thulean route to Eurasia might have been restricted to very high latitudes until the Late Paleocene (60–54 Ma) and Early Eocene when a land connection was established across South Greenland (Morley 2003). This relatively short-lived land connection is thought to have occurred at around the time of the Late Paleocene/Early Eocene thermal maximum (~55 Mya) (Zachos et al. 2001; Brikiatis 2014), thus providing a route for the dispersal of animals that depend on a warmer climate (Morley 2003). Other bees are thought to have used the Thulean route during a time window between 69 and 47 Mya (Praz and Packer 2014). It is also possible that stingless bees migrated to Europe from Asia. A migration route between Europe and Asia was probably available during the Paleocene (66–56 Mya), until the climatic change from the Eocene to Oligocene (50–35 Mya) removed this option (Rasmussen and Cameron 2010). Meliponini could have reached the African continent via Asia too. One possible time period for a dispersal is the middle Eocene climate optimum (~40 Mya) (Bohaty et al. 2009). There is evidence that primates, rodents and other Asian land mammal groups dispersed from Asia to Africa during this time period (Chaimanee et al. 2012).

Northern Extinctions and the "Grande Coupure"

Fossils show that stingless bees were present in the northern hemisphere during the Eocene until a slow but sustained climatic change from warm and humid weather with predominantly humid forest habitats to cooler climate with arid, and more open savannah-like habitats during the Oligocene (50–35 Mya) (Zachos et al. 2001) is likely to have driven the Palearctic stingless bee species to extinction (Grimaldi and Engel 2005; Rasmussen and Cameron 2010). This global cooling is thought to have caused considerable extinction of other corbiculate lineages as well (Engel 2001a, b; Grimaldi and Engel 2005). One of these affected groups was the now extinct tribe Melikertini, the closest known relatives of the Meliponini. This tribe consisted of several morphologically diverse genera of eusocial bees inhabiting Europe. They differed from stingless bees, for example, in having a complete wing venation and a well-developed sting (Fig. 2.4) (Engel 2001a). Melikertine bees appear to have gone extinct around the time of the Eocene–Oligocene transition, coinciding with the extinction event called "grande coupure" (great break) (Engel 2001a).

Stingless bees did not share the fate of the Melikertini, but their distribution changed dramatically during this period of global cooling. The reasons why stingless bees are unable to inhabit cooler habitats, like for example bumble bees or honey bees, are not well understood, but it is speculated that their thermoregulatory abilities are not sufficient to maintain optimal conditions for the brood in cooler conditions (Chap. 3) or that suitable nesting sites and nesting material are rare outside tropical and sub-tropical zones (Ortiz-Mora et al. 1995). This latter explanation seems unlikely given their diversity in nesting habits (Chap. 3). Stingless bees probably recolonised southern parts of North America via Central America as early as 15–20 mya (Rasmussen and Cameron 2010). *Proplebeia dominicana* (Fig. 2.4), a species that is similar to extant *Plebeia* (Moure and Camargo 1978) and found in Dominican and Mexican amber might be the result of this recolonisation of North America.

Colonisation of Madagascar

How stingless bees colonised Madagascar remains an intriguing question. There are currently seven endemic species described, all belonging to the genus *Liotrigona*, which is also found in continental Africa (Pauly et al. 2001; Koch 2010; Pauly and Anguilet 2013). It is not currently known whether a radiation from a single ancestral species occurred on the island or if Madagascar was colonised multiple times by colonies from different *Liotrigona* species coming from mainland Africa (Koch 2010). What makes the colonisation of Madagascar puzzling is that the two landmasses are separated since about 160 million years (Yoder and Nowak 2006). Hence, vicariance cannot explain the presence of stingless bees on Madagascar (Fuller et al. 2005). Furthermore, the ~400 km distance between Madagascar and mainland Africa has been roughly the same during the last 120 million years (Yoder and Nowak 2006). McCall (1997) suggested that a land bridge may have connected Africa and Madagascar between 45 and 26 Mya, but this has been questioned by others, e.g. because the channel between Africa and Madagascar is quite deep (Rogers et al. 2000; Yoder and Nowak 2006). A rafting hypothesis has been proposed to explain the occurrence of allodopine bees on Madagascar (Fuller et al. 2005): bees might have travelled in dead stems and branches or with rafts of vegetation washed into the Mozambique Channel. However, allodapines use a very different mode of dispersal that makes rafting a more feasible option for this group of bees (Fuller et al. 2005). The rafting island hypothesis is currently considered the most plausible explanation for the arrival of several groups of vertebrates (Yoder and Nowak 2006). A recent analysis of the mitochondrial DNA of *Apis mellifera* sub-species has suggested the Comoro islands as possible stepping stones that allowed honey bees to cross form continental Africa to Madagascar as recently as 20,000–30,000 years ago (Techer et al. 2017). Could this route have been used by stingless bees in earlier times when water levels were lower? Detailed phylogenetic comparisons with *Liotrigona* species from the African mainland would be highly instructive to establish when the colonisation of Madagascar occurred.

Colonisation of Australia

Australia is home to two genera (*Austroplebeia* and *Tetragonula*) that appear to come from different waves of migration. *Austroplebeia* is more closely related to Afrotropical species than to *Tetragonula* (Fig. 2.2), which suggests that they colonised Australia from Africa via Asia (Rasmussen and Cameron 2007). A similar dispersal scenario from Africa to Australasia has been suggested for *Braunsapis* (Apidae: Xylocopinae) (Fuller et al. 2005), which originated in tropical Africa during the early Miocene, dispersed into Asia about 17 mya and arrived in Australia during the late Miocene (Fuller et al. 2005).

The seven species of *Tetragonula* found in Australia (Dollin et al. 1997; Franck et al. 2004) seem to originate from three different dispersal events to Australia from the Indo-Malayan Region (Franck et al. 2004). Some species are thought to have colonised Australia more recently when periodic Pleistocene land bridges connected Australia via the Cape York Peninsula and New Guinea, the last of which disappeared around 10,000 years ago (Dollin et al. 1997; Franck et al. 2004). How and when these different migrations to Australia occurred is difficult to know due to the complex geological history of the Malay Archipelago (Michaux 2010; Van Welzen et al. 2011). Sea levels have fluctuated greatly in the region and these fluctuations had a considerable impact on the area and location of exposed land surface.

2.4 List of Genera and Species

2.4.1 Neotropical Species

Aparatrigona Moure, 1951

Small (4–5 mm worker body length), relatively dark stingless bees with striking yellow markings, similar to *Paratrigona* (considered to be *Paratrigona* by Michener (2007), but elevated to genus status by Camargo and Moure (1994)). Occur from Costa Rica in the north to Brazil in the south, two species (Camargo and Pedro 2013):

- *A. impunctata* (Ducke, 1916)
- *A. isopterophila* (Schwarz, 1934)

Camargoia Moure, 1989

Medium-sized bees (7–8 mm body length), yellowish lower half of the face, closely related to *Tetragona* and *Ptilotrigona* (Camargo 1996; Michener 2007). Mainly in the northern parts of Brazil and French Guiana, ground nesting; three species (Camargo and Pedro 2013):

- *C. camargoi* (Moure, 1989)
- *C. nordestina* (Camargo, 1996)

- *C. pilicornis* (Ducke, 1910)

Celetrigona Moure, 1950

Small bees (3–5 mm body length), mainly found in the Amazonian rainforest area, closely related to the *Leurotrigona–Trigonisca* group (Camargo and Pedro 2009; Rasmussen and Cameron 2010). Four species (Camargo and Pedro 2013):

- *C. euclydiana* (Camargo & Pedro, 2009)
- *C. hirsuticornis* (Camargo & Pedro, 2009)
- *C. longicornis* (Friese, 1903)
- *C. manauara* (Camargo & Pedro, 2009)

Cephalotrigona Schwarz, 1940

Relatively large bees (6–10 mm body length), sister group of the large genus *Trigona*, distributed from Mexico to Argentina (Schwarz 1948; Michener 2007; Rasmussen and Cameron 2010; Camargo and Pedro 2013). Notable are a large head and large food pots. Mostly tree cavity nesting, though may also nest in the ground (Schwarz 1948; Michener 2007).

- *C. capitata* (Smith, 1854)
- *C. eburneiventer* (Schwarz, 1948)
- *C. femorata* (Smith, 1854)
- *C. oaxacana* (Ayala, 1999)
- *C. zexmeniae* (Cockerell, 1912)

Duckeola Moure, 1944

Relatively large (8–9 mm body length) and robust bees, distributed mainly in the Amazon basin (Michener 2007; Camargo and Pedro 2013). Closely related to *Trichotrigona*, *Frieseomelitta* and *Tetragonisca* (Rasmussen and Cameron 2010). Two species (Camargo and Pedro 2013):

- *D. ghilianii* (Spinola, 1853)
- *D. pavani* (Moure, 1963)

Friesella Moure, 1946

Very small and shy bees (c. 3 mm body length), found in southern Brazil (Camargo and Pedro 2013). Closely related to *Plebeia* (Rasmussen and Cameron 2010). One species:

- *F. schrottkyi* (Friese, 1900)

Frieseomelitta Ihering, 1912

Small to mid-sized, slender bees (c. 4–6.5 mm body length), from Mexico in the north to Brazil and Bolivia in the south (Michener 2007; Camargo and Pedro 2013). They build clustered brood cells and elongated storage pots (Chap. 3). Some species have guards of distinct colour (Grüter et al. 2017). Closely related to *Trichotrigona*, *Duckeola* and *Tetragonisca* (Rasmussen and Cameron 2010). Sixteen species:

- *F. dispar* (Moure, 1950)
- *F. doederleini* (Friese, 1900)
- *F. flavicornis* (Fabricius, 1798)
- *F. francoi* (Moure, 1946)
- *F. freiremaiai* (Moure, 1963)
- *F. languida* (Moure, 1990)
- *F. lehmanni* (Friese, 1901)
- *F. longipes* (Smith, 1854)
- *F. meadewaldoi* (Cockerell, 1915)
- *F. nigra* (Cresson, 1878)
- *F. paranigra* (Schwarz, 1940)
- *F. paupera* (Provancher, 1888)
- *F. portoi* (Friese, 1900)
- *F. silvestrii* (Friese, 1902)
- *F. trichocerata* (Moure, 1990)
- *F. varia* (Lepeletier, 1836)

Geotrigona **Moure, 1943**

Medium-sized, robust bees (c. 5–6.5 mm body length), from Mexico in the north to northern Argentina in the south (Michener 2007; Camargo and Pedro 2013). Closely related to *Tetragona* (Rasmussen and Cameron 2010). Nests are typically in cavities in the ground (Chap. 3). Twenty-two species:

- *G. acapulconis* (Strand, 1919)
- *G. aequinoctialis* (Ducke, 1925)
- *G. argentina* (Camargo & Moure, 1996)
- *G. chiriquiensis* (Schwarz, 1951)
- *G. fulvatra* (Camargo & Moure, 1996)
- *G. fulvohirta* (Friese, 1900)
- *G. fumipennis* (Camargo & Moure, 1996)
- *G. joearroyoi* (Gonzalez & Engel, 2012)
- *G. kaba* (Gonzalez & Sepúlveda, 2007)
- *G. kraussi* (Schwarz, 1951)
- *G. kwyrakai* (Camargo & Moure, 1996)
- *G. leucogastra* (Cockerell, 1914)
- *G. lutzi* (Camargo & Moure, 1996)
- *G. mattogrossensis* (Ducke, 1925)
- *G. mombuca* (Smith, 1863)
- *G. subfulva* (Camargo & Moure, 1996)
- *G. subgrisea* (Cockerell, 1920)
- *G. subnigra* (Schwarz, 1940)
- *G. subterranea* (Friese, 1901)

- *G. tellurica* (Camargo & Moure, 1996)
- *G. terricola* (Camargo & Moure, 1996)
- *G. xanthopoda* (Camargo & Moure, 1996)

Lestrimelitta **Friese, 1903**

Small to medium-sized, black, shiny and robust bees (c. 4–7 mm body length), from Mexico in the north to northern Argentina in the south (Marchi and Melo 2006; Michener 2007; Gonzalez and Griswold 2012; Camargo and Pedro 2013). Exclusively cleptoparasitic lifestyle (Chap. 7). Closely related to *Plebeia* and *Friesella* (Rasmussen and Cameron 2010). Twenty-three species:

- *L. catira* (Gonzalez & Griswold, 2012)
- *L. chacoana* (Roig Alsina, 2010)
- *L. chamelensis* (Ayala, 1999)
- *L. ciliata* (Marchi & Melo, 2006)
- *L. danuncia* (Oliveira & Marchi, 2005)
- *L. ehrhardti* (Friese, 1931)
- *L. glaberrima* (Oliveira & Marchi, 2005)
- *L. glabrata* (Camargo & Moure, 1989)
- *L. guyanensis* (Roubik, 1980)
- *L. huilensis* (Gonzalez & Griswold, 2012)
- *L. limao* (Smith, 1863)
- *L. maracaia* (Marchi & Melo, 2006)
- *L. monodonta* (Camargo & Moure, 1989)
- *L. mourei* (Oliveira & Marchi, 2005)
- *L. nana* (Melo, 2003)
- *L. niitkib* (Ayala, 1999)
- *L. opita* (Gonzalez & Griswold, 2012)
- *L. rufa* (Friese, 1903)
- *L. rufipes* (Friese, 1903)
- *L. similis* (Marchi & Melo, 2006)
- *L. spinosa* (Marchi & Melo, 2006)
- *L. sulina* (Marchi & Melo, 2006)
- *L. tropica* (Marchi & Melo, 2006)

Leurotrigona **Friese, 1903**

Minute bees (2–3 mm body length) that are closely related to other minute bees of the genera *Celetrigona and Trigonisca* (Camargo and Pedro 2005; Rasmussen and Cameron 2010). From Colombia and French Guiana in the north to Paraguay in the south (Camargo and Pedro 2013). Paraphyletic group according to Rasmussen and Cameron (2010) (Fig. 2.2). Engel et al. (2019) suggested the new subgenus name *gona* for *L. crispula* and *L. pusilla*:

- *L. crispula* (Pedro & Camargo, 2009)
- *L. gracilis* (Pedro & Camargo, 2009)

- *L. muelleri* (Friese, 1900)
- *L. pusilla* (Moure & Camargo, in Moure et al., 1988)

Melipona Illiger, 1806

Large, robust bees (8–15 mm), typically living in relatively small colonies (Chap. 1; Michener 2007). The largest species, *Melipona fuliginosa* and *M. titania* (together with *M. fallax* they form the *M. fuliginosa* species group), are comparable in size to the giant honeybee *Apis dorsata*. Distribution from Mexico in the north to Argentina in the south. A unique feature is that queens are reared in worker-sized cells. Queen determination is likely to have a genetic component (Chap. 5). With about 74 species, *Melipona* is the largest stingless bee genus. Some authors further divide the species into four subgenera based on morphological traits (Camargo and Pedro 2013; but see Michener 2007). Molecular data suggest that three subgenera are monophyletic (*Michmelia*, *Melikerria* and *Melipona*), whereas one (*Eomelipona*) is polyphyletic (Ramírez et al. 2010; Rasmussen and Cameron 2010).

Melipona (Eomelipona)

- *M. amazonica* (Schulz, 1905)
- *M. asilvai* (Moure, 1971)
- *M. bicolor* (Lepeletier, 1836)
- *M. bradleyi* (Schwarz, 1932)
- *M. carrikeri* (Cockerell, 1919)
- *M. concinnula** (Cockerell, 1919)
- *M. illustris* (Schwarz, 1932)
- *M. marginata* (Lepeletier, 1836)
- *M. micheneri* (Schwarz, 1951)
- *M. obscurior* (Moure, 1971)
- *M. ogilviei* (Schwarz, 1932)
- *M. puncticollis* (Friese, 1902)
- *M. schwarzi* (Moure, 1963)
- *M. torrida* (Friese, 1916)
- *M. tumupasae* (Schwarz, 1932)

*Known from only one specimen collected in Venezuela and could be a senior synonym of *M. ogilviei*, according to Schwarz (1932, p. 419) and Camargo and Pedro (2013).

Melipona (Melikerria)

- *M. ambigua* (Roubik & Camargo, 2012)
- *M. beecheii* (Bennett, 1831)
- *M. compressipes* (Fabricius, 1804)
- *M. fasciculata* (Smith, 1854)
- *M. grandis* (Guérin, 1844)
- *M. insularis* (Roubik & Camargo, 2012)
- *M. interrupta* (Latreille, 1811)

- *M. quinquefasciata* (Lepeletier, 1836)
- *M. salti* (Schwarz, 1932)
- *M. triplaridis* (Cockerell, 1925)

Melipona (Melipona)
- *M. baeri* (Vachal, 1904)
- *M. favosa* (Fabricius, 1798)
- *M. fuscata* (Lepeletier, 1836)
- *M. lunulata* (Friese, 1900)
- *M. lupitae* (Ayala, 1999)
- *M. mandacaia* (Smith, 1863)
- *M. orbignyi* (Guérin, 1844)
- *M. peruviana* (Friese, 1900)
- *M. phenax* (Cockerell, 1919)
- *M. quadrifasciata* (Lepeletier, 1836)
- *M. subnitida* (Ducke, 1910)
- *M. variegatipes* (Gribodo, 1893)
- *M. yucatanica* (Camargo, Moure & Roubik, 1988)

Melipona (Michmelia)
- *M. apiformis* (Buysson, in Du Buysson and Marshall, 1892)
- *M. belizeae* (Schwarz, 1932)
- *M. boliviana* (Schwarz, 1932)
- *M. brachychaeta* (Moure, 1950)
- *M. captiosa* (Moure, 1962)
- *M. capixaba* (Moure & Camargo, 1994)
- *M. colimana* (Ayala, 1999)
- *M. costaricensis* (Cockerell, 1919)
- *M. cramptoni* (Cockerell, 1920)
- *M. crinita* (Moure & Kerr, 1950)
- *M. dubia* (Moure & Kerr, 1950)
- *M. eburnea* (Friese, 1900)
- *M. fallax* (Camargo & Pedro, 2008)
- *M. fasciata* (Latreille, 1811)
- *M. flavolineata* (Friese, 1900)
- *M. fuliginosa* (Lepeletier, 1836)
- *M. fulva* (Lepeletier, 1836)
- *M. fuscopilosa* (Moure & Kerr, 1950)
- *M. illota* (Cockerell, 1919)
- *M. indecisa* (Cockerell, 1919)
- *M. lateralis* (Erichson, 1848)
- *M. melanoventer* (Schwarz, 1932)
- *M. mimetica* (Cockerell, 1914)
- *M. mondury* (Smith, 1863)
- *M. nebulosa* (Camargo, 1988)

- *M. nigrescens* (Friese, 1900)
- *M. nitidifrons* (Benoist, 1933)
- *M. panamica* (Cockerell, 1912)
- *M. paraensis* (Ducke, 1916)
- *M. rufescens* (Friese, 1900)
- *M. rufiventris* (Lepeletier, 1836)
- *M. scutellaris* (Latreille, 1811)
- *M. seminigra* (Friese, 1903)
- *M. solani* (Cockerell, 1912)
- *M. titania* (Gribodo, 1893)
- *M. trinitatis* (Cockerell, 1919)

Meliwillea Roubik, Lobo and Camargo, 1997

Small to medium-sized bees (5–6 mm body length), just one species that is closely related to *Scaptotrigona*, found in mountainous regions in Central America (Roubik et al. 1997).

- *M. bivea* (Roubik, Lobo and Camargo, 1997)

Mourella Schwarz, 1946

Medium-sized, dark bees (5–6 mm body length), genus with just one species, which is closely related to *Schwarziana* (Rasmussen and Cameron 2010), found in central South America (Camargo and Pedro 2013).

- *M. caerulea* (Friese, 1900)

Nannotrigona Cockerell, 1922

Small (3.5–5 mm), shy, dark bees, very common in the Neotropics. This group is also successful in urbanised and disturbed areas (Rasmussen and Gonzalez 2017). Closely related to *Mourella* and *Schwarziana* (Rasmussen and Cameron 2010). Ten species (Rasmussen and Gonzalez 2017).

- *N. camargoi* (Rasmussen & Gonzalez, 2017)
- *N. chapadana* (Schwarz, 1938)
- *N. dutrae* (Friese, 1901)
- *N. melanocera* (Schwarz, 1938)
- *N. mellaria* (Smith, 1862)
- *N. perilampoides* (Cresson, 1878)
- *N. punctata* (Smith, 1854)
- *N. schultzei* (Friese, 1901)
- *N. testaceicornis* (Lepeletier, 1836)
- *N. tristella* (Cockerell, 1922)

Nogueirapis **Moure, 1953**

Small (3.5–5.5 mm body length) bees with conspicuous yellow markings, closely related to *Partamona*. Four species, probably all nesting in the ground. Distribution from Mexico to northern South America (Michener 2007; Rasmussen and Cameron 2010; Ayala and Engel 2014).

- *N. butteli* (Friese, 1900)
- *N. costaricana* (Ayala & Engel, 2014)
- *N. minor* (Moure & Camargo, 1982)
- *N. mirandula* (Cockerell, 1917)

Oxytrigona **Cockerell, 1917**

Medium-sized bees (5–6 mm body length), workers with a conspicuous red head and large mandibular glands for the secretion of caustic defence substances (Chap. 7). Closest relatives are *Scaptotrigona* (Rasmussen and Cameron 2010). Genus contains 11 species and is widely distributed in the Neotropics (Camargo and Pedro 2013).

- *O. chocoana* (Gonzalez & Roubik, 2008)
- *O. daemoniaca* (Camargo, 1984)
- *O. flaveola* (Friese, 1900)
- *O. huaoranii* (Gonzalez & Roubik, 2008)
- *O. ignis* (Camargo, 1984)
- *O. isthmina* (Gonzalez & Roubik, 2008)
- *O. mediorufa* (Cockerell, 1913)
- *O. mellicolor* (Packard, 1869)
- *O. mulfordi* (Schwarz, 1948)
- *O. obscura* (Friese, 1900)
- *O. tataira* (Smith, 1863)

Parapartamona **Schwarz, 1948**

Medium-sized bees, closely related to *Partamona* (Rasmussen and Cameron 2010). Known only from the Andes in Columbia, Ecuador and Peru where they are found at altitudes of up to 3400 m. Currently with seven recognised species, but species identities remain controversial (Gonzalez and Smith-Pardo 2003; Camargo and Pedro 2013).

- *P. brevipilosa* (Schwarz, 1948)
- *P. caliensis* (Schwarz, 1948)
- *P. fumata* (Moure, 1995)
- *P. imberbis* (Moure, 1995)
- *P. tungurahuana* (Schwarz, 1948)
- *P. vittigera* (Moure, 1995)
- *P. zonata* (Smith, 1854)

Paratrigona **Moure, 1951**

Relatively small to medium-sized bees (4–6 mm body length) with conspicuous yellow markings on the thorax and the face (Camargo and Moure 1994; Michener 2007; Gonzalez and Griswold 2011). Species-rich genus (32 species) with a distribution from Mexico to Argentina. Closely related to *Aparatrigona* (Rasmussen and Cameron 2010).

- *P. anduzei* (Schwarz, 1943)
- *P. catabolonota* (Camargo & Moure, 1994)
- *P. compsa* (Camargo & Moure, 1994)
- *P. crassicornis* (Camargo & Moure, 1994)
- *P. eutaeniata* (Camargo & Moure, 1994)
- *P. euxanthospila* (Camargo & Moure, 1994)
- *P. femoralis* (Camargo & Moure, 1994)
- *P. glabella* (Camargo & Moure, 1994)
- *P. guatemalensis* (Schwarz, 1938)
- *P. guigliae* (Moure, 1960)
- *P. haeckeli* (Friese, 1900)
- *P. incerta* (Camargo & Moure, 1994)
- *P. lineata* (Lepeletier, 1836)
- *P. lineatifrons* (Schwarz, 1938)
- *P. lophocoryphe* (Moure, 1963)
- *P. lundelli* (Schwarz, 1938)
- *P. melanaspis* (Camargo & Moure, 1994)
- *P. myrmecophila* (Moure, 1989)
- *P. nuda* (Schwarz, 1943)
- *P. onorei* (Camargo & Moure, 1994)
- *P. opaca* (Cockerell, 1917)
- *P. ornaticeps* (Schwarz, 1938)
- *P. pacifica* (Schwarz, 1943)
- *P. pannosa* (Moure, 1989)
- *P. peltata* (Spinola, 1853)
- *P. permixta* (Camargo & Moure, 1994)
- *P. prosopiformis* (Gribodo, 1893)
- *P. rinconi* (Camargo & Moure, 1994)
- *P. scapisetosa* (Gonzalez & Griswold, 2011)
- *P. subnuda* (Moure, 1947)
- *P. uwa* (Gonzalez & Vélez, 2007)
- *P. wasbaueri* (Gonzalez & Griswold, 2011)

Paratrigonoides **Camargo and Roubik, 2005**

Morphologically very similar to *Aparatrigona* and *Paratrigona* and considered a sister group (Michener 2007). One species known from Colombia (Camargo and Pedro 2013):

- *P. mayri* (Camargo & Roubik, 2005)

Partamona Schwarz, 1938

Small to medium-sized bees (4.5–7 mm) that often build elaborate entrance structures (e.g. the “toad mouth”, Chap. 3) (Camargo and Pedro 2003). Closely related to *Parapartamona* (Rasmussen and Cameron 2010). Species-rich genus (32 species) found from Mexico to Argentina (Camargo and Pedro 2013).

- *P. aequatoriana* (Camargo, 1980)
- *P. ailyae* (Camargo, 1980)
- *P. auripennis* (Pedro & Camargo, 2003)
- *P. batesi* (Pedro & Camargo, 2003)
- *P. bilineata* (Say, 1837)
- *P. chapadicola* (Pedro & Camargo, 2003)
- *P. combinata* (Pedro & Camargo, 2003)
- *P. criptica* (Pedro & Camargo, 2003)
- *P. cupira* (Smith, 1863)
- *P. epiphytophila* (Pedro & Camargo, 2003)
- *P. ferreirai* (Pedro & Camargo, 2003)
- *P. grandipennis* (Schwarz, 1951)
- *P. gregaria* (Pedro & Camargo, 2003)
- *P. helleri* (Friese, 1900)
- *P. littoralis* (Pedro & Camargo, 2003)
- *P. mourei* (Camargo, 1980)
- *P. mulata* (Moure, in Camargo, 1980)
- *P. musarum* (Cockerell, 1917)
- *P. nhambiquara* (Pedro & Camargo, 2003)
- *P. nigrior* (Cockerell, 1925)
- *P. orizabaensis* (Strand, 1919)
- *P. pearsoni* (Schwarz, 1938)
- *P. peckolti* (Friese, 1901)
- *P. rustica* (Pedro & Camargo, 2003)
- *P. seridoensis* (Pedro & Camargo, 2003)
- *P. sooretamae* (Pedro & Camargo, 2003)
- *P. subtilis* (Pedro & Camargo, 2003)
- *P. testacea* (Klug, 1807)
- *P. vicina* (Camargo, 1980)
- *P. vitae* (Pedro & Camargo, 2003)
- *P. xanthogastra* (Pedro & Camargo, 1997)
- *P. yungarum* (Pedro & Camargo, 2003)

Plebeia Schwarz, 1938

Small bees (3–6 mm) forming a species-rich (ca. 40 species) polyphylectic group (Rasmussen and Cameron 2010; Alvarez et al. 2016) ranging from Mexico to Argentina (Camargo and Pedro 2013).

- *P. alvarengai* (Moure, 1994)
- *P. catamarcensis* (Holmberg, 1903)
- *P. cora* (Ayala, 1999)
- *P. droryana* (Friese, 1900)
- *P. emerina* (Friese, 1900)
- *P. emerinoides** (Silvestri, 1902)
- *P. flavocincta* (Cockerell, 1912)
- *P. franki* (Friese, 1900)
- *P. fraterna* (Laroca & Rodriguez-Parilli, 2009)
- *P. frontalis* (Friese, 1911)
- *P. fulvopilosa* (Ayala, 1999)
- *P. goeldiana* (Friese, 1900)
- *P. grapiuna* (Melo & Costa, 2009)
- *P. guazurary* (Alvarez, Rasmussen & Abrahamovich, 2016)
- *P. jatiformis* (Cockerell, 1912)
- *P. julianii* (Moure, 1962)
- *P. kerri* (Moure, 1950)
- *P. llorentei* (Ayala, 1999)
- *P. lucii* (Moure, 2004)
- *P. malaris* (Moure, 1962)
- *P. manantlensis* (Ayala, 1999)
- *P. margaritae* (Moure, 1962)
- *P. melanica* (Ayala, 1999)
- *P. meridionalis* (Ducke, 1916)
- *P. mexica* (Ayala, 1999)
- *P. minima* (Gribodo, 1893)
- *P. molesta* (Puls, in Strobel, 1868)
- *P. mosquito* (Smith, 1863)
- *P. moureana* (Ayala, 1999)
- *P. nigriceps* (Friese, 1901)
- *P. parkeri* (Ayala, 1999)
- *P. peruvicola* (Moure, 1994)
- *P. phrynostoma* (Moure, 2004)
- *P. poecilochroa* (Moure & Camargo, 1993)
- *P. pugnax* (Moure, *in litt.*)
- *P. pulchra* (Ayala, 1999)
- *P. remota* (Holmberg, 1903)
- *P. saiqui* (Friese, 1900)
- *P. tica* (Wille, 1969)
- *P. tobagoensis* (Melo, 2003)
- *P. variicolor* (Ducke, 1916)
- *P. wittmanni* (Moure & Camargo, 1989)

P. emerinoides has been considered a junior synonym of *P. nigriceps* by Camargo and Pedro (2013), but is considered a valid species by Roig-Alsina and Alvarez (2017).

Plectoplebeia **Melo, 2016**

Small to medium-sized bees (5.5–6 mm) with one species (formerly *Plebeia intermedia*) (Melo 2016). Known from Argentina, Bolivia and Peru (Flores et al. 2015; Alvarez et al. 2016; Melo 2016).

- *P. nigrifacies* (Friese, 1900)

Ptilotrigona **Moure, 1951**

Relatively large bees (8–10 mm), closest relatives are *Tetragona* (Rasmussen and Cameron 2010). Colonies store little or no honey (Camargo and Pedro 2004). From Costa Rica in the north to northern parts of Brazil in the south, cavity nesting, three species (Camargo and Pedro 2013).

- *P. lurida* (Smith, 1854)
- *P. occidentalis* (Schulz, 1904)
- *P. pereneae* (Schwarz, 1943)

Scaptotrigona **Moure, 1942**

Small to mid-sized robust bees (5–7 mm), closely related to *Oxytrigona* (Rasmussen and Cameron 2010). About 23 cavity nesting species (Michener 2007; Camargo and Pedro 2013; Hurtado-Burillo et al. 2017). From Mexico to Argentina. Note that the species herein treated as *Scaptotrigona depilis* (Moure, 1942) refers to the taxon frequently called *Scaptotrigona* aff. *depilis* in the São Paulo region. A taxonomic revision is needed to better assess the species boundaries of *Scaptotrigona depilis*.

- *S. affabra* (Moure, 1989)
- *S. barrocoloradensis* (Schwarz, 1951)
- *S. bipunctata* (Lepeletier, 1836)
- *S. depilis* (Moure, 1942)
- *S. emersoni* (Schwarz, 1938)
- *S. fulvicutis* (Moure, 1964)
- *S. hellwegeri* (Friese, 1900)
- *S. jujuyensis* (Schrottky, 1911)
- *S. limae* (Brèthes, 1920)
- *S. luteipennis* (Friese, 1902)
- *S. mexicana* (Guérin, 1844)
- *S. ochrotricha* (Buysson, in Du Buysson and Marshall, 1892)
- *S. panamensis* (Cockerell, 1913)
- *S. pectoralis* (Dalla Torre, 1896)
- *S. polysticta* (Moure, 1950)
- *S. postica* (Latreille, 1807)

- *S. subobscuripennis* (Schwarz, 1951)
- *S. tricolorata* (Camargo, 1988)
- *S. tubiba* (Smith, 1863)
- *S. turusiri* (Janvier, 1955)
- *S. wheeleri* (Cockerell, 1913)
- *S. xanthotricha* (Moure, 1950)

Scaura Schwarz, 1938

Small bees (3–6 mm) (Schwarz 1948), currently belonging to ten species (Rasmussen and Cameron 2010; Camargo and Pedro 2013; Nogueira et al. 2019). Distribution from Mexico to the south of Brazil. Two species were previously in the genus *Schwarzula*, but Rasmussen and Cameron (2010) recommended synonymising the two genera under *Scaura* to create a monophyletic genus (see also Michener 2007, p. 812). Nogueira et al. (2019) suggest synonymising *S. tenuis* and *S. latitarsis*, but this would create a polyphyletic species according to the molecular phylogeny of Rasmussen and Cameron (2010) and is, thus, not followed here.

- *S. amazonica* (Nogueira, Oliveira & Oliveira, 2019)
- *S. argyrea* (Cockerell, 1912)
- *S. aspera* (Nogueira & Oliveira, 2019)
- *S. atlantica* (Melo, 2004)
- *S. cearensis* (Nogueira, Santos Júnior & Oliveira, 2019)
- *S. latitarsis* (Friese, 1900)
- *S. longula* (Lepeletier, 1836)
- *S. tenuis* (Ducke, 1916)
- *S.* (formerly *Schwarzula*) *coccidophila* (Camargo amazonica Pedro, 2002)
- *S.* (formerly *Schwarzula*) *timida* (Silvestri, 1902)

Schwarziana Moure, 1943

Medium-sized bees (6–7.5 mm), restricted to eastern Brazil, Paraguay and northern Argentina (Camargo and Pedro 2013; Melo 2015). Closely related to *Mourella* (Rasmussen and Cameron 2010). Colonies nest in the ground and dwarf queens exist (Chaps. 3 and 5).

- *S. bocainensis* (Melo, 2015)
- *S. chapadensis* (Melo, 2015)
- *S. mourei* (Melo, 2003)
- *S. quadripunctata* (Lepeletier, 1836)

Tetragona Lepeletier & Audinet-Serville, 1828

Medium-sized bees (5–8 mm), ranging from Mexico to Argentina (Camargo and Pedro 2013; Roig-Alsina et al. 2013). Closely related to *Ptilotrigona* and *Camargoia* (Michener 2007; Rasmussen and Cameron 2010). Currently 13 species:

- *T. beebei* (Schwarz, 1938)
- *T. clavipes* (Fabricius, 1804)
- *T. dissecta* (Moure, 2000)
- *T. dorsalis* (Smith, 1854)
- *T. essequiboensis* (Schwarz, 1940)
- *T. goettei* (Friese, 1900)
- *T. handlirschii* (Friese, 1900)
- *T. kaieteurensis* (Schwarz, 1938)
- *T. mayarum* (Cockerell, 1912)
- *T. perangulata* (Cockerell, 1917)
- *T. quadrangula* (Lepeletier, 1836)
- *T. truncata* (Moure, 1971)
- *T. ziegleri* (Friese, 1900)

Tetragonisca Moure, 1946

Small yellowish bees (4–5 mm), ranging from Mexico to Argentina (Camargo and Pedro 2013). Closest relatives are *Duckeola*, *Frieseomelitta* and *Trichotrigona* (Rasmussen and Cameron 2010). *T. angustula* and *T. fiebrigi* have a soldier caste (Grüter et al. 2012, 2017; Segers et al. 2015). This genus includes cavity nesting, ground nesting and exposed-nesting species (Chap. 3). Currently four species are described, but note that *T. angustula* and *T. fiebrigi* have been considered sub-species that are in the process of speciation by Francisco et al. (2014).

- *T. angustula* (Latreille, 1811)
- *T. buchwaldi* (Friese, 1925)
- *T. fiebrigi* (Schwarz, 1938)
- *T. weyrauchi* (Schwarz, 1943)

Trichotrigona Camargo & Moure, 1983

Small bees (4–5 mm) with black and yellow markings, rare and so far only found in the Amazon region. Two species have been described, they do not appear to store food and are hypothesised to live in a cleptobiotic association with *Frieseomelitta* (Camargo and Moure 1983; Pedro and Cordeiro 2015).

- *T. camargoiana* (Pedro & Cordeiro, 2015)
- *T. extranea* (Camargo & Moure, 1983)

Trigona Jurine, 1807

Highly diverse group, from small to large workers (5.5–11 mm), ranging from Mexico to Argentina (Camargo and Pedro 2013). Colonies tend to be large (Chap. 1, Table 1.3); some species nest in the ground, others in cavities, in exposed nests or in termite or ant nests (Chap. 3). Currently 32 described species.

- *T. albipennis* (Almeida, 1995)
- *T. amalthea* (Olivier, 1789)

- *T. amazonensis* (Ducke, 1916)
- *T. branneri* (Cockerell, 1912)
- *T. braueri* (Friese, 1900)
- *T. chanchamayoensis* (Schwarz, 1948)
- *T. cilipes* (Fabricius, 1804)
- *T. corvina* (Cockerell, 1913)
- *T. crassipes* (Fabricius, 1793)
- *T. dallatorreana* (Friese, 1900)
- *T. dimidiata* (Smith, 1854)
- *T. ferricauda* (Cockerell, 1917)
- *T. fulviventris* (Guérin, 1844)
- *T. fuscipennis* (Friese, 1900)
- *T. guianae* (Cockerell, 1910)
- *T. hyalinata* (Lepeletier, 1836)
- *T. hypogea* (Silvestri, 1902)
- *T. lacteipennis* (Friese, 1900)
- *T. muzoensis* (Schwarz, 1948)
- *T. necrophaga* (Camargo & Roubik, 1991)
- *T. nigerrima* (Cresson, 1878)
- *T. pallens* (Fabricius, 1798)
- *T. pampana* (Strand, 1910)
- *T. pellucida* (Cockerell, 1912)
- *T. permodica* (Almeida, 1995)
- *T. recursa* (Smith, 1863)
- *T. sesquipedalis* (Almeida, 1984)
- *T. silvestriana* (Vachal, 1908)
- *T. spinipes* (Fabricius, 1793)
- *T. truculenta* (Almeida, 1984)
- *T. venezuelana* (Schwarz, 1948)
- *T. williana* (Friese, 1900)

Trigonisca Moure, 1950

Minute bees (2–4 mm body length), closely related to other minute bees of the genera *Celetrigona* and *Leurotrigona* (Camargo and Pedro 2005; Michener 2007; Rasmussen and Cameron 2010; Engel et al. 2019). Ten species were previously grouped in the genus *Dolichotrigona*, but Rasmussen and Cameron (2010) recommended synonymising them under *Trigonisca* to create a monophyletic genus (see Fig. 2.2). Often perceived as a nuisance because of their tendency to fly into eyes, ears and the nose of humans to collect human secretions (Camargo and Pedro 2005). Currently 37 described species, ranging from Mexico to Argentina (Camargo and Pedro 2013; Roig-Alsina et al. 2013; Alvarez and Lucia 2018; Engel et al. 2019).

- *T. atomaria* (Cockerell, 1917)
- *T. azteca* (Ayala, 1999)

- *T. bidentata* (Albuquerque & Camargo, 2007)
- *T. browni* (Camargo & Pedro, 2005)
- *T. buyssoni* (Friese, 1902)
- *T. ceophloei* (Schwarz, 1938)
- *T. chachapoya* (Camargo & Pedro, 2005)
- *T. clavicornis* (Camargo & Pedro, 2005)
- *T. discolor* (Wille, 1965)
- *T. dobzhanskyi* (Moure, 1950)
- *T. duckei* (Friese, 1900)
- *T. extrema* (Albuquerque & Camargo, 2007)
- *T. flavicans* (Moure, 1950)
- *T. fraissei* (Friese, 1901)
- *T. graeffei* (Friese, 1900)
- *T. hirticornis* (Albuquerque & Camargo, 2007)
- *T. intermedia* (Moure, 1990)
- *T. longitarsis* (Ducke, 1916)
- *T. martinezi* (Brèthes, 1920)
- *T. maya* (Ayala, 1999)
- *T. mepecheu* (Engel & Gonzalez, 2019)
- *T. mendersoni* (Camargo & Pedro, 2005)
- *T. meridionalis* (Albuquerque & Camargo, 2007)
- *T. mixteca* (Ayala, 1999)
- *T. moratoi* (Camargo & Pedro, 2005)
- *T. nataliae* (Moure, 1950)
- *T. pediculana* (Fabricius, 1804)
- *T. pipioli* (Ayala, 1999)
- *T. rondoni* (Camargo & Pedro, 2005)
- *T. roubiki* (Albuquerque & Camargo, 2007)
- *T. sachamiski* (Alvarez & Lucia, 2018)
- *T. schulthessi* (Friese, 1900)
- *T. tavaresi* (Camargo & Pedro, 2005)
- *T. townsendi* (Cockerell, 1911)
- *T. unidentata* (Albuquerque & Camargo, 2007)
- *T. variegatifrons* (Albuquerque & Camargo, 2007)
- *T. vitrifrons* (Albuquerque & Camargo, 2007)

2.4.2 Afrotropical Species

Apotrigona Moure, 1950

Small to mid-sized bees (5–7 mm body length) bees. Currently with one species that has been referred to by several names (e.g. *Meliponula nebulata* or *Apotrigona infuscata*) (Eardley 2004; Rasmussen and Cameron 2007). Michener (2007) suggests synonymy with *Plebeiella*, but molecular data suggests that this would create a non-monophyletic group (Rasmussen and Cameron 2007). Reported in

Sierra Leone, Ivory Coast, Nigeria, Gabon, Congo and Uganda (Anguilet et al. 2015; Ascher and Pickering 2018).

- *A. nebulata* (Smith, 1854)

Axestotrigona Moure, 1961

Medium-sized bees (5.5–7.5 mm body length) (Eardley 2004; Rasmussen and Cameron 2007). Several species with unclear status, sometimes with different colour morphs (Eardley 2004; Rasmussen and Cameron 2007; Michener 2007; Pauly and Anguilet 2013; Ndungu et al. 2017). Currently contains about seven species (Eardley 2004; Pauly and Anguilet 2013; Ndungu et al. 2017). Reported from Gambia to Kenya, in the south to South Africa (Michener 2007; Ascher and Pickering 2018):

- *A. cameroonensis* (Friese, 1900)
- *A. erythra* (Schletterer, 1891)
- *A. ferruginea** (Lepeletier, 1841)
- *A. simpsoni* (Moure, 1961)
- *A. richardsi* (Darchen, 1981)
- *A. togoensis* (Stadelmann, 1895)

*Two morphs, reddish brown and black, most likely representing two species (e.g. Ndungu et al. 2017).

Cleptotrigona Moure, 1961

Small robust, black bees (3.5–4 mm body length), obligate robbers invading the nests of other stingless bees, mainly *Hypotrigona* (Chap. 7) (Eardley 2004). Currently one valid species, but Moure (1961) reported two. Probably a sister group of *Liotrigona* (Michener 2007). Reported from Liberia to Tanzania, in the south to Angola and South Africa (Michener 2007; Ascher and Pickering 2018):

- *C. cubiceps* (Friese, 1912)

Dactylurina Cockerell, 1934

Slender, medium-sized bees (5–7 mm body length) that are unusual in that they build vertical brood combs and exposed nests (Eardley 2004). Two species, closely related to *Meliponula* (Rasmussen and Cameron 2007). Widespread in tropical Africa, from Kenya and Tanzania to Congo, Angola and Liberia (Michener 2007; Ascher and Pickering 2018).

- *D. schmidti* (Stadelmann, 1895)
- *D. staudingeri* (Gribodo, 1893)

Hypotrigona Cockerell, 1934

Minute bees (2–4 mm body length) that are difficult to separate using morphological traits (Eardley 2004; Michener 2007). Widespread and abundant in tropical Africa, from Somalia in the east to Senegal in the west and from Sudan in the north to South Africa in the south (Michener 2007; Ascher and Pickering 2018). Currently about five species (Eardley 2004; Michener 2007).

- *H. araujoi* (Michener, 1959)
- *H. braunsi* (Kohl, 1894)
- *H. gribodoi* (Magretti, 1884)
- *H. squamuligera** (Benoist, 1937)
- *H. ruspolii* (Magretti, 1898)

**Hypotrigona penna* (Eardley, 2004) is considered a junior synonym of *H. squamuligera* by Pauly and Anguilet (2013).

Liotrigona Moure, 1961

Minute bees (2–4 mm body length), closely related to the other genus of minute bees, *Hypotrigona* (Rasmussen and Cameron 2007). With 12 species the most species-rich African genus (Koch 2010; Pauly et al. 2001; Pauly and Anguilet 2013; Anguilet et al. 2015). Widely distributed in sub-Saharan Africa and the only stingless bee genus found on Madagascar (seven endemic species) (Pauly et al. 2001; Michener 2007; Koch 2010).

- *L. baleensis* (Pauly & Hora, 2013)
- *L. betsimisaraka* (Pauli, 2001)
- *L. bitika* (Brooks & Michener, 1988)
- *L. bottegoi* (Magretti, 1895)
- *L. bouyssoui** (Vachal, 1903)
- *L. chromensis* (Pauly, 2001)
- *L. gabonensis* (Pauly & Anguilet, 2013)
- *L. kinzelbachi* (Koch, 2010)
- *L. madecassa* (Saussure, 1890)
- *L. mahafalya* (Brooks & Michener, 1988)
- *L. nilssoni* (Michener, 1990)
- *L. voeltzkovi* (Friese, 1900)

*Considered a *Hypotrigona* by Eardley (2004), but Pauly and Anguilet (2013) consider it a senior synonym of *Liotrigona parvula* (Darchen, 1971).

Meliplebeia Moure, 1961

Small to medium-sized bees (4–7 mm body length) (Eardley 2004; Michener 2007). Distribution from equatorial Africa to South Africa (Michener 2007; Ascher and Pickering 2018). Currently four species:

- *M. beccarii* (Gribodo, 1879)
- *M. gambiana* (Moure, 1961)
- *M. ogouensis* (Vachal, 1903)

- *M. roubiki* (Eardley, 2004)

Meliponula **Cockerell, 1934**

Largest African stingless bees (c. 6.5–8 mm body length) with yellowish body parts, thus easy to identify (Eardley 2004; Michener 2007). One species, from Senegal in the west to Kenya in the east and to Botswana and Namibia in the south (Eardley 2004; Ascher and Pickering 2018).

- *M. bocandei* (Spinola, 1853)

Plebeiella **Moure, 1961**

Two small (c. 4–5.5 mm body length) ground nesting species (Eardley 2004; Estienne et al. 2017). From Togo to Kenya and to Namibia and Zambia in the south (Eardley 2004; Ascher and Pickering 2018).

- *P. griswoldorum* (Eardley, 2004)
- *P. lendliana* (Friese, 1900)

Plebeina **Moure, 1961**

Small bees (c. 3–5 mm body length) living in terrestrial termite nests (Eardley 2004). From Senegal to Kenya and to South Africa in the south (Eardley 2004; Ascher and Pickering 2018). Several different names have been used for what might be a variable species or several species (Michener 2007; Kajobe and Roubik 2018). *Plebeina hildebrandti* (Friese, 1900) and *P. denoiti* (Vachal, 1903) are considered junior synonyms of *P. armata* (Kajobe and Roubik 2018):

- *P. armata* (Magretti, 1895)

2.4.3 Indo-Malayan and Australasian Species[7]

Austroplebeia **Moure, 1961**

Small, robust bees (ca. 3–4 mm body length) with distinct yellow markings on the scutellum and often in the face (Michener 2007; Dollin et al. 2015). They are most closely related to *Lisotrigona* and, interestingly, to the African genus *Liotrigona*. A dispersal event from Africa to Australia via the Indo-Malayan region seems most likely (Rasmussen and Cameron 2010). Found in the northern and eastern parts of Australia and in New Guinea (Dollin et al. 2015). Unusual for building clustered brood combs with spherical cells (Chap. 3), ~5 species (Rasmussen 2008; Rasmussen et al. 2017):

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[7] See the Appendix of Rasmussen et al. (2017) for a list of junior synonyms of Indo-Malayan and Australasian species.

- *A. australis* (Friese, 1898)
- *A. cassiae* (Cockerell, 1910)
- *A. cincta* (Mocsáry in Friese, 1898)
- *A. essingtoni* (Cockerell, 1905)
- *A. magna* (Dollin, Dollin, & Rasmussen, 2015)

Geniotrigona **Moure, 1961**

Robust, medium-sized bees (c. 5–7 mm body length) of black and dark brown colouration. From Myanmar and Laos to Sumatra and Kalimantan (Indonesia) (Rasmussen 2008; Rasmussen et al. 2017; Ascher and Pickering 2018). Two species:

- *G. lacteifasciata* (Cameron, 1902)
- *G. thoracica* (Smith, 1857)

Heterotrigona **Schwarz, 1939**

Relatively small to mid-sized bees (c. 4–7 mm body length) with a distribution from Thailand to Indonesia and the Philippines (Engel and Rasmussen 2017; Rasmussen et al. 2017; Ascher and Pickering 2018):

- *H. bakeri* (Cockerell, 1919)
- *H. erythrogastra* (Cameron, 1902)
- *H. itama* (Cockerell, 1918)

Homotrigona **Moure, 1961**

Medium-sized to large and robust bees (c. 7–8 mm body length) with a distribution from Thailand and Vietnam to Indonesia (Rasmussen 2008; Rasmussen et al. 2017; Ascher and Pickering 2018). Four species:

- *H. aliceae* (Cockerell, 1929)
- *H. anamitica* (Friese, 1909)
- *H. fimbriata* (Smith, 1857)
- *H. lutea* (Bingham, 1897)

Lepidotrigona **Schwarz, 1939**

Small to medium-sized bees (c. 4–5.5 mm body length) with a distribution from India to Taiwan and the Philippines and, in the south, to Java (Michener 2007). Thirteen species (Rasmussen 2008; Rasmussen et al. 2017; Attasopa et al. 2018):

- *L. arcifera* (Cockerell, 1929)
- *L. doipaensis* (Schwarz, 1939)
- *L. flavibasis* (Cockerell, 1929)
- *L. hoozana* (Strand, 1913)
- *L. javanica* (Gribodoo, 1891)
- *L. latebalteata* (Cameron, 1902)
- *L. latipes* (Friese, 1900)

- *L. nitidiventris* (Smith, 1857)
- *L. palavanica* (Cockerell, 1915)
- *L. satun* (Attasopa & Bänziger, 2018)
- *L. terminata* (Smith, 1878)
- *L. trochanterica* (Cockerell, 1920)
- *L. ventralis* (Smith, 1857)

Lisotrigona Moure, 1961

Minute to small bees (c. 2.5–4 mm body length) with a distribution from India and Sri Lanka to Malaysia (Rasmussen 2008). About six species (Engel 2000b; Rasmussen 2008; Viraktamath and Jose 2017):

- *L. cacciae* (Nurse, 1907)
- *L. carpenteri* (Engel, 2000)
- *L. chandrai* (Viraktamath & Sajan Jose, 2017)
- *L. furva* (Engel, 2000)
- *L. mohandasi* (Jobiraj & Narendran, 2004)
- *L. revanai* (Viraktamath & Sajan Jose, 2017)

Lophotrigona Moure, 1961

Medium-sized to large and robust bee (7–8 mm body length), dominant at food sources, distribution from Myanmar and Thailand to Indonesia (Rasmussen 2008). One species:

- *L. canifrons* (Smith, 1857)

Odontotrigona Moure, 1961

Medium-sized, black bees (~6 mm body length), found in Malaysia (Rasmussen 2008; Ascher and Pickering 2018). One species:

- *O. haematoptera* (Cockerell, 1919)

Papuatrigona Michener & Sakagami, 1991

One species of small to mid-sized bees (c. 4.5–5 mm body length), found in New Guinea and Indonesia (Michener 2007; Rasmussen 2008):

- *P. genalis* (Friese, 1908)

Pariotrigona Moure, 1961

Minute bees (c. 2.5–3 mm body length). From Thailand in the north to Borneo and Sumatra in the south (Michener 2007). One described species, closely related to *Hypotrigona* (Michener 2007; Rasmussen and Cameron 2010; Rasmussen et al. 2017):

- *P. pendleburyi* (Schwarz, 1939)

Platytrigona **Moure, 1961**

Medium-sized bees, found in Brunei, Indonesia, Malaysia and Papua New Guinea (Rasmussen 2008; Rasmussen et al. 2017; Ascher and Pickering 2018). Five described species:

- *P. flaviventris* (Friese, 1908)
- *P. hobbyi* (Schwarz, 1937)
- *P. keyensis* (Friese, 1901)
- *P. lamingtonia* (Cockerell, 1929)
- *P. planifrons* (Smith, 1865)

Sahulotrigona **Engel & Rasmussen, 2017**

Small to medium sized bees (~5–6 mm body length), found in New Guinea. Two species:

- *S. atricornis* (Smith, 1865)
- *S. paradisaea* (Engel & Rasmussen, 2017)

Sundatrigona **Inoue & Sakagami, 1993**

Small, shiny and black bees (3–4 mm body length). Two species found in Indonesia and Malaysia (Schwarz 1937; Rasmussen 2008):

- *S. lieftincki* (Sakagami & Inoue, 1987)
- *S. moorei* (Schwarz, 1937)

Tetragonilla **Moure, 1961**

Medium sized bees (c. 5–6), closely related to *Tetragonula*. From Myanmar and Thailand to Malaysia and Indonesia. Pronounced dark wings with white tips, similar to *Tetrigona* and some Neotropical *Frieseomelitta*, four species:

- *T. atripes* (Smith, 1857)
- *T. collina* (Smith, 1857)
- *T. fuscibasis* (Cockerell, 1920)
- *T. rufibasalis* (Cockerell, 1918)

Tetragonula **Moure, 1961**

Small to medium sized bees (c. 3–7 mm body length). Wide distribution in tropical Asia and Australia. Abundant and species-rich genus with approx. 34 species (Rasmussen 2008; Rasmussen et al. 2017; Shanas and Faseeh 2019). Three species have been allocated to the subgenus *Flavotetragonula* (Shanas and Faseeh 2019). Species show great biological diversity, e.g. in their nesting behaviour (see Chap. 3).

- *T. bengalensis* (Cameron, 1897)
- *T. biroi* (Friese, 1898)

- *T. carbonaria* (Smith, 1854)
- *T. clypearis* (Friese, 1909)
- *T. dapitanensis* (Cockerell, 1925)
- *T. davenporti* (Franck, 2004)
- *T. drescheri* (Schwarz, 1939)
- *T. fuscobalteata* (Cameron, 1908)
- *T. geissleri* (Cockerell, 1918)
- *T. hirashimai* (Sakagami, 1978)
- *T. hockingsi* (Cockerell, 1929)
- *T. iridipennis* (Smith, 1854)
- *T. laeviceps* (Smith, 1857)
- *T. malaipanae* (Engel, Michener, & Boontop, 2017)
- *T. melanocephala* (Gribodo, 1893)
- *T. melina* (Gribodo, 1893)
- *T. mellipes* (Friese, 1898)
- *T. minangkabau* (Sakagami & Inoue, 1985)
- *T. minor* (Sakagami, 1978)
- *T. pagdeni* (Schwarz, 1939)
- *T. pagdeniformis* (Sakagami, 1978)
- *T. penangensis* (Cockerell, 1919)
- *T. perlucipinnae* (Faseeh & Shanas, 2019)
- *T. reepeni* (Friese, 1918)
- *T. ruficornis* (Smith, 1870)
- *T. sapiens* (Cockerell, 1911)
- *T. sarawakensis* (Schwarz, 1937)
- *T. sirindhornae* (Michener & Boongird, 2004)
- *T. testaceitarsis* (Cameron, 1901)
- *T. travancorica* (Shanas & Faseeh, 2019)
- *T. zucchii* (Sakagami, 1978)

Tetragonula (Flavotetragonula)
- *T. calophyllae* (Shanas & Faseeh, 2019)
- *T. gressitti* (Sakagami, 1978)
- *T. praeterita* (Walker, 1860)

Tetrigona Moure, 1961
Medium-sized to large bees (c. 6–8 mm body length). Distribution from Myanmar, Laos and Vietnam in the north to Indonesia and Timor in the south (Rasmussen 2008). Five species:

- *T. apicalis* (Smith, 1857)
- *T. binghami* (Schwarz, 1937)
- *T. melanoleuca* (Cockerell, 1929)
- *T. peninsularis* (Cockerell, 1927)
- *T. vidua* (Lepeletier, 1836)

Wallacetrigona Engel & Rasmussen, 2017

Medium-sized bees (c. 5–7 mm body length). Currently one species known from Indonesia, east of the Wallace Line (Rasmussen et al. 2017):

- *W. incisa* (Sakagami & Inoue, 1989)

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Nesting Biology

3

The nest is the place where bees spend most of their time. Only towards the end of a worker's life will she start to leave the nest regularly to forage for resources or guard the nest entrance (Chaps. 6 and 8). In the complete darkness of the nest cavity, bees are in frequent contact with nest-mates, share food and exchange chemical and tactile information about the numerous needs of the colony. The nest is also the place where the collected resources are stored, where brood is reared and where bees find refuge from enemies. Given these important roles of the nesting cavity, it seems evident that its location, architectural features and the materials and resources it harbours are critical for the success of a colony. For instance, nest location and architecture influence the predation intensity (e.g. Fowler 1979; Slaa 2006) or the climatic conditions experienced by the brood (Jones and Oldroyd 2006). Finding a suitable nest-site and building the required structures might be even more important in stingless bees than in honey bees because stingless bee colonies are unable to swap an unsuitable nest for a better one (see Chap. 4).

Stingless bee nesting traits show a great diversity and do not seem to follow evolutionary relationships. Closely related species can differ in key aspects of nesting, such as the arrangement of the brood cells (e.g. combs vs. clusters) or the type of cavities used (e.g. the Neotropical genus *Tetragonisca* contains a species that nests in the ground, two that nest in cavities and one the builds exposed nests) and in some species, traits such as the presence of protective sheets around the brood nest (involucrum) vary within species.

3.1 Nesting Sites

As a group, stingless bees have adapted to a wide range of nesting sites, from living in ant and termite nests above and below ground, cavities in trees, branches, rocks or human constructions to largely self-built, exposed nests in trees (see Fig. 3.1; Table 3.1) (Schwarz 1948; Wille and Michener 1973; Michener 1974; Roubik 1983, 2006).

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Fig. 3.1 Different nesting styles. (**a**) *Tetragonisca angustula* nesting in a metal pipe (c. 3 cm diameter). (**b**) *Lestrimelitta limao* nest in a house wall (Photo: Lucas von Zuben). (**c**) Exposed nest of *Trigona spinipes* in a tree. (**d**) *Trigona hyalinata* nest attached to a wall. (**e**) *Partamona helleri* mud nest with the typical "toad mouth" entrance in an indentation of a wall. (**f**) *Frieseomelitta varia* colony living in an electricity box. This species covers the surfaces surrounding the nest with a layer of dark resin, here on the wall of the building. The function of this behaviour is not yet known (Photos **a** and **c**–**f** were taken in Ribeirão Preto, Brazil, by the author)

3.1.1 Above Ground, Cavity-Nesting Species

The majority of stingless bees nest in pre-existing cavities in tree trunks or branches (Fig. 3.2), which are entered through a relatively small entrance hole

Table 3.1 Nesting habits of 145 species from Central America

Nesting habits Either exclusively or commonly	% of species (*N*)
Ground	11.7% (17)
Cavities in trees[a]	65.5% (95)
In active termite nests[b]	9% (13)
In active ant nests	2.1% (3)
Exposed or partly exposed	11.7% (17)

Data taken from Table 2 in Wille and Michener (1973)

[a] These often also nest in artificial cavities

[b] Both below and above ground

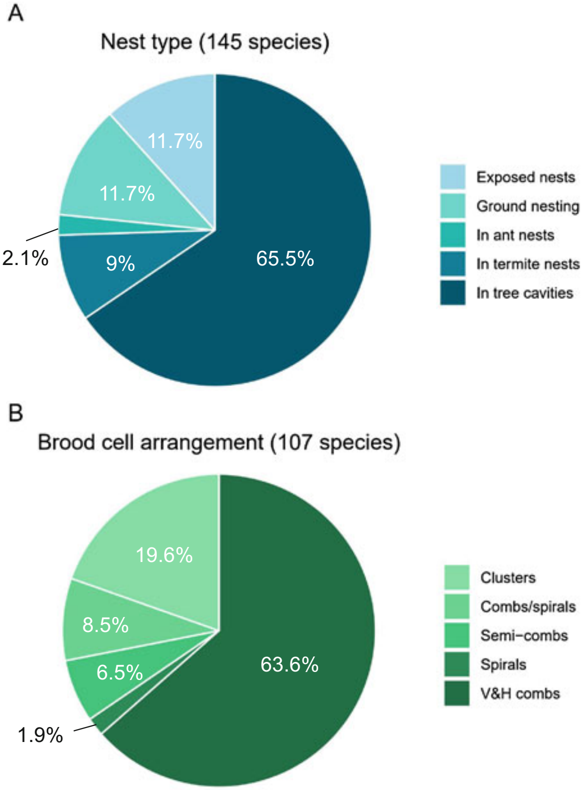

Fig. 3.2 (**a**) The nesting habits of 145 species from Central America, data taken from Table 3.1. (**b**) The brood cell arrangement of 107 species listed in Table 1.3. The most common types are brood combs, either vertical or horizontal (V & H). The category "semi-combs" contain some plastic species (see Table 1.3)

(Fig. 3.3). The selected trees are usually large (>50 cm diameter at breast height) and alive (Roubik 1983; Eltz et al. 2003; Samejima et al. 2004; Siqueira et al. 2012; Silva and Ramalho 2014; Arena et al. 2018) and there is a trend for larger bees to nest in

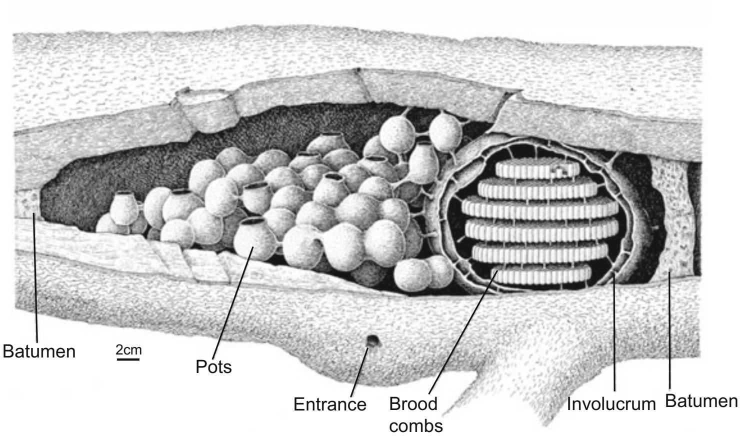

Fig. 3.3 A nest of *Melipona grandis* in a tree branch (from Camargo 1970). See text for description of the different functional parts

larger trees (Hubbell and Johnson 1977).[1] Colonies of some species, however, commonly nest in dead trees, e.g. the Neotropical *Frieseomelitta* spp. and *Trichotrigona extranea* or the Asian *Tetragonula melanocephala* and *Tetragonilla rufibasalis* (Camargo and Moure 1983; Samejima et al. 2004). If nests are in trunks, they are usually relatively close to the ground (<5 m height) (Kerr et al. 1967; Eltz et al. 2003; Cortopassi-Laurino et al. 2009; Silva and Ramalho 2014). However, some species, *Trigona* and *Oxytrigona* in particular, often have nests at 10–25 m height (Roubik 1983). Other species, like *Melipona nigra,* nest at the foot of a tree in root cavities or between roots (Camargo 1970; Michener 1974; Eltz et al. 2003; Rasmussen and Camargo 2008). Nesting height is likely to have implications for the predation pressure and the microclimate experienced by a colony. In *Tetragonisca angustula*, for example, founding colonies were less likely to die if they were nesting higher up in trees, possibly because attacks by ants are less common (Slaa 2006).

Most stingless bee species do not seem to be very specific in their choice of tree species and opportunistically exploit available nesting sites (Hubbell and Johnson 1977; Fowler 1979; Eltz et al. 2003; Siqueira et al. 2012; Tornyie and Kwapong 2015; Silva and Ramalho 2014). A survey of 716 inhabited trees in Brazil found that the utilised trees belonged to 56 different families (Cortopassi-Laurino et al. 2009; see also Eltz et al. 2003; Siqueira et al. 2012; Silva and Ramalho 2014). Some species, however, seem to show a preference. For example, 46 of 48 examined *Melipona quadrifasciata* colonies in a Brazilian “Cerrado” (savannah) habitat were

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[1] Hubbell and Johnson (1977) highlight that the link between tree size and presence of large-bodied bee species may not only explain the absence of large-bodied bees in areas without large trees but will also determine the ability of large-bodied bee species to recolonise secondary-growth habitats. The recolonisation of secondary forest would thus follow a sequence of bee species that is linked to bee size. This, in turn, may affect forest management practices (see also Samejima et al. 2004).

found in one tree species, *Caryocar brasiliense,* even though many other potential nesting sites were available (Antonini and Martins 2003; see also Martins et al. 2004). Likewise, Kerr (1984, cited in Roubik 1989, p. 198) reports that colonies nested in only 12 of 200 available tree species in Mato Grosso in Brazil.[2]

The cavity size requirements are species-specific and, in a study including 30 species in Panamá, ranged from about 0.1 L (e.g. *Plebeia minima*) to more than 30 L (e.g. *Cephalotrigona zexmeniae*) (Roubik 1983). The Asian *Lophotrigona canifrons* can be found in huge cavities that exceed 300 L (Inoue et al. 1993). If cavities are too large, colonies can limit the inhabited space using sheets (or plates) of batumen (Michener 1974), i.e. hard, protective layers that consist of cerumen (see below), resin, mud, faeces or plant material and enclose the nest cavity (Ihering 1903; Schwarz 1948; Michener 1974; Wille 1983).

Many cavity-nesting species can take advantage of human constructions and nest under roofs, in hollow spaces in walls, electricity boxes and even metal tubes (Fig. 3.1). The small Neotropical *Tetragonisca angustula*, in particular, shows what Schwarz (1948, pp. 17) called an “impish perversity in converting into a nest hollow what man had intended for other ends”. Colonies of this species have been found in a letter box, metal cans, a sewing machine and in the valve of a water pipe (Schwarz 1948; pers. obs.). The colony shown in Fig. 3.1a has moved into a long metal pipe of not more than 3 cm diameter and lived there for several years. This remarkable ability to exploit a wide range of natural and human-made cavities might be one of the reasons why *T. angustula* is the most common stingless bee in many Neotropical areas (Freitas 2001; Slaa 2006; Velez-Ruiz et al. 2013).

Occasionally, colonies take advantage of abandoned cavities made by other animals, e.g. by birds or beetle and moth larvae (Schwarz 1948; Darchen 1969; Michener 1974; Camargo and Pedro 2002). Abandoned mud nests of the bird *Furnarius rufus* are occasionally used, e.g. by *Tetragonisca angustula* (pers. obs.), and the stingless bee *Paratrigona pacifica* seems to reuse these bird nests on a regular basis (Schwarz 1948; Camargo and Moure 1994).

3.1.2 Ground-Nesting Species

A sizable minority of species, including species belonging to the American *Camargoia*, *Geotrigona*, *Melipona*, *Mourella,* *Nogueirapis*, *Paratrigona,* *Partamona,* *Schwarziana* and *Tetragonisa* or the African *Meliplebeia*, *Plebeiella* and *Plebeina*, take advantage of cavities in the ground, often abandoned nests of ants, termites or rodents (da Silva et al. 1972; Wille 1966; Michener 1974; Fowler 1979; Camargo 1996; Camargo and Pedro 2003; Eardley 2004; Njoya 2009; Vossler et al. 2010; Barbosa et al. 2013; Njoya et al. 2017; Galaschi-Teixeira et al. 2018).

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[2] Roubik (1989) did not specify the number of colonies that were examined by Kerr, which makes it difficult to determine whether this report indeed indicates a strong preference for certain tree species.

Geotrigona mombuca, Schwarziana quadripunctata and *Paratrigona lineata*, for example, are thought to mostly use abandoned nests of leaf-cutter ants (*Atta*) (Schwarz 1948; Michener 1974; Wille 1983), whereas *Tetragonisca buchwaldi* uses cavities made by small mammals (Wille 1966). It thus seems that stingless bees do not excavate their own cavity, but they may enlarge an existing cavity (Michener 1974; I have watched a *Scaptotrigona bipunctata* colony remove significant amounts of earth to make space for their nest at the base of house). Occasionally, natural cracks in rocks or cavities formed by rain water can be used (Wille 1966; Bänziger et al. 2011; Barbosa et al. 2013).

Most nests are less than 1 m below ground: *Nogueirapis mirandula* colonies usually nest at a depth of 25–30 cm and *Tetragonisca buchwaldi* nests are found at a depth of 15–35 cm (Wille 1966). Estienne et al. (2017) studied 73 nests of the African *Plebeiella lendliana* and found that nest chambers were at an average depth of 49.8 ± 19.8 cm (range 14–117 cm). *Paratrigona lineata*, *Geotrigona subterranean* or *G. argentina*, on the other hand, frequently nests at 1 m and more below the surface (Wille 1983; Vossler et al. 2010; Barbosa et al. 2013) and nests of *Geotrigona subterranea* and *Schwarziana quadripunctata* were found at a depth of ~3 m (Schwarz 1948). Having nests at greater depth might not necessarily increase protection against mammalian predators, at least for nests at less than 1 m depth. On the other hand, a non-vertical entrance tube can make nests harder to find (Estienne et al. 2017) (see also Chap. 7).

A prominent feature of many nests in the ground is a vertical projection exiting from the floor of the nest, probably functioning as a drain duct to get rid of excess moisture and water (Fig. 3.4) (Portugal-Araujo 1963; Wille 1966; Camargo 1970; Wille and Michener 1973; Njoya 2009; Njoya et al. 2017). Occasionally, nests have more than one duct, whereas *Geotrigona subterranea, G. mombuca* and *Tetragonisca buchwaldi* seem to lack a drain duct, but nests have openings at the bottom, which presumably have the same purpose (Wille 1966; Barbosa et al. 2013). The drainage channel itself is probably created by the bees themselves. Drainage outlets can also be found in some tree nesting species, e.g. *Trigona* or *Tetragona* (Roubik 2006). Ground nesting species have additional ways to prevent water from entering the nest: *Schwarziana* and *Geotrigona* colonies, for instance, build an involucrum (see Fig. 3.3 and below) with furrows that prevent water from entering the brood area (Roubik 1989).

3.1.3 Nesting in Active Ant or Termite Nests

Numerous species live in walled-off parts of ant (e.g. *Azteca, Camponotus* or *Crematogaster*) or termite nests (often *Nasutitermes*, but also *Constrictotermes, Macrotermes*, *Microcerotermes, Odontotermes* or *Pseudocanthotermes*), both below and above ground (e.g. Wille and Michener; Roubik 2006; Michael Hrncir, pers. commun.). Wille and Michener (1973) found that 9% of all species they studied can nest with termites (Table 3.1), with most of them being entirely reliant on termites for nesting. Examples include species from the American genera *Aparatrigona, Partamona, Scaura* or *Trigona* (Schwarz 1948; Roubik 1983; Wille

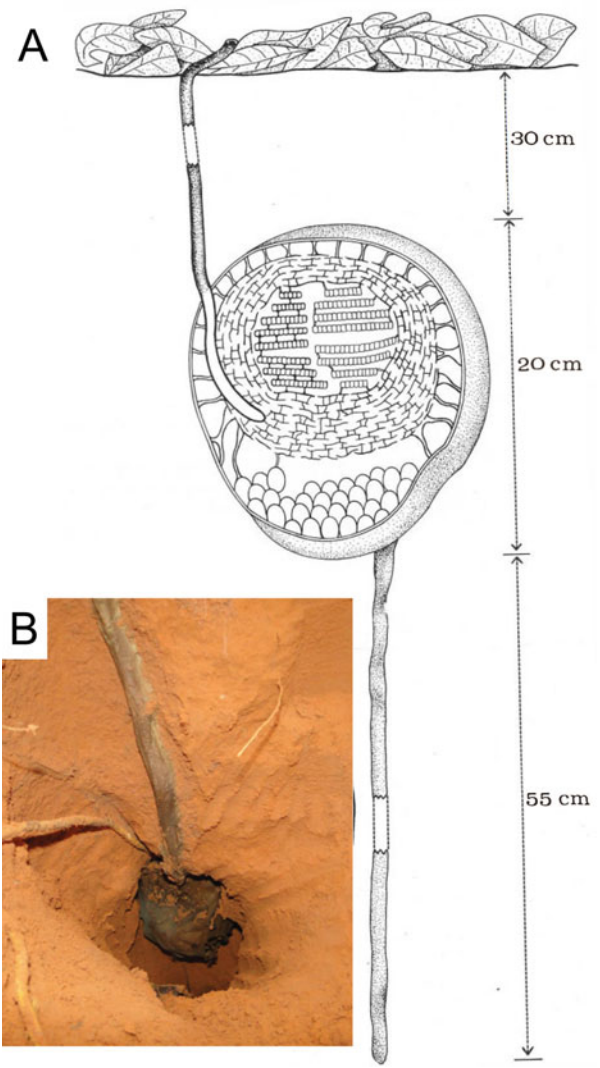

Fig. 3.4 (**a**) Underground nest of *Nogueirapis mirandula* with vertical drain duct (from Wille 1966). This nest was studied in the rain forest of Costa Rica. (**b**) Underground nest of *Geotrigona subterranea* in Brazil (from Barbosa et al. 2013)

1983; Camargo and Pedro 2003; Rasmussen 2004; Rasmussen and Camargo 2008) or the African genera *Apotrigona, Axestotrigona* or *Plebeina* (Namu 2008; Darchen 1969; Namu and Wittmann 2016). Figure 3.4 shows nests of different *Partamona* species nesting in active or abandoned termite nests. Colonies of some species, e.g. *Partamona peckolti* and *Trigona amalthea*, live in termite nests in some areas, but not in others (Schwarz 1948; Camargo and Pedro 2003). While many colonies probably take advantage of pre-existing cavities, *Aparatrigona isopterophila, Apotrigona nebulata* and *Scaura* are suspected to excavate into the termite nest, displacing the termites from that part of the nest (Darchen 1969; Michener 1974; Roubik 1983).

Fig. 3.5 *Partamona* colonies nesting in termite nests (drawings from Camargo and Pedro 2003). (**a**) *P. auripennis* in an abandoned termite nest, Pará state, Brazil. (**b**) Aggregation of nests of *P. batesi* in an active nest of *Nasutitermes acangussu* in Amazonia state, Brazil. Eleven different entrances can be seen. (**c**) *Partamona gregaria* (two lower entrances) and a *Partamona vicina* nest (upper entrance) in an active termite nest attached to the roof of a house

Darchen (1972) speculates that termite soldiers aggressively defend their nest against bee invaders and suggests that the presence of a bee colony might often be a case of involuntary acceptance of an intruder, rather than of a beneficial association (see also Schwarz 1948). Bees that only require small cavities, like *Scaura latitarsis*, are unlikely to impose significant costs to the termites, but in the case of termite nests hosting multiple *Partamona* nests (Fig. 3.5d, e), one might wonder whether termite colonies lose valuable living space to the bees. A possible benefit to termites could be that the often aggressive *Partamona* colonies provide protection against common enemies (Roubik 1989).

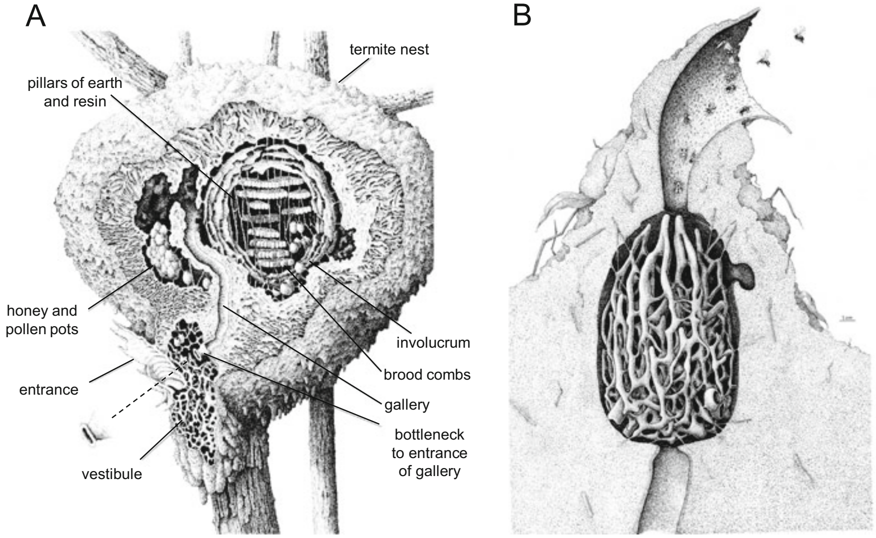

Fig. 3.6 Details of *Partamona* nest architecture. (**a**) *Partamona pearsoni* nest cut longitudinally, in an active nest of *Nasutitermes* cf. *peruanus* in Amazonia state, Brazil. On the lower left, a detailed drawing of the bottleneck leading to the connection gallery to the brood nest (drawing from Camargo and Pedro 2003). (**b**) *P. testacea* vestibule adjacent to the nest entrance (drawing from Camargo 1970)

In some *Partamona* species, structures of unclear function can be found adjacent to the entrance: a vestibule chamber containing intricate structures made from mud and resin and empty storage pots built by workers (Fig. 3.6). It has been speculated that these structures allow guards to rest or that they might delay attackers, such as robber bees (see Chap. 7) or discourage vertebrate predators like anteaters (Camargo and Pedro 2003; Roubik 2006). Occasionally, more elaborate “false nests” can be found, which are also likely to function as a distraction for attackers (Camargo and Pedro 2003).

Associations between stingless bees and ants are more puzzling given that ants pose significant risks to stingless bees (Chap. 7) (Schwarz 1948; Slaa 2006). There are even species that nest exclusively with ants (Table 3.1) (Wille and Michener 1973; Sakagami et al. 1989). *Camponotus* nests, in particular, can be popular nesting sites (Schwarz 1948; Kerr et al. 1967). Examples of stingless bees associated with *Camponotus* include a *Trigona braueri* colony sharing a tree cavity with a *Camponotus* sp. colony (Ihering 1903, pp. 218) or *Scaptotrigona bipunctata* living with *C. sericeiventris* (Schwarz 1948). *Trigona chanchamayoensis* is reported to regularly associate with *C. senex* (Camargo and Posey 1990). *Paratrigona peltata,* too, is reported to often inhabit aerial *Camponotus* nests (Wille and Michener 1973). *Partamona testacea*, on the other hand, was found to be exclusively associated with either bullet ants (*Paraponera clavata*) or leafcutter ants (*Atta*) in a rainforest habitat in Peru (Bordoni et al. 2020). Other reported associations involved a *Paratrigona* *opaca* colony co-inhabiting a former *Azteca* ant nest together with a colony of the aggressive ant *Dolichoderus bispinosus* (Schwarz 1948; Kerr et al. 1967; Camargo and Moure 1994). *Trigona cilipes* has a preference to nest with *Azteca* or *Crematogaster* colonies (Kerr et al. 1967), but has also been found in an active nest of the paper wasp *Epipona tatua* (Rasmussen 2004).[3] It is not clear how much contact usually exists between bees and ants that share a nest, but Roubik (1989) observed that *Nannotrigona mellaria* lives in such close association with their ant neighbours where frequent contact was unavoidable. He hypothesised that bees might use chemical camouflage to avoid being attacked. The potential for conflict is highlighted by the example of *Paratrigona opaca* living in *Camponotus senex* nests: guards positioned inside the entrance tube aggressively defended their nest entrance and killed worker ants that came too close (Kerr et al. 1967). The entrance tube of one examined bee nest was covered with large amounts of sticky resin, which probably served to prevent ants from walking on the tube and entering the nest (see also Chap. 7). *Partamona testacea* and bullet ants, on the other hand, seem to show low levels of aggression towards each other and Bordoni et al. (2020) hypothesise that the members of each party learn the odours of their associates in order to show tolerance to them.

We still know little about how nesting associations between stingless bees and other social insects are initiated and maintained. Sakagami et al. (1989) provide a rare description involving the Asian stingless bee *Sundatrigona moorei*, which nests with *Crematogaster*: a new nest was initiated by bees depositing resin on vegetation near the host nest, before an entrance tube is built on the surface of the ant nest. The entrance is then extended inside the ant nest and an ant-free area is created by building resinous walls. The size is then slowly increased by a continuous replacement of the cavity walls (Sakagami et al. 1989). The rapid construction of resin walls to create an initially small, protected area seems to be a critical stage for the establishment of a new nest inside an existing ant or termite nest (Kerr et al. 1967). Stingless bees potentially benefit from their associations with ants and termites as the hosts provide protection against enemies. Furthermore, nesting inside the nests of another species could help with climate control and homeostasis (see below).

3.1.4 Exposed Nests

Numerous stingless bee species live in exposed or partly exposed nests (Table 3.1). The African *Dactylurina* builds nests hanging from the undersides of large branches, which protect the colonies from bad weather (Darchen and Pain 1966; Michener 1974). Several American *Trigona* species (e.g. *T. corvina*, *T. spinipes* and *T. nigerrima*) and *Tetragonisca weyrauchi* build fully exposed nests (Fig. 3.1) (Michener 1974; Roubik 2006; Rasmussen and Camargo 2008). To compensate

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[3] This species was later considered to be *Trigona lacteipennis* (Rasmussen and Camargo 2008).

for the lack of protection from walls, workers encase the colony with hard layers of batumen (also called *laminate batumen*) consisting of cerumen, plant material, earth or faeces (both vertebrate and bee faeces) (Wille 1983; Wille and Michener 1973). In *Trigona corvina,* the combined batumen layers can be 24 cm thick (Michener 1974) and nests can weigh up to 140 kg (Roubik and Patiño 2009). The largest stingless bee nests are probably built by *T. amazonensis*: their exposed nests can be as high as 6 m and 1 m wide, weighing a few hundred kilograms (Roubik 1989). The outermost layer is thin and often brittle. Another large structure that is occasionally described in exposed *Trigona* nests (e.g. *T. amalthea*, *T. corvina, T. dallatorreana, T. nigerrima or T. spinipes*) and partially exposed *Partamona* nests (*P. cupira* and *P. testacea*) is the scutellum[4] (Ihering 1903; Schwarz 1948; Nogueira-Neto 1962; Wille and Michener 1973; Sakagami 1982; Wille 1983; Rasmussen and Camargo 2008, see their Fig. 16). Schwarz (1948, pp. 25) describes the scutellum as a bowl-shaped mass of earthen material mixed with wax and resin. Wille (1983) describes it as depositions in the batumen on one side of the nest, whereas Roubik and Patiño (2009) refer to the entire shield encasing a colony as the scutellum and found that in *T. corvina*, it mainly consists of pollen exines in bee faeces (unlike honey bees, stingless bee workers defaecate inside the nest, see Chap. 6). This is consistent with Nogueira-Neto's (1962) observation that the scutellum consists of bee faeces, dead bees, cerumen, resin and remains of brood cocoons. It is not currently known whether the scutellum has a defensive function (Roubik 2006), serves as structural support for the nest (Nogueira-Neto 1962) or is simply accumulated waste (Rasmussen and Camargo 2008).

3.1.5 Intra-specific Variation in Nesting Habits

Some species fall into more than one category in terms of their nesting habits, either showing variation within a population (*Trigona fulviventris* nests in both tree cavities and in the ground in Mexico) (Buchwald and Breed 2005) or showing different nesting behaviours in different areas. For example, while Schwarz (1948) suggests that *Plebeia minima* has a preference for tree cavities and vacant beetle burrows, Roubik (1983) found nests to be partly exposed and surrounded by a thick wall of resin on the side of a palm tree or palm hut. *Trigona cilipes* builds nests next to termite colonies in hollow trees in South America, but is not associated with termites in Central America (Roubik 1983). Another interesting case is the meat eating *T. hypogea*, whose nests are found in the ground in some areas of Brazil (Schwarz 1948), but is only found in tree cavities in other parts of Brazil and in Panamá (Roubik 1983 and Gonzalez et al. 2018 discuss more examples of variation

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[4] von Ihering (1940) (cited in Nogueira-Neto 1962) describes how in Northeastern Brazil, the scutellum is used for fishing. The structure is ground to a powder, cooked and placed in a basket that is then put into water. The powder kills fish due to its toxicity, possibly because of a high content of acetylcholine.

in nesting habits). From these descriptions, it is, however, not clear whether the diverging reports indicate an intra-specific behavioural plasticity in nesting habits or whether the different authors may describe different species. *Plebeia minima*, for example, is likely to represent a species complex (Chap. 2) and the differences in nesting habits between black and reddish brown morphed *Axestotrigona ferruginea* colonies is further indication that these morphs represent two different species (Ndungu et al. 2017).

3.2 Nest Architecture, Building Materials and Building Behaviour

Nests of stingless bees are mainly made of a substance called cerumen, which is bee-produced wax mixed with resin and other sticky plant substances (cerumen might also contain saliva secreted by workers, Massaro et al. 2011). The addition of resin has at least two advantages: resin in combination with wax provides a firmer building material than wax alone and collected resin has been shown to have antimicrobial properties, reducing the growth of fungi and bacteria (Messer 1985; Velikova et al. 2000; Chapuisat et al. 2007; Muli et al. 2008; Simone-Finstrom and Spivak 2010; Choudhari et al. 2012; Massaro et al. 2014; Torres et al. 2018; Lavinas et al. 2019). This could be particularly important in the warm and humid environments inhabited by stingless bees.

Stingless bee wax is simpler in composition than honey bee wax and has a lower melting point (Blomquist et al. 1985; Milborrow et al. 1987; Koedam et al. 2002). It contains a much higher percentage of hydrocarbons (e.g. ~60–70% in *Trigonisca atomaria* and *T. buyssoni*, Blomquist et al. 1985; 90% in *Austroplebeia australis*, Milborrow et al. 1987 vs. ~14% in *Apis mellifera*, Winston 1987). The second most important compounds are esters (from ~6% in *A. australis* to ~25% in *T. atomaria* and *T. buyssoni*, Blomquist et al. 1985; Milborrow et al. 1987 vs. ~35% in *Apis mellifera*, Winston 1987). The amount of resin in the cerumen varies among species and the different functional parts of the nest, but can be more than 40% (Schwarz 1945; Roubik 2006). Smaller bees that nest in stems and branches, e.g. *Trigonisca* or *Plebeia*, have been reported to use less resin and relatively more wax (Roubik 2006).

Wax is taken from a wax deposit or directly from a wax-secreting worker (Fig. 3.7a) and combined with resin taken from one of the several resin piles (Fig. 3.7b) that can be distributed throughout the nest, but are often near the entrance. In some species (e.g. *Melipona* or *Partamona*), mud and faeces are also important building materials, either by themselves or mixed with wax, resin or plant material (some *Melipona* has been observed to add little stones and plant seeds; Garcia et al.

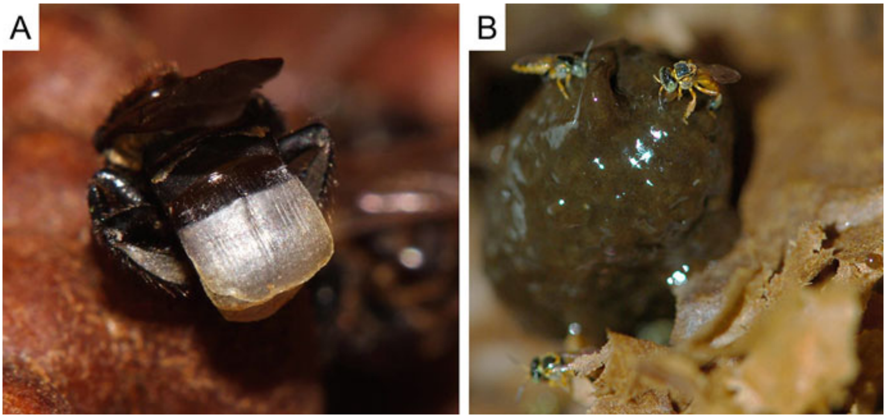

Fig. 3.7 (**a**) *Scaptotrigona depilis* worker secreting wax scales from the dorsal part of the abdomen (Photo: Cristiano Menezes). (**b**) *Tetragonisca angustula* worker bee on resin pile near the nest entrance. Several resin deposits can be found throughout the nest (Photo: C. Grüter)

1992; Roubik 2006).[5] This leads to the creation of batumen of varying composition.[6] Since resin is such an important component of nests (and for defence, Chap. 7), foragers collect it in large quantities, particularly species that build exposed nests and require resin for the protective layers (Michener 1974).

As mentioned before, nests are usually surrounded by batumen for protection (Schwarz 1948; Michener 1974). A distinction is sometimes made between thick *batumen plates* that separate the nest from parts of the natural cavity (see Fig. 3.1) and the *lining batumen*, a thin layer on the walls of the nest (e.g. Wille and Michener 1973). In ground nesting species, the batumen layers are probably important for waterproofing (Wille 1966). Colonies can increase the size of the cavity by relocating batumen walls if an increased colony size requires this (Michener 1974).

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[5] Because it consists of different materials, cerumen can vary in colour from almost white, e.g. in *Plebeia mosquito* to black, e.g. in *Melipona fasciculata*. Cerumen of stingless bees has been put to various uses by indigenous people, including to make candles (see also Chap. 1) (Schwarz 1945).

[6] "Propolis" is a term used by honey bee researchers and beekeepers, either for bee-collected resin (e.g. Winston 1987) or for resin to which bee-derived secretions are added (e.g. Simone-Finstrom and Spivak 2010), which is used by bees as a building and repair material. Stingless bee researchers occasionally use "propolis" (or "geopropolis") as a synonym of cerumen (e.g. Massaro et al. 2011) or an equivalent of "batumen" (Greco et al. 2010). Due to this ambiguity and the diversity of materials used by stingless bees, I avoid the term "propolis" for stingless bees, but refer to resin (or resin-like substances), cerumen or batumen, depending on the composition.

3.2.1 The Entrance

The entrance to stingless bee nests varies from small, inconspicuous openings the size of a bee head (Fig. 3.8) (e.g. in *Frieseomelitta*[7] and some *Melipona*) to large elaborate and ornate tubes of more than 50 cm length (e.g. in *Lestrimelitta*, *Geniotrigona* or *Scaptotrigona*, see e.g. Wille and Michener 1973). Camargo and Pedro (2003), for example, describe an entrance tube of 1.2 m length in a *Trigona cilipes* colony nesting together with an ant nest. Nest entrances of *Lepidotrigona terminata* colonies living in limestone cavities in Thailand can be longer than 1.5 m (Bänziger et al. 2011). Due to their weight, long tubes can periodically fall off (Roubik 2006; pers. obs.). It is often possible to identify the species living in a cavity based on the features of the entrance (e.g. Roubik 2006; Ndungu et al. 2019).

The relative size of the entrance hole varies greatly between stingless bee species (Biesmeijer et al. 2007; Couvillon et al. 2008). Entrances are an important visual beacon for returning bees (Camargo and Pedro 2003) and the entrance is often the first structure that is built at a new nest site (Wille and Michener 1973). Having larger nest entrances will facilitate foraging traffic, but may require more entrance guards to guarantee an adequate defence (Biesmeijer et al. 2007; Couvillon et al. 2008). A curious entrance architecture is found in some *Partamona* species: *P. helleri* workers build a large outer mud entrance that leads to a small, adjacent entrance. This architecture allows foragers to enter with high speed and bounce off the ceiling of the outer entrance towards the small inner entrance (Fig. 3.1) (Couvillon et al. 2008; Shackleton et al. 2019). Due to the peculiar appearance of this entrance, some *Partamona* species are locally called "boca-de-sapo" or "toad mouth" (Fig. 3.1).

Entrance tubes vary from being hard and brittle to soft and flexible. Often, only the part near the opening is soft and flexible, which helps workers to close the entrance at night (Chap. 7) (Wille and Michener 1973; Roubik 1989; Grüter et al. 2011). The tube can be perforated and coated with small droplets of resin, which probably helps to stop ants or spiders from entering (Wille and Michener 1973; Roubik 2006). Dead ants can occasionally be seen partially covered with cerumen or resin on entrance tubes (e.g. in *Tetragonisca angustula*, Fig. 7.2). While most entrances are made from cerumen, many *Melipona* species use mud to build the entrance (Schwarz 1948). *M. fuliginosa* uses small seeds mixed with resin (Roubik 1989).

The entrance tube often continues inside the nest, leading to the vicinity of the food storage area (Michener 1974; Roubik 1983). This internal entrance tube tends to be thicker than the external entrance tube. Occasionally, nests have two or more external entrances, particularly in some *Partamona* species (Schwarz 1948). *Plebeia jatiformis* and *P. droryana* normally have two or even three entrance

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[7] Some *Frieseomelitta* species, e.g. *F. varia*, have a habit of covering the area surrounding the entrance with a thin layer of dark resin (see Fig. 3.1f). This area can extend to more than 1 m^2 and might provide a visual or olfactory signal for orientation or may play a role in colony defence, e.g. by keeping ants or spiders at bay.

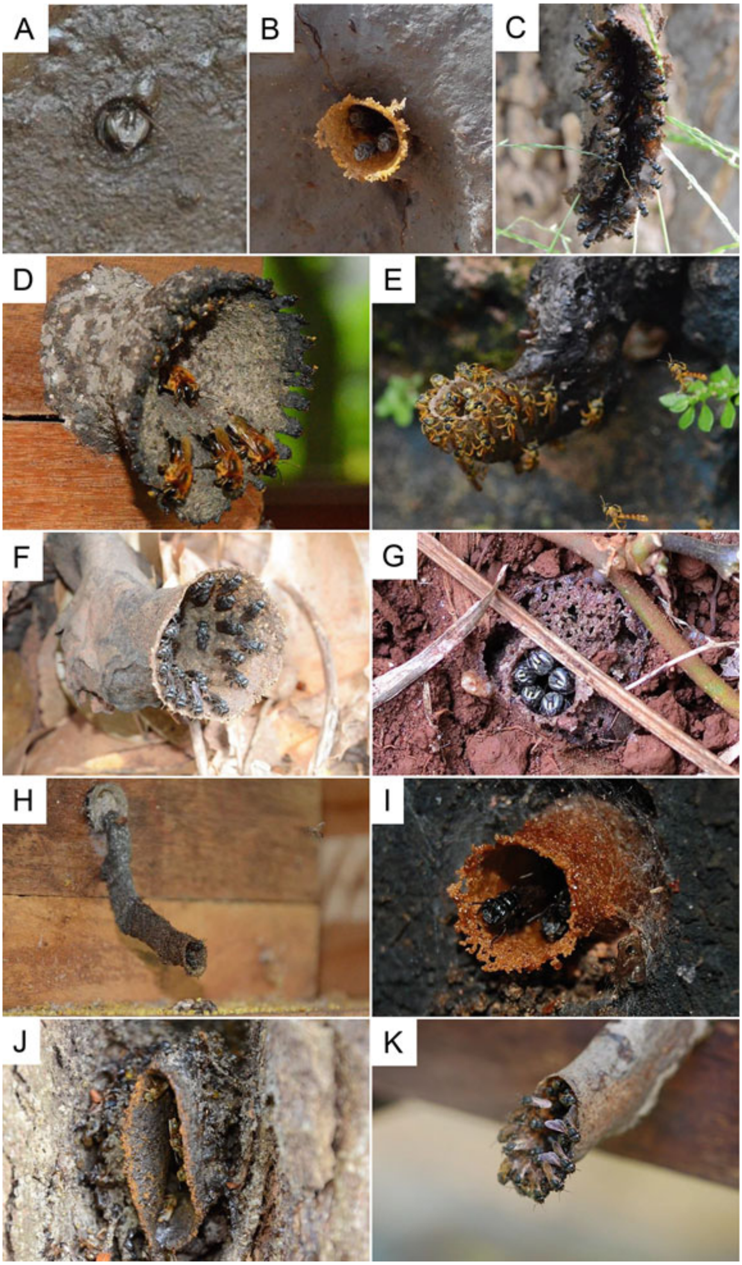

Fig. 3.8 Nest entrances of Neotropical species. (**a**) *Frieseomelitta languida*. (**b**) *Friesella schrottki*. (**c**) *Lestrimelitta limao*. (**d**) *Melipona seminigra*. (**e**) *Tetragonisca angustula*. (**f**) *Scaptotrigona bipunctata*. (**g**) *Paratrigona lineata* entrance in the ground. (**h**) *Leurotrigona muelleri* with waste pile just below the entrance. (**i**) *Scaura longula*. (**j**) *Tetragona clavipes*. (**k**) *Scaptotrigona depilis*. (All photos taken in Brazil by the author)

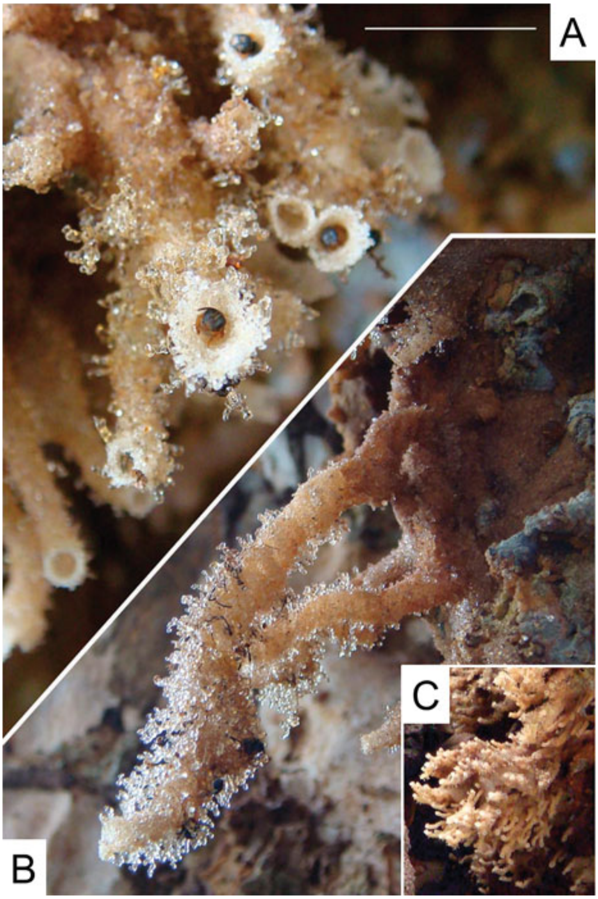

Fig. 3.9 Nest entrances of *Pariotrigona pendleburyi* consist of many tublets (**a** and **b**) that form coral-like entrance clumps (**c**) (from Bänziger et al. 2011). Many entrances have one guard bee inside

holes (Roubik 1983). In *P. droryana*, the two holes differ in size, with the smaller one being a couple of centimetres above the larger one. The function of the smaller opening is unclear as it is not usually used by foragers, but is occupied by guards. It is possible that it provides additional space for entrance guards or plays a role in air circulation (Paulo Nogueira-Neto, pers. commun.). Probably, the most elaborate entrances are built by *Pariotrigona pendleburyi* colonies nesting in limestone in Thailand. Here, dozens of small tubes are joined in coral-like clumps (Fig. 3.9) (Bänziger et al. 2011). Many tubes are occupied by a single guard.

3.2.2 Brood Chamber

The Involucrum

In many species, mainly the comb building species (see below), the brood is surrounded by the involucrum, a protective structure that consists of several soft layers of cerumen separating the brood chamber from the rest of the nest (Fig. 3.3) (Schwarz 1948; Wille 1983; Michener 2007).[8] In some species, like the Australian *Austroplebeia australis* and *A. cincta*, or in some American *Plebeia* species, there is intra-specific variation as to whether the involucrum is present, incomplete or absent (Michener 1961; Kerr et al. 1967; Drumond et al. 1995; van Benthem et al. 1995). Kerr et al. (1967) attribute the absence of the involucrum in some *Melipona* species in the Amazonas region of Brazil to the constant and high temperatures. The idea that the need for constant brood conditions favours the construction of an involucrum could also explain why the comb building *Scaura latitarsis, Trigona cilipes* and *Plebeia wittmanni* do not build an involucrum: the former two nests in active termite and ant nests (Kerr et al. 1967; Camargo 1970), whereas the latter lives in cracks of granite blocks (Wittmann 1989). These habitats are likely to create more constant temperatures, thus making an involucrum unnecessary. The number of cerumen layers of the involucrum also depends on the season. In the Asian *Lepidotrigona ventralis*, the involucrum consists of ~4 times more layers in winter than in summer (Chinh et al. 2005). *Austroplebeia australis* and *Plebeia remota* colonies only build an involucrum during the coldest months of the year (van Benthem et al. 1995; *A. australis* may have a very thin layer of involucrum during the summer, Halcroft et al. 2013). The strength of the involucrum can further depend on the amount of wax that is available in the nest (Kolmes and Sommeijer 1992). The involucrum is not closed, but contains passageways that allow workers to enter and leave the brood chamber (Schwarz 1948). Another function of the involucrum might be to keep parasitic flies or bees away from the brood (Roubik 2006).

Brood Cells and Their Arrangement

Brood cells are made of soft cerumen and each cell is built to rear one individual bee. The number of brood cells in a nest varies greatly and can range from a few, e.g. in the Asian *Lisotrigona carpenteri*, to more than 80,000 in some American *Trigona* nests (Michener 1946). Males and workers are reared in cells of identical size. Queen cells are usually larger than worker cells (Engels and Imperatriz-Fonseca 1990). Rarely, giant males are found in these queen cells (Nogueira-Neto 1997; Alves et al. 2009). In *Melipona* and *Trichotrigona,* queens are reared in worker-sized cells and in some species, colonies produce both dwarf-queens in worker-sized cells and large queens in larger cells (see Chap. 5) (Camargo 1974; Wenseleers et al. 2005; Ribeiro et al. 2006; Camargo and Pedro 2007). In *Tetragonisca angustula*, cells in the centre of the combs are slightly larger and are used to rear the larger soldiers (see Chaps. 6 and 7) (Segers et al. 2015).

There are three main types of cell arrangements: horizontal combs, vertical combs and clustered cells. However, several variations and intermediate forms exist (Fig. 3.2).

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[8] Colonies normally have just one brood chamber, but nests with several brood chambers have been observed (e.g. in *Plebeia frontalis*, Wille and Michener 1973, their Fig. 16).

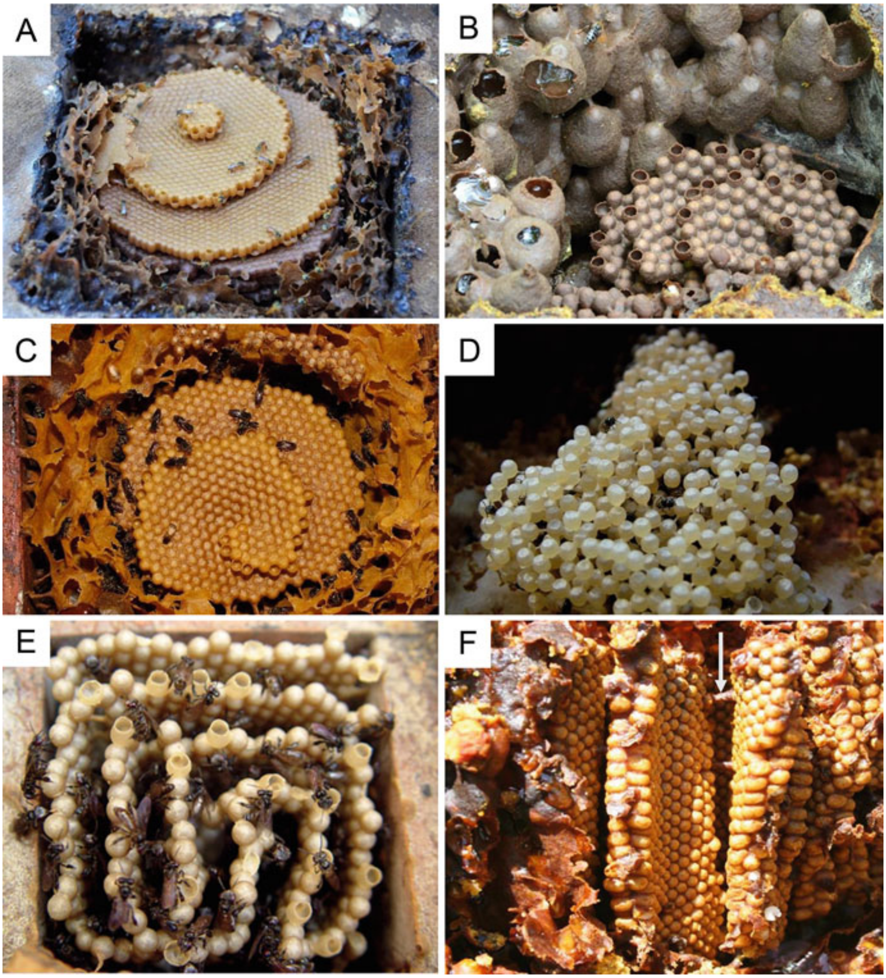

Fig. 3.10 (**a**) Horizontal brood combs of *Tetragonisca fiebrigi* (Photo: C. Grüter). (**b**) *Duckeola ghiliani* builds brood combs of a more irregular arrangement ("semi-combs") and without the protection of an involucrum. The much larger food pots are well separated from the brood area (Photo: C. Grüter). (**c**) *Nannotrigona testaceicornis* brood combs arranged as a spiral (Photo: C. Menezes). (**d**) Clustered brood cells of *Leurotrigona muelleri* (Photo: C. Menezes). (**e**) *Scaura longula* builds vertical brood combs consisting of one layer of cells (from Cortopassi-Laurino and Nogueira-Neto 2018). (**f**) Vertical brood combs in the African *Dactylurina staudingeri,* consisting of two layers of cells each (see also Fig. 3.11). The arrow indicates a cerumen pillar separating the combs (from Njoya 2009)

Horizontal Brood Combs

Most species build horizontal brood combs (Table 1.3, Figs. 3.3 and 3.10) (Wille 1983). Each comb consists of a single layer of cells that open upwards. Thin pillars of solid cerumen keep the horizontal combs in place (Fig. 3.11). The number of

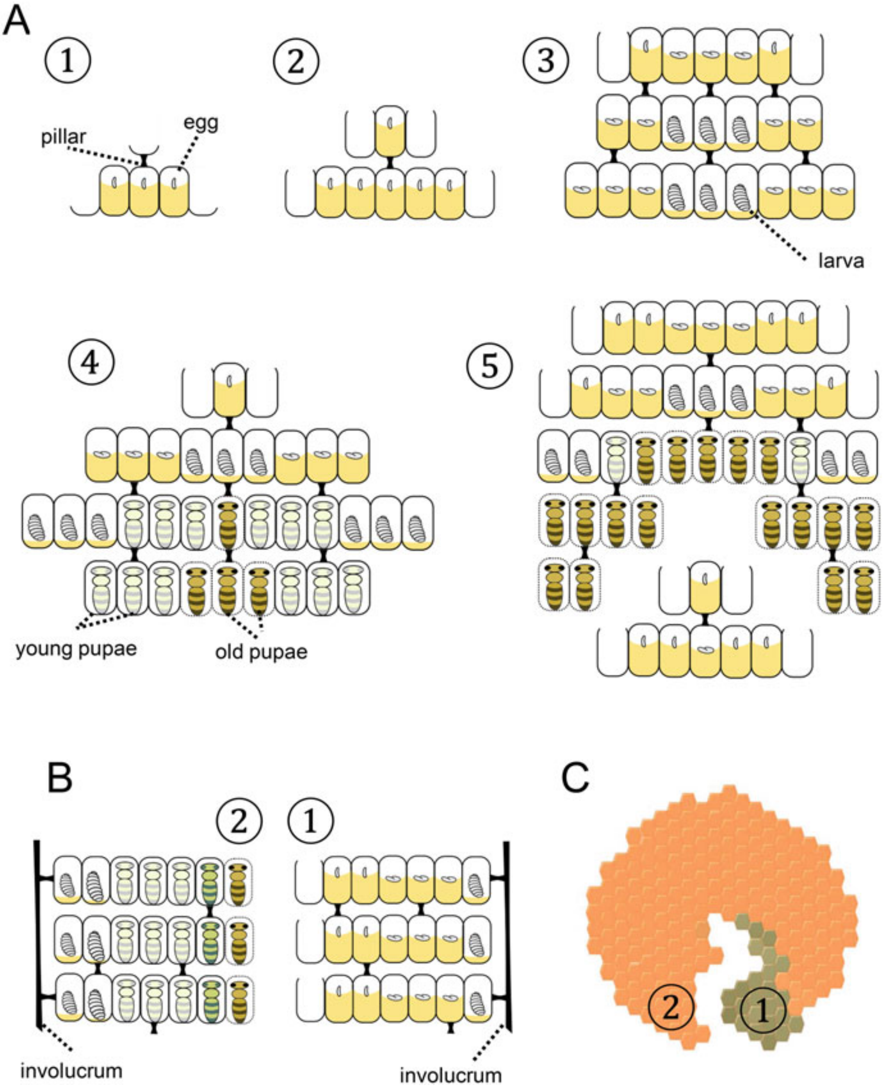

Fig. 3.11 The building of brood combs. (**a**) A new comb is started when workers build a single cell on top of an already existing comb. Subsequently, cells are built adjacent to the first cell (1–4). If there is no space left to build upwards, the new combs are built in the space vacated by the emerging older brood (5) (modified from Sakagami 1982). (**b**, **c**) In some African species, combs are built in a spiralling fashion. New cells are built along the radius of the comb (C1, green cells), as older brood emerge along the lagging edge (C2)

brood combs varies considerably between species, colonies and seasons and can range from less than 5 to 40 combs (Schwarz 1948; Wille and Michener 1973; Barbosa et al. 2013). Stronger colonies tend to produce larger combs, i.e. combs with a larger diameter (van Benthem et al. 1995; Alves et al. 2009). Occasionally, e.g. in the "firebee" *Oxytrigona tataira*, *Tetragona clavipes* or in the Australian "sugarbag" bee *Tetragonula carbonaria*, combs are connected to form a spiral (Table 1.3, Fig. 3.10) (Michener 1974; Souza et al. 2007; Duarte et al. 2016). The spiral pattern is not always a species-specific trait, but may be present in some colonies of a species but not in others (Wille 1983; Drumond et al. 1995), or the same colony may build spirals occasionally (e.g. in *Melipona*, *Plebeia*, *Plebeina*, *Nannotrigona*, *Trigona* and *Tetragona*) (Kerr 1948; Roubik 2006). There are even cases, e.g. in *Oxytrigona mellicolor*, where spiralled combs and discs can be found in the same colony at the same time (Schwarz 1948). This suggests that only minor modifications in building behaviour are required to switch between discs and spirals (Oldroyd and Pratt 2015; Cardoso et al. 2020), but it is not unknown why bees sometimes build discs and at other times spirals.

Cells are mass-provisioned, i.e. before the queen lays an egg, and several workers regurgitate brood food into the cell until it is about 2/3 full (see Fig. 5.1a, b). After oviposition, the cell is closed without delay and remains sealed until the bee is ready to emerge (Chap. 5). As soon as the developing larvae spin their cocoons inside the cell, workers start to remove most of the cerumen of the cells to reuse it in other locations (Kerr 1948; Michener 1974). Thus, unlike in honey bees, each brood cell is used only once in stingless bees. Occasionally, bees leave the cerumen floor of the cells intact after the brood has emerged, thereby creating a solid cerumen platform from which to build the new cells. This cerumen plate, called *trochoblast* (Ihering 1903; van Benthem et al. 1995), can be fortified with new material or consists of the leftovers of the old cell floors.

Workers start to build a new brood comb on top of an existing brood comb by making a thin wax pillar in the middle of the existing brood comb, on top of which a single cell is built (Fig. 3.11a). This first cell is cylindrical, but is constrained into the typical hexagonal shape as soon as the six surrounding cells are built. Unlike in honey bees, new cells are usually built sequentially (Sakagami 1982). Some species, however, simultaneously build multiple cells at different locations of the brood comb (Sakagami and Zucchi 1974). Each cell is constructed by several workers. The finishing touch consists of adding a soft cerumen collar, which is used to seal the cell after oviposition (Cepeda 2006). Workers often start to build a new comb before the previous comb is finished (Figs. 3.10 and 3.11), which takes about 3–10 days (*Paratrigona opaca*, Schwarz 1948; *Tetragonisca angustula*, Segers et al. 2015). The result of this building strategy is that brood in the centre is always older than brood in the periphery and, thus, emerges first (Fig. 3.11).

When there is no space left to build upwards, workers start to build a new comb on the bottom of the brood chamber using the space that is created when the bees from the older, lower brood combs emerge (Fig. 3.11). *Melipona bicolor* (and possibly *Paratrigona subnuda*) is an exception because new combs are always built on top. Nogueira-Neto (1997, p. 54) speculates that the brood combs slowly sink downwards due to their weight, thus constantly creating new space for the next comb to be built on top. The African *Plebeiella lendliana* and *Plebeina armata* (= *P. hildebrandti*) represent further exceptions to the patterns usually observed in comb-building species (Portugal-Araujo 1963). They build combs with an advancing edge of new cells along the radius of the comb, followed by a lagging edge where bees emerge, leading to combs to behave like a slowly moving spiral (Fig. 3.11b, c). Thus, instead of building new combs on top of each other, combs are continuously being reconstructed.

We know little about how workers know where to build next in order to achieve the species-specific nest designs. Other social insects do not seem to simply follow an innate blueprint, but use seemingly simple behavioural rules in combination with information provided by the existing structures themselves, a process called stigmergy (Camazine et al. 2001; Oldroyd and Pratt 2015; Khuong et al. 2016; Perna and Theraulaz 2017; Cardoso et al. 2020). Michener (1961), for example, observed the building behaviour in *Tetragonula carbonaria* and described that bees carrying bits of cerumen were wandering around, apparently in random fashion, until they happened to encounter an elevated point, to which they add the cerumen. The higher the point, the more likely it was that a bee would deposit a load of building material. This resulted in the building of pillars. He argues that other nest construction activities might be similarly guided and stimulated by existing structures (Michener 1974, pp. 62).

Clustered Cells

The second most commonly found type of brood cell arrangement is cell clusters, often held together with thin cerumen connections. A clustered arrangement of brood cells can be found in numerous distantly related genera (e.g. the American *Trigonisca*, *Frieseomelitta*, *Leurotrigona*, the Australian *Austroplebeia* or the African *Hypotrigona*) (Fig. 3.10). Cluster building species tend to be of smaller body size and mostly lack an involucrum (*Austroplebeia australis* and *A. cincta* are exceptions) (Michener 1961; Camargo 1970; Wille and Michener 1973; Roubik 2006). These species often nest in irregular, tubular and small cavities (Wille and Michener 1973; Michener 1974), which could make it difficult to build an involucrum around the brood cells. Furthermore, this style of brood cell arrangement might allow colonies to exploit irregular cavities that are unsuitable for comb building species.

Species differ in whether cells are nearly spherical (e.g. *Austroplebeia* or *Leurotrigona*) or vertically elongated (e.g. *Frieseomelitta*). In some species, cells touch each other (e.g. *Austroplebeia*), whereas in others, they are well separated (e.g. *Frieseomelitta*) (Wille and Michener 1973). Furthermore, cells can open in various directions in *Austroplebeia* and *Trigonisca*, but open only upwards in others, e.g. *Frieseomelitta*. New cells are added to the periphery of the cluster or in the hollow space in the centre that is created by emerging bees (Michener 1961). Building can advance in all directions, but tends to advance from the centre to the periphery.

Semi-Combs

In some species, e.g. the American *Duckeola ghiliani, Friesella schrottkyi, Plebeia julianii* or the Australian *Tetragonula hockingsi*, the arrangement of the brood cells is much more irregular (*D. ghiliani*, *F. schrottkyi* and *P. julianii* also do not have an involucrum) (Fig. 3.10) (Kerr et al. 1967; Drumond et al. 1998; Sakagami et al. 1973; Njoya 2009; Brito et al. 2012; Oldroyd and Pratt 2015; Pedro and Cordeiro 2015). The brood cells are still connected in groups, but otherwise arranged in such an irregular fashion that they appear to be intermediate between a comb and a cluster ("semi-combs").

In the Asian *Tetragonula iridipennis*, colonies from different populations differ in their cell arrangement, from cluster-like to irregular semi-comb-like as in *T. hockingsi* (bees from these populations also differ in their morphology, suggesting that they might represent closely related species) (Francoy et al. 2016). Variation in the cell arrangement, from more to less irregular, can be found in the same colonies in *Friesella schrottkyi* (Sakagami et al. 1973). These observations suggest—as was the case with spiralled brood combs—that the transition between semi-combs and combs or clusters might require only minor modifications in behaviour. It has been suggested that these behavioural differences could be linked to the nature of interactions between the queen and workers during the provisioning and oviposition process (POP, see Chap. 5), but this requires further study (Oldroyd and Pratt 2015).

Vertical Combs

The African genus *Dactylurina* and the American *Scaura longula* are the only stingless bees that construct vertical combs. Combs consist of two layers of horizontally oriented cells in *Dactylurina* and of one layer of cells in *Scaura* (Figs. 3.10 and

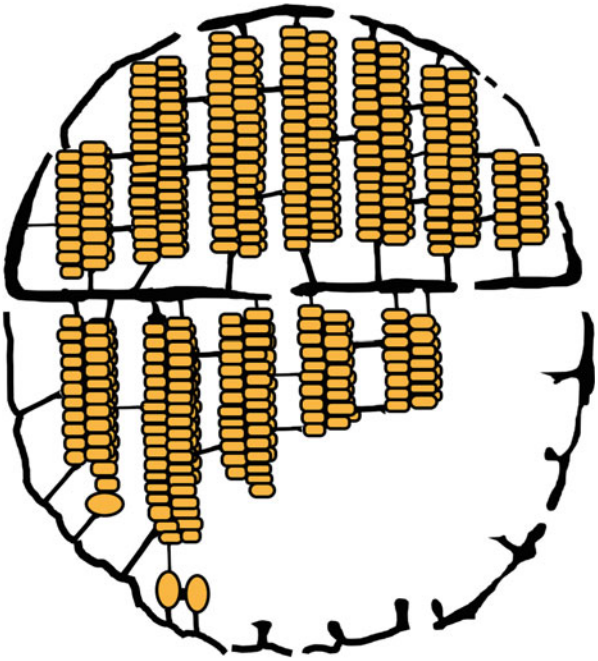

Fig. 3.12 Vertical combs of *Dactylurina staudingeri* (modified from Darchen and Pain 1966). Cells are in two rows and combs are connected to each other and to the surrounding involucrum via cerumen pillars. In the lower part, three larger queen cells are shown

3.12) (Darchen and Pain 1966; Nogueira-Neto 1997; Eardley 2004; Njoya et al. 2016). In *Dactylurina*, cells are built from top to bottom (Wille and Michener 1973) and the brood combs are surrounded by an involucrum. The brood layers are held together by cerumen pillars (Fig. 3.10) (Njoya et al. 2016). Interestingly, the brood area in *Dactylurina* is divided into an upper and a lower half, separated by a cerumen sheet (Fig. 3.12) (Darchen and Pain 1966; Michener 1974; Njoya et al. 2016). Each half contains about 6–10 double combs. The function of the separation between the upper and lower portion is not known, but could improve the structural stability of the vertical arrangement of brood combs.

3.2.3 Food Storage

Unlike in honey bees and bumble bees, food storage pots are clearly differentiated from brood cells in location and shape (Figs. 3.3 and 3.10). The storage pots are made from soft cerumen and, depending on the time of year, colonies can contain from a few to hundreds of pots (Schwarz 1948). The amount of stored honey varies greatly among and within species and can range from a few grams to several kilograms (usually <5 kg) (Chap. 1) (see Roubik 1983, Table 3, for more detailed information). Colonies of *Trichotrigona* do not appear to store any food in their nest, but may instead steal food from other colonies (Camargo and Pedro 2007; Pedro and Cordeiro 2015).

In species that build an involucrum, food pots are typically outside the involucrum (Figs. 3.3 and 3.4), but there are some exceptions (Camargo 1970). Pots can be directly attached to the lining batumen or connected via pillars. Storage pots are larger than brood cells and, in some of the larger species, e.g. *Melipona* or *Cephalotrigona*, can reach the size of a chicken egg. In some species, honey and pollen pots are separated; in others, they are mixed. Normally, honey and pollen pots are of the same shape but in the American *Frieseomelitta*, the two types of pots differ in shape and size. The honey pots are rounder and small, whereas pollen pots are larger and elongated (Fig. 3.13) (Kerr et al. 1967). The size of the pots can also vary considerably in the same colony (Michener 1974). In the cleptoparasitic

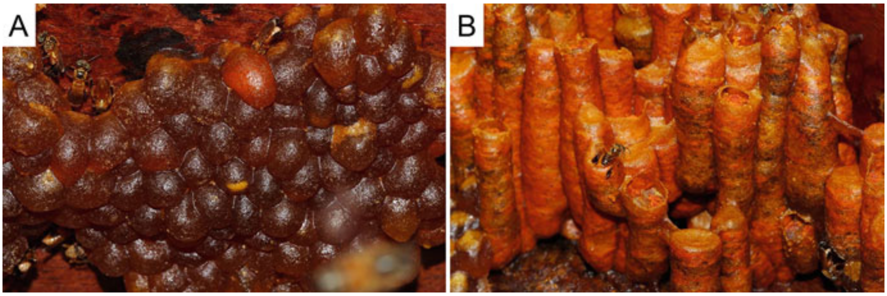

Fig. 3.13 *Frieseomelitta varia* honey (**a**) and pollen (**b**) pots. Unlike in many other species, the pots for the two resources differ in both size and shape (photos: Cristiano Menezes)

Lestrimelitta and *Cleptotrigona*, pollen, honey and brood food that have been raided from other colonies are stored as a mixture in the same pots (Michener 1974; Roubik 2006).

3.2.4 Imprisonment Chambers

Several species, including *Plebeia* spp., *Frieseomelitta varia, F. silvestrii, Friesella schrottki, Schwarziana quadripunctata* and *Tetragonisca angustula*, build queen "imprisonment chambers" (see also Chap. 4) (Fig. 3.14) (Juliani 1962; Sakagami 1982; Roubik 2006; Engels and Imperatriz-Fonseca 1990). In some cases, they are reused empty food pots (Juliani 1962). Colonies can contain more than one imprisonment chamber, each containing an unmated queen. The cells protect unmated queens from being killed by workers when there is still an ovipositing queen in the nest. These royal chambers are often not completely sealed, but have openings that allow workers to enter or queens to receive food from workers via trophallaxis (Drumond et al. 1995). In *Plebeia subnuda* and *P. droryana*, queens occasionally leave the chamber to go on excursions (Imperatriz-Fonseca 1977; Imperatriz-Fonseca and Zucchi 1995). This suggests that unmated queens remain in their chamber voluntarily to avoid aggression by workers or the egg-laying queen. The idea of a self-imposed confinement is further supported by the observation that, occasionally, the unmated queens themselves build the royal chamber (Sakagami 1982; Imperatriz-Fonseca and Zucchi 1995). Queens can stay inside a chamber for months (Drumond et al. 1995). In some cases, e.g. *Frieseomelitta varia*, nurse-aged workers have been observed to guard the chamber (Sakagami 1982). In *Leurotrigona muelleri* and *Celetrigona longicornis*, a shield of guarding workers entirely replaces the chamber walls (Terada 1974, cited in Imperatriz-Fonseca 1977; Sakagami 1982).

Fig. 3.14 Queen "imprisonment" chamber in *Plebeia droryana* (Photo: Cristiano Menezes)

3.3 Control of Climate Conditions

Maintaining relatively constant climatic conditions inside the nest is important for colony functioning in many social insects (Jones and Oldroyd 2006). Brood rearing, for example, requires the temperature to stay within certain limits: in the honey bee *Apis mellifera,* the brood requires constant temperatures ranging from 33 to 36 °C for optimal development. Both passive (e.g. nest site selection) and active measures (shivering of fight muscles, ventilation and evaporative cooling) allow honey bees to achieve very constant conditions (Heinrich 1993; Bujok et al. 2002; Tautz et al. 2003; Jones and Oldroyd 2006). Stingless bee brood also benefits from constant conditions. In *Scaptotrigona depilis*, for example, brood mortality increases dramatically if brood is reared outside a 26–34 °C temperature range (Vollet-Neto et al. 2015). In the African *Plebeina armata,* brood dies if the temperature surpasses 35.5 °C (Moritz and Crewe 1988), whereas a temperature of ≥34 °C leads to a substantial increase in brood mortality in the American *Melipona interrupta* (Becker et al. 2018). Males of *Melipona marginata* produce polyploid sperm if reared below 21 °C (Kerr 1972). In *Austroplebeia australis*, on the other hand, a species whose distribution includes warm temperate regions in Australia, colonies continue to produce brood during the cold season and brood is able to survive sub-zero temperatures (Halcroft et al. 2013). It remains unknown, however, whether sub-optimal rearing temperatures have more subtle, sub-lethal consequences, e.g. adverse effects on the behavioural performance of worker bees as has been shown in honey bees (Tautz et al. 2003; Jones et al. 2005).

Stingless bees are often thought to lack efficient climate control and it has been hypothesised that this explains why this tribe is largely restricted to tropical and sub-tropical areas (Wille 1983; Engels et al. 1995). However, even in the tropics and sub-tropics, temperatures vary greatly and, depending on location, altitude, time of year or time of day, may range from less than 0 °C to more than 40 °C (Darchen 1973; Halcroft et al. 2013; Sung et al. 2011). Thus, thermoregulation is likely to be important for stingless bee colonies. The species that have been tested so far seem to differ greatly in their ability to maintain climatic homeostasis. For example, while thermoregulation is almost non-existent in the African *Apotrigona nebulata* (Darchen 1969), the American *Trigona spinipes* matches *Apis mellifera* in their ability to keep the brood nest at a constant temperature (Zucchi and Sakagami 1972). Zucchi and Sakagami (1972) measured the temperature in various parts of a *T. spinipes* nest during a 24-h period and while the external temperature fluctuated between 15.5 and 28 °C, the temperature in the brood nest was considerably higher and varied between 34.1 and 36.0 °C. Other species with good thermoregulatory abilities are the American *Melipona fuliginosa, M. rufiventris, M. seminigra*, *Partamona cupira*, *Scaptotrigona depilis* and *S. postica* or the Asian *Lepidotrigona ventralis*, whereas in *Austroplebeia essingtoni*, *Frieseomelitta varia*, *Leurotrigona muelleri* and *Tetragonula laeviceps*, nest temperature is more or less similar to ambient temperature (Zucchi and Sakagami 1972; Wille and Orozco 1975; Wille 1976; Roubik and Peralta 1983; Engels et al. 1995; Sung et al. 2008; Sakagami et al. 1983; Ayton et al. 2016). The latter group of species has clustered brood cells and no involucrum, which might explain why internal conditions fluctuate more. On the other hand, *Austroplebeia essingtoni* does show a capacity to maintain the relative humidity of the hive at ~65%, which means that during the hot and wet summer months, humidity inside the nest stays below ambient conditions, which could reduce fungal growth (Ayton et al. 2016). The authors of this study hypothesise that the waste piles might absorb moisture. Removing the waste from the colony would then remove excess water.

3.3.1 Active Climate Control

In many species, cooling is achieved by fanning, which creates air flow (Nogueira-Neto 1948; Wille 1983). Workers are thought to position themselves in different parts of the nest in a way that facilitates air leaving the nest through the nest entrance, often in rows of several workers (Sakagami and Oniki 1963; Michener 1974; Roubik 2006). Bees often direct their head towards the entrance rather than their abdomen (Sakagami and Oniki 1963; Sakagami and Zucchi 1967). When studying two African species, *Hypotrigona gribodoi* and *Plebeina armata*, Moritz and Crewe (1988) found that fanning caused an exchange of the entire nest air within 1–7 h. The number of fanning bees positively is correlated with ambient temperature in *Scaptotrigona depilis* (Vollet-Neto et al. 2015) and in the Amazonian *Melipona crinita*, workers do not start ventilating at the nest entrance before the internal temperature surpasses 37 °C (Cortopassi-Laurino and Nogueira-Neto 2003).

Darchen (1972), studying African meliponini in the Ivorian savanna, observed that during the hottest hours of the day, workers make numerous small holes into the outer layers of the nest, possibly to improve air circulation. The same strategy might be used by workers of the exposed-nesting *Trigona spinipes* (Roubik 2006). Another way to avoid overheating is the withdrawal of workers from the brood area, which was observed in *Scaptotrigona postica* in response to high temperatures (Engels et al. 1995). Nogueira-Neto (1948) observed that fanning also occurs during colder winter days and he suggested that this is more likely to help in humidity regulation than in cooling. He argued that ventilation is important to reduce the growth of mould. Furthermore, the exchange of air might also be important to replenish oxygen (Nogueira-Neto 1948; Moritz and Crewe 1988).

Honey bees are known to use evaporative cooling as a method of temperature regulation (Lindauer 1954) when ambient temperatures are high. To do so, bees collect water and deposit small amounts on surfaces throughout the nest or they perform a behaviour called “tongue-lashing” to evaporate regurgitated water even faster (Winston 1987). Conclusive evidence that stingless bees collect water for evaporative cooling is still missing, but Macías-Macías et al. (2011) found that the workers of *Melipona colimana* colonies that inhabit Mexican highland areas regurgitated water and fanned their wings in response to high temperatures. Thus, this species might use water evaporation to cool the colony when facing heat. Further evidence for the use of water for cooling comes from the observation that *Scaptotrigona depilis* workers increase water collection if the temperature in the brood area reaches critical levels (Vollet-Neto et al. 2015). The authors found that during the hottest times of the day, the temperature in the brood nest was ~1 °C lower than in an empty control box, whereas during cooler periods, brood nest temperature was higher than in the control box. These findings raise the possibility that some species might use evaporative cooling, but observations of workers regurgitating water in response to heat inside the nest or the deposition of water droplets in the brood nest area would further corroborate the existence of evaporative cooling. On the other hand, several species do not seem to use water to cool the nest (Roubik and Peralta 1983; Engels et al. 1995; Roubik 2006).

As mentioned earlier, stingless bees inhabiting certain regions frequently experience low ambient temperatures and behavioural strategies that increase the nest temperature would improve conditions for the brood (Engels et al. 1995; Halcroft et al. 2013). Honey bees generate heat by shivering their wing muscles (Winston 1987; Heinrich 1993) and there is evidence that some stingless bee species might use the same strategy (Roldão-Sbordoni et al. 2019). When exposed to low temperatures, workers of the Mexican highland species *Melipona colimana* consumed syrup and increased their thorax temperature (Macías-Macías et al. 2011), whereas the closely related lowland species *Melipona beecheii*, which experiences less extreme temperature ranges, did not show this behaviour (Macías-Macías et al. 2011). An interesting behaviour that might play a role in thermoregulation was observed in *Scaptotrigona postica*. Workers of colonies exposed to cool temperatures started to cover the brood cells, possibly to warm the cells using their flight muscles (Engels et al. 1995), as was observed in honey bees (Bujok et al. 2002).

3.3.2 Passive Climate Control

Selecting an appropriate nest cavity in combination with architectural features appears to be the most common mechanisms for keeping nest temperatures optimal in many stingless bee species (Jones and Oldroyd 2006). The insulation provided by wood, batumen, cerumen, earth or stone guarantees that colonies do not easily overheat. Furthermore, physical properties of the nest and the cavity (e.g. location, size and shape) will determine to what extent ambient air currents enter and circulate in the nest (Roubik 2006). The retention or loss of warmth in the brood area might also depend on the brood cell arrangement as packed layers of cells in comb building species are likely to retain heat better than the loose, isolated cells in some cluster building cells (Oldroyd and Pratt 2015).

3.4 Evolution of Nest Architecture

Variation in nest architecture often seems unrelated to stingless bee phylogeny, which makes it challenging to determine whether particular nesting traits are ancestral or derived. Several authors have suggested that clustered cells, which show similarities with the brood cell arrangement in bumble bees and some orchid bees, represent the ancestral state (Michener 1964; Camargo 1970; Wille and Michener 1973). This would imply that horizontal combs evolved from clustered cells. However, the phylogenetic relationships among stingless bee species suggest that reversions from combs to clusters occurred repeatedly. Wille and Michener (1973) argued that the rather spherical cells that open in various directions are found in the cluster building Australian *Austroplebeia* or some Neotropical *Trigonisca* represent the ancestral condition, whereas the oval and upward-oriented cells in clusters of *Frieseomelitta* spp. are derived. Indeed, the phylogenetic position of *Frieseomelitta* and *Trichotrigona* (Fig. 2.2) in relation to their closest Neotropical relatives (mostly comb-building genera) suggests that, in their case, cell clusters are derived from brood combs.

Michener (1961) argued that the simple involucrum found in *Austroplebeia*, often consisting of one sheet, represents the ancestral condition. Multi-layered, laminated involucra would have appeared later to improve climate control in the brood area. Ancestral nests were also likely to have featured a single layer of batumen lining the nest cavity (Wille and Michener 1973). The building of exposed nests and the mixing of cerumen and batumen with plant or faecal material, as is frequently found in the large Neotropical genus *Trigona*, are most likely derived characters (Michener 1964; Sakagami 1982; Rasmussen and Camargo 2008). Exposed nesting allowed stingless bee colonies to become independent of finding suitable cavities (see also Chap. 1) (Rasmussen and Camargo 2008). However, formal analyses that build on our improved understanding of stingless bee phylogeny are needed to test hypotheses about the evolutionary origins of and the transitions between different nesting traits (see Rasmussen and Camargo 2008 for such an analysis in *Trigona*).

3.5 Stingless Bee Communities and Associations with Other Organisms

3.5.1 Colonial Communities and Colony Densities

The natural density of stingless bee colonies is highly variable, ranging from a few to as many as 1600 colonies per square kilometre (Fowler 1979; Oliveira et al. 1995; Breed et al. 1999; Eltz et al. 2002; Batista et al. 2003; Samejima et al. 2004; Kajobe and Roubik 2006; Slaa 2006; Siqueira et al. 2012; Tornyie and Kwapong 2015; Bobadoye et al. 2017). The factors that affect nesting density and species diversity are relatively poorly understood, but three factors that are likely to play a key role are (1) food source availability (Hubbell and Johnson 1977; Eltz et al. 2002; Arena et al. 2018), (2) nest site availability (Michener 1974; Eltz et al. 2002; Inoue et al. 1993), and (3) human alterations of the environment (Kajobe and Roubik 2006; Slaa 2006; Pioker-Hara et al. 2014; Cairns et al. 2005; Brosi 2009; Freitas et al. 2009). These three factors are, to some degree, inter-related and connected to other key aspects of habitat quality, such as plant species diversity and forest cover. Different studies contrast with respect to the importance that they attribute to the different factors in shaping stingless bee communities. This highlights that the importance of nest site abundance, food source availability and human impact is likely to vary among habitats and stingless bee community.

Food Source Availability

Hubbell and Johnson (1977) and Eltz et al. (2002) found that food source availability was the best predictor of stingless bee nest density in a Central American and a Southeast Asian habitat, whereas nest predation or the number of available nesting sites seemed less important for the species they studied. Likewise, food availability was positively associated with species richness and abundance in an Andean forest in Colombia (Gutiérrez-Chacón et al. 2018). Plant diversity and, thus, continuous food availability were also the best predictor of colony growth and colony number in *Tetragonula carbonaria* in Australia (Kaluza et al. 2018). Other studies, however, found no strong connection between food source availability per se and nest density (Kajobe and Roubik 2006; Brosi 2009).

A clumped nest distribution is expected when food sources are clustered (Michener 1946), whereas a uniform food source distribution could lead to a more uniform nest dispersion (Hubbell and Johnson 1977; Eltz et al. 2002). Furthermore, a more uniform distribution with similar distances between nests might also be expected if competition for food is intense among colonies (see Chap. 8). Accordingly, Hubbell and Johnson (1977) found that mass-recruiting and highly competitive species had a more uniform distribution, whereas non-recruiting species were more randomly distributed.[9] Some species (e.g. *Trigona fulviventris*) seem to directly affect the distribution of nests by aggressively preventing the establishment of conspecific rival nests in the vicinity of their own nest (Hubbell and Johnson 1977).

Nest Site Availability

There are circumstances when nest density and distribution depend on the availability of suitable nesting sites (Michener 1974, pp. 333; Eltz et al. 2002). For example, an experimental increase in nesting sites increased colony density by 250% in the Asian *Tetragonula minangkabau* (Inoue et al. 1993). Ants, in particular, appear to be important competitors for nesting sites (Nogueira-Neto 1954; Inoue et al. 1993). In Costa Rica, swarming frequency of stingless bees was positively correlated with the availability of empty nesting sites and a reduction of nesting sites by deforestation leads to a decrease in nest density (Slaa 2006). Similarly, Lichtenberg et al. (2017) found that species that rely less on nesting cavities in trees persist better in highly deforested areas.

There are numerous reports of extremely high nest densities in locations with clustered nesting sites (Starr and Sakagami 1987; Roubik 1989; Eltz et al. 2003;

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[9] The impact of food source competition is highlighted by the finding that, in *Tetragonisca angustula*, moving colonies from areas with lower nest densities to areas with higher densities of stingless bee colonies caused the moved colonies to produce smaller workers and have a reduced biomass (Segers et al. 2016).

Leonhardt et al. 2010). Aggregations of more than 20–30 colonies (often belonging to more than one species) in a single tree have been observed (Schwarz 1948; Roubik 1983, 1989; Camargo and Pedro 2002). Starr and Sakagami (1987) found 84 colonies of two *Tetragonula* species in a bamboo farmhouse in the Philippines. Several to dozens of nests of the Asian *Tetragonilla collina* were found nesting among the roots of a *Ficus* tree (Roubik 1989) and the American *Partamona cupira* can form aggregations of more than 100 nests on cliff sides (Roubik 1983). In *Scaptotrigona luteipennis*, *S. postica* and the African *Axestotrigona ferruginea*, several colonies can occasionally be found sharing one continuous cavity, divided by batumen plates (Schwarz 1948; Roubik 1983). It is likely that such clusters are, at least partly, the result of a clustered occurrence of potential nesting sites rather than due to high food source availability.

Human Impact

There is increasing evidence that anthropogenic activities negatively affect the abundance and diversity of stingless bees (see also Chap. 1). The consequences of human disturbances vary greatly among species. Some species do relatively well in disturbed, deforested or urban environments, e.g. those taking advantage of small cavities in human constructions or competitively dominant species (Michener 1946; Batista et al. 2003; Jaffé et al. 2016; Lichtenberg et al. 2017; Kiatoko et al. 2018; Cely-Santos and Philpott 2019), whereas others require natural forests with little human interference (Brown and Albrecht 2001; Batista et al. 2003; Pioker-Hara et al. 2014; Brosi 2009; Brown and de Oliveira 2014). Interestingly, Lichtenberg et al. (2017) found that species with a broader diet were more affected by deforestation, possibly because deforested areas contain fewer resources and their predominantly solitary foraging strategy means that they are competitively inferior to socially foraging species (see also Chap. 8).

Despite the observation that certain species can do well in disturbed areas (e.g. *Tetragonisca angustula* and *Trigona spinipes* in the Neotropics or *Axestotrigona ferruginea* (reddish brown morph) and *Hypotrigona gribodoi* in Kenya) (e.g. Giannini et al. 2015; Jaffé et al. 2016; Kiatoko et al. 2018), overall nest density and species diversity are negatively impacted by deforestation and the conversion of natural habitats into agricultural and urban landscapes (Fig. 1.15) (Klein et al. 2002; Cairns et al. 2005; Slaa 2006; Brosi et al. 2007, 2008; Brosi 2009; Freitas et al. 2009; Venturieri 2009; Ramírez et al. 2013; Pioker-Hara et al. 2014; Kennedy et al. 2013; Brown and de Oliveira 2014; Kiatoko et al. 2018; Arena et al. 2018; Smith and Mayfield 2018). For example, when Cairns et al. (2005) compared stingless bee abundance and diversity in different locations in Mexico, they found that stingless bee diversity was negatively associated with the degree of anthropogenic disturbance. A similar pattern was found in two different Brazilian biomes, the "Cerrado" (savanna) (Pioker-Hara et al. 2014) and the Amazon basin (Brown and Albrecht 2001), where the abundance and diversity of *Melipona* species were substantially reduced in more disturbed areas. Biodiverse forests, in particular, are important for stingless bee communities because they provide a more diverse range of nesting options and a more constant supply of food sources (Brosi et al. 2007;

Kaluza et al. 2017, 2018; Smith and Mayfield 2018).[10] This can explain why lower levels of forest cover and a reduced plant species richness were negatively associated with stingless bee abundance and species richness in Costa Rica and Colombia (Brosi et al. 2008; Brosi 2009; Gutiérrez-Chacón et al. 2018). Similarly, a lower plant biodiversity and small forest fragment size led to reduced colony growth and nest densities in *Tetragonula carbonaria* in Australia (Kaluza et al. 2018; Smith and Mayfield 2018). Stingless bee diversity and abundance were also lower at greater distances from forested areas in Costa Rica (honey bees showed the opposite pattern) (Brosi et al. 2007). Both good and bad news can be gained from these studies. While Brown and Albrecht (2001) suggest that already a moderate level of deforestation has a negative impact on meliponine diversity, the data of Brosi (2009), Kaluza et al. (2018) and Arena et al. (2018) suggest that providing even small fragments of forest and natural vegetation have positive effects on stingless bees.

3.5.2 Close Associations Between Colonies

Mixed colonies, i.e. colonies consisting of two stingless bee species, have been found in nature and can be created artificially. In these cases, brood and emerging workers from one species are accepted by the other (Michener 1974 described a nest consisting of *Scaptotrigona postica* and *Melipona marginata* worker). However, mixed colonies are probably very rare in nature (Roubik 1981). In the case of a nest shared by *Melipona fuliginosa* and *M. fasciata*, the first had built the nest, whereas the latter added its own structures, such as storage pots. The brood combs of the two species were in separate locations inside the nest (Roubik 1981). The *M. fasciata* workers were less numerous and were seen transferring food to *M. fuliginosa* workers at the entrance, possibly to be allowed to enter. Menezes et al. (2009b) reported a case of a spontaneously formed mixed-colony consisting of a *Scaptotrigona depilis* colony that was invaded by *Nannotrigona testaceicornis*. In this case, no *N. testaceicornis* queen was found during the study period and it is possible that the association was the result of *N. testaceicornis* workers entering the *S. depilis* colony by mistake (Menezes et al. 2009b).

A fascinating, yet poorly understood association seems to exist between *Trichotrigona* and *Frieseomelitta* colonies in the Amazon region. *Trichotrigona extranea* and *T. camargoiana* colonies do not appear to store any food, but always seem to nest in close proximity to *Frieseomelitta* colonies (Camargo and Pedro 2007; Pedro and Cordeiro 2015), a closely related genus (Rasmussen and Cameron 2010). Colonies may share the same cavity, separated only by batumen plates (Camargo and Pedro 2007). Due to this arrangement, it has been suspected that

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[10] An alternative explanation could be that species in deforested areas suffer more from diseases, parasites and predators (Brosi et al. 2007).

Trichotrigona consumes the food stored in *Frieseomelitta* colonies and is, thus, an obligate cleptoparasite (Camargo and Pedro 2007; Pedro and Cordeiro 2015).

3.5.3 Inquilines

Given our limited knowledge about most stingless bee species, it is not surprising that even less is known about the organisms that live inside stingless bee nests. However, inquilines seem to be common. Salt (1929), in a preliminary account, identified arthropods from 10 orders, 19 families, 30 genera and 37 species in stingless bee nests, with beetles being particularly common. Others have reported that in the Neotropical genera *Geotrigona, Trigona* and *Melipona*, several arthropod taxa live in the area of the waste deposits, including collembolans, earwigs, woodlice, myriapods, pseudoscorpions and mites (Roubik 2006; Barbosa et al. 2013). They are mainly feeding on the decomposing material and fungi (Roubik 2006; Barbosa et al. 2013). In *Melipona*, beetles feed on pollen, faecal waste and fungi growing in the more humid parts of the nest (Roubik and Wheeler 1982; Roubik 2006). Their role may be mutualistic as they reduce waste material and fungal growth.

Portugal-Araujo (1963) mentioned that natural subterranean nests of two African species contained larvae of a small beetle, possibly the small hive beetle (SHB) *Aethina tumida*,[11] a species that has become an emerging pest in honey bees in the last years (Neumann and Elzen 2004). The small hive beetle has also been found in *Dactylurina staudingeri* nests, an exposed-nesting species (Mutsaers 2006, cited in Halcroft et al. 2011). This beetle causes little or no damage in healthy stingless bee colonies, but can destroy weakened colonies by feeding on the food stores (Portugal-Araujo 1963). Greco et al. (2010) report that these invasive beetles can now also be found in nests of the Australian *Tetragonula carbonaria*. Another beetle that is associated with several Asian stingless bees is *Haptoncus luteolus*. This beetle feeds on pollen and, like the small hive beetle, has the potential to damage and destroy heavily infested colonies (Krishnan et al. 2015). *Tetragonula minangkabau* colonies are occasionally devastated by larvae of the beetle *Procoryphaeus wallacei* (Inoue et al. 1993). *Cleidostethus meliponae* seems to be entirely adapted to live parasitically in the nests of African stingless bees (Schwarz 1948). Beetle larvae from the genus *Amphicrossus* mainly live in *Apotrigona nebulata* nests in Africa where they eat pollen and honey (Darchen 1969). It is again in weakened colonies where these parasites can have devastating effects. Beetles of some species can disperse by hitch-hiking on the hind legs of worker bees (Roubik and Wheeler 1982). Beetles from the family *Leiodidae* might even switch host species at mud collection sites (Roubik and Wheeler 1982), whereas others disperse during inter-colony food transfer (mainly robbing) (Roubik 1989).

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[11] Portugal-Araujo states that the beetle belonged to the genus *Aecthina*, but given the description of the damage caused by the larvae, the similarity with the genus name of the small hive beetle (*Aethina*) and that I was unable to find a beetle genus called *Aecthina* I suspect that he was describing the small hive beetle.

Scaura (formerly *Schwarzula*) *coccidophila* colonies house and tend soft-scale insects from the genus *Cryptostigma*, occasionally up to 200 individuals (Camargo and Pedro 2002). Farming these guests is likely to be of great benefit as the bees obtain not only honey dew from them but also wax. Due to these wax contributing insects, the *S. coccidophila* colonies examined by Camargo and Pedro (2002) were able to store large amounts of wax in deposits, an otherwise unusual observation in stingless bee nests. Another rare feature in these bees is that they use pure wax without the addition of resin as building material for their nest (Camargo and Pedro 2002). The scale insects, on the other hand, might benefit from this partnership due to enhanced protection against enemies.

Michiliid flies consume faecal waste and occur in large numbers in nests of *Melipona capixaba* (Melo 1996). Phorid flies (several genera of the Phoridae) often oviposit on the waste piles, in open pollen pots and on damaged brood, which can become a problem if workers do not manage to remove the waste or repair damage, as may be the case in weakened colonies (see Sect. 7.1) (Sakagami and Zucchi 1967; Nogueira-Neto 1997) and phorid fly larvae are known to kill stingless bee colonies within days (Nogueira-Neto 1997). Due to the potential damage, stingless bee workers have been observed to remove the larvae of different fly or beetle species (Schwarz 1948; Halcroft et al. 2011). An alternative to the removal of bothersome inquilines is covering them with wax and resin ("mummification"), as was observed in the case of fly larvae inhabiting *Tetragonula carbonaria* nests and beetles found in *T. carbonaria* or *Tetragonisca angustula* nests (Schwarz 1948; Greco et al. 2010).

Mites can represent a major health problem for honey bees (best known is *Varroa destructor*) and numerous mite species have been found in stingless bee nests (particularly from the families Acaridae, Blattisociidae, Laelapidae and Pyemotidae) (Schwarz 1948; Delfinado-Baker et al. 1983; Fain and Heard 1987; Delfinado-Baker and Baker 1988; Baker et al. 1984; Menezes et al. 2009a; Vijayakumar and Jayaraj 2013; Radhakrishnan and Ramaraju 2017; Da-Costa et al. 2019). The risks they pose are not yet well understood and are likely to vary from case to case. Some mite species are considered beneficial, e.g. because they eat nematodes or detrimental fungi and contain their growth (Roubik 1989). There is evidence, for instance, that mites of the species *Neotydeolus therapeutikos*, which were found inside brood cells of *Scaptotrigona* nests, considerably reduce brood mortality (Roubik 1989). In other cases, mites are found near food pots or inside brood cells, where they are attached to larvae or pupae (similar to *Varroa destructor* mites parasitising honey bees) (Salt 1929; Vijayakumar and Jayaraj 2013; Roubik 1989; Menezes et al. 2009a). Mites have also been found on the bodies of adult workers (e.g. Schwarz 1948; Radhakrishnan and Ramaraju 2017). Schwarz (1948) described a *Partamona testacea* colony where ~25% of all workers were mite infested. Since many of them were found on or adjacent to the hind tibia (a phenomenon also observed in *Trigona corvina*), he suspected that they might eat pollen grains from the body of the bee. *Pyemotes* sp. have been found on *Tetragonula iridipennis* queens and were suspected to kill heavily infested queens (Vijayakumar and Jayaraj 2013). In the Neotropics, *Pyemotes tritici* can be particularly problematic and has been reported to kill colonies of several *Melipona* species, *Tetragonisca angustula* and *Frieseomelitta varia* (Kerr et al. 1996; Nogueira-Neto 1997; Menezes et al. 2009a). These mites are likely to spread to other colonies when workers from neighbouring colonies steal food from infested nests or because of beekeeping work.

3.5.4 Microorganisms

Stingless bee nests are home to many microorganisms (Rosa et al. 2003; Promnuan et al. 2009; Menezes et al. 2013; Morais et al. 2013; Cambronero-Heinrichs et al. 2019). The functional relationships between stingless bees and microorganisms, mainly bacteria, yeasts and moulds, are still poorly understood (Menezes et al. 2013). In species that store food for longer time periods, such as stingless bees, mutualisms that help prevent food from spoiling can be very beneficial. Indeed, microorganisms appear to play an important role in honey maturation and in biochemical modifications of stored pollen ("pot-pollen"), honey ("pot-honey") or brood food (Vit et al. 2013, 2018). In *Melipona* and *Trigona*, for example, bacteria from the genus *Bacillus* have been identified in food pots and larval food and are suspected to participate in the metabolic conversion and fermentation of food through the secretion of enzymes (see also Chap. 1) (Gilliam et al. 1985,1990; Morais et al. 2013). These enzymes appear to cause a softening of the exine wall of pollen before it is eaten by bees and they may also reduce the susceptibility of pollen to harmful microorganism (Villegas-Plazas et al. 2018). Interestingly, *Scaptotrigona depilis* workers prefer fermented pollen from old storage pots over freshly collected pollen when given the choice (Vollet-Neto et al. 2017). The bacterium *Paenibacillus polymyxa* was found in the larval food of *Melipona scutellaris,* where it seems to offer protection against entomopathogenic fungi (Menegatti et al. 2018). Treatment of colonies with an antibiotic to kill all bacteria has led to colony death, suggesting that bacteria play a vital role in colony survival (Machado 1971; Gilliam et al. 1990).

Ptilotrigona stingless bees (*P. lurida* and *P. pereneae*) appear to rely on yeast (*Candida* sp.) to promote desiccation and, therefore, long-term preservation of stored pollen (Camargo and Pedro 2004) and various other yeast species is suspected to convert and conserve food via enzyme secretion inside stingless bee nests (Rosa et al. 2003; Menezes et al. 2013). For example, *Starmerella meliponinorum* can be found throughout the nests of *Tetragonisca angustula* and *Frieseomelitta varia* and this yeast is suspected to improve the nutritional quality of stored food (Rosa et al. 2003; Morais et al. 2013; see Villegas-Plazas et al. 2018 for a review). However, not all species of yeast isolated from storage pots are likely to be beneficial for stingless bees and some might cause honey or pollen to spoil (Rosa et al. 2003).

Recently, Menezes et al. (2015) and Paludo et al. (2018) provided an intriguing case of an obligate relationship between a stingless bee and a microorganism. *Scaptotrigona depilis* requires the presence of a fungus from the genus *Zygosaccharomyces* to survive (see also Chap. 5). The fungus grows inside the brood cells where larvae feed on it (Fig. 5.1c). Most larvae reared without the fungus die. The primary function of the fungus does not seem to be nutritional, but it seems to supply bee larvae with essential steroid precursors (Paludo et al. 2018). The same fungus has also been found in other species and may more commonly be associated with stingless bees (Menezes et al. 2013).

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Swarming and Mating

4

To start a new colony, stingless bee queens need the help of workers. The first tasks of these workers are finding and inspecting potential nest sites, building nest-structures at the selected site, gathering food and defending the new home. Most details of this process are still poorly understood and there has been little research on stingless bee swarming and collective nest site selection since the pioneering work of Paulo Nogueira-Neto (1954). As a result, the internal and external conditions that lead to swarming, as well as the signals that coordinate this process, remain largely unknown. Existing studies do, however, show that stingless bees and honey bees have found different solutions for the same problem of establishing a daughter colony. Three major differences are the (1) reproductive status and age of the queen that leaves the nest, (2) the temporal dynamics of colony foundation and (3) the communication processes involved in nest site selection. The last two differences, i.e. the progressive colony foundation over an extended time period (vs. a single migratory movement of a compact swarm in honey bees) and the lack of an obvious recruitment signal (the waggle dance in honey bees), make swarming and nest site selection challenging to study in stingless bees.

4.1 Swarming in Stingless Bees

In *Apis mellifera*, the mother queen and a swarm of several thousand workers move to a new home after workers have started to rear replacement queens (Winston 1987; Seeley 2010). Hockings (1883) was probably the first to recognise that in stingless bees, it is the unmated (“virgin”) queen that leaves the nest, while the mother queen stays behind in the mother nest (Nogueira-Neto 1954). One reason for this difference is that mated stingless bee queens are largely unable to fly due to the increased size of their abdomen (and ovaries) after mating (Hockings 1883), a phenomenon called physogastrism or physogastry (Chap. 1; some examples in Fig. 4.1). In *Scaptotrigona postica*, for example, total queen weight increases about 250% from emergence to full physogastry (Engels 1987). Another reason for the poor flight

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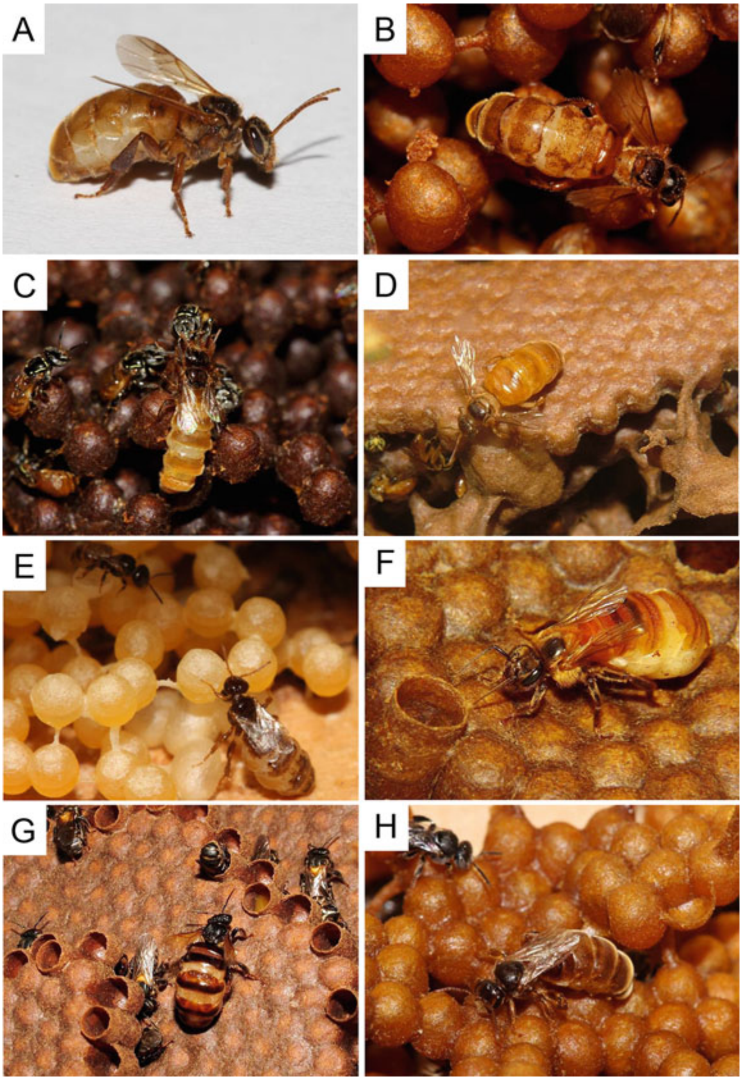

Fig. 4.1 Physogastric queens of different Neotropical stingless bees: (**a**) *Frieseomelitta flavicornis*, (**b**) *F. varia*, (**c**) *Plebeia minima*, (**d**) *Tetragonisca angustula*, (**e**) *Leurotrigona muelleri*, (**f**) *Melipona flavolineata*, (**g**) *Scaptotrigona depilis* and (**h**) *Friesella schrottkyi* (Photos: Cristiano Menezes)

ability of physogastric queens is that older queens often have severely damaged wings (Michener 1974), presumably caused by the frequent ritualised wing beating during the provisioning and oviposition process (POP) (Chap. 5). This also means that stingless bee colonies are unable to abandon their nest and migrate to a new location—a process called absconding—if the old nest suddenly becomes uninhabitable. Absconding, e.g. due to predator attacks, resource scarcity, exposure to bad weather conditions or fire, is a common strategy in honey bees (Winston 1987; Oldroyd and Wongsiri 2006). However, there are exceptions to the rule and Inoue et al. (1984a) observed the successful absconding of a colony, including the queen, in the Asian *Tetragonula laeviceps*. Absconding of workers without the queen as a response to stressors was also observed (Portugal-Araujo 1963; Ribeiro and Bego 1994).[1] In the two cases involving *Frieseomelitta silvestrii* observed by Ribeiro and Bego (1994), workers joined nearby conspecific colonies.

An alternative scenario is that mated stingless bee queens do not leave the nest for other reasons and that this has allowed physogastry to evolve (Peters et al. 1999). Peters et al. (1999), for example, argued that honey bee colonies benefit from the old queen leaving prior to the emergence of daughter queens because this avoids potentially deadly confrontations between mother and daughter queens. Due to higher relatedness among females in stingless bees compared to honey bees (see below), mother queens might be more tolerant towards the presence of daughter queens in stingless bees (note, however, that the tolerance towards unmated queens varies among species, see below). This, in turn, sets the stage for a situation where daughter queens are sometimes allowed to emerge and leave the nest to start a new colony.

4.1.1 Swarming Phases

Swarming has mainly been described in cavity nesting species and the following descriptions will focus on these accounts. The swarming process can roughly be divided into four overlapping phases:

Reconnaissance and Preparation

Scouts inspect new nest sites and their surroundings, often for several days (Nogueira-Neto 1954; Inoue et al. 1984b; van Veen and Sommeijer 2000a). In one instance of the swarming of a *Partamona* colony (originally called *Trigona cupira*, but possibly *P. peckolti* or *P. bilineata*), more than 100 scouts were seen entering and leaving a potential nest site one day before material transfer started (Wille and Orozco 1975). It is likely that these scouts acquire information about the suitability of the nest sites, e.g. based on the size of the entrance and the cavity or the presence of enemies. Colonies sometimes do not move into cavities despite initial reconnaissance activities (Nogueira-Neto 1954; Inoue et al. 1993) and colonies were seen preparing two cavities simultaneously before abandoning one (van Veen and Sommeijer 2000a). This suggests that scouts evaluate the suitability of a potential nest site before swarming, as is well documented in honey bees (Lindauer 1955;

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[1] Schwarz (1948, p. 62) mentions the reports of Goudot that he was told by Natives in Colombia of the habit of a species, possibly belonging to the genus *Tetragona*, to commonly abscond nests provided to them.

Visscher 2007; Seeley 2010). However, the criteria used by workers to determine whether a nest site is suitable have not yet been investigated experimentally in stingless bees. If a nest site receives a positive evaluation, workers start cleaning their new home (Nogueira-Neto 1954; van Veen and Sommeijer 2000a). Chemical marks may be left near the nest site (observed in *Scaptotrigona postica* and *Trigona fulviventris*) to help nestmates locate the new place (Kerr et al. 1962; Hubbell and Johnson 1977). Scouts from different colonies occasionally fight over the same nest site (Hubbell and Johnson 1977). In species that live in termite or ant nests, such as *Scaura* spp., workers start the swarming process by building a nest entrance on the outside of the termite nest, before starting to excavate into the nest of their hosts to create a cavity (see also Chap. 3) (Roubik 1989; Sakagami et al. 1989).

Transport of Building Material and Food

Workers begin to seal cracks in the cavity using resin, batumen or mud. Soon afterwards, they start to build an entrance tube (Ihering 1903; Michener 1946; Nogueira-Neto 1954; Wille and Orozco 1975), which may function as a visual beacon for nestmate workers (Sakagami et al. 1989; Camargo and Pedro 2003). Guards can soon be seen next to the nest entrance (*Partamona bilineata*, Wille and Orozco 1975; *Tetragonisca angustula*, pers. observation). The first food pots are built and filled with honey. This requires an increasing number of bees transporting cerumen and honey from the mother nest (Nogueira-Neto 1954). To confirm that the honey is indeed brought from the mother colony, Nogueira-Neto (1954) added artificial colouring to honey that he provided at the mother nest. He soon found the coloured honey in the daughter nest. Pollen is also brought from the mother nest, either carried in the pollen baskets or in a semi-liquid state in the crop (Kerr 1951; Nogueira-Neto 1954; Wille and Orozco 1975). The latter strategy probably requires the mixing of pollen with honey or nectar. Other activities during this stage include the building of the inner entrance tube, cerumen sheets and an incipient involucrum (see Chap. 3 for a detailed description of these nest structures). The first brood cells are being constructed while or after the involucrum is built (Nogueira-Neto 1954).

The progressive establishment of the daughter nest requires that mother and daughter colony remain connected via workers travelling back and forth between the two nests. The duration of this social link varies greatly among species and colonies: nests were connected for 2–3 days in *Frieseomelitta varia*, ~7–20 days in *Tetragonula laeviceps*, ~1 month in *Plebeia mosquito* and *Tetragonisca angustula*, ~2 months in *Melipona orbignyi* and *Plebeia poecilochroa*, ~3–4 months in *Trigonisca* sp. and ~6 months in *Partamona bilineata* and another *Tetragonisca angustula* colony (Nogueira-Neto 1954; Terada 1972; Wille and Orozco 1975; Sakagami 1982; Inoue et al. 1984b; Drumond et al. 1995; van Veen and Sommeijer 2000a). Workers often continue to bring resources from the mother nest, while the daughter colony is already producing its own brood (Nogueira-Neto 1954). As a result, daughter nests increase in biomass, while mother colonies lose mass (Darchen 1977). On the other hand, daughter colonies do not solely rely on resources from the mother nests while nests are connected as foragers from the daughter nest start to collect nectar, pollen and resin from nearby plants (Wille and Orozco 1975; Inoue et al. 1984b; van Veen and Sommeijer 2000a). Wille and Orozco (1975), for instance, estimated that in *Partamona bilineata*, the dependence of daughter colonies on mother colonies in terms of resource requirements was less than 50% and only relatively small amounts of food were transported from the mother to the daughter nest in *Tetragonula laeviceps* (Inoue et al. 1984b). It is unknown why the link breaks up, but the death of workers with knowledge of both nests is likely to contribute to a complete social separation of the nests.

It has been reported that stingless bee colonies prefer to move into cavities that had been used by other colonies before and still contain building material and nest structures from the previous occupiers (Nogueira-Neto 1954; Wille and Orozco 1975). It is unclear whether colonies prefer such cavities because they contain valuable resources and are, thus, cost-effective or because the cavity itself is of higher quality and, thus, chosen successively by different colonies. It is also possible that scouts are more likely to find such cavities because the existing nesting material emits an attractive odour (Michener 1946; Nogueira-Neto 1954). Honey bees, likewise, prefer to swarm into cavities that already contain wax (Seeley 2010). An extreme case is the usurpation of inhabited nests, which requires the killing of the resident colony. Such usurpations were observed in the Australian *Tetragonula hockingsi* usurping a *T. carbonaria* colony (Cunningham et al. 2014), the American *Scaptotrigona postica* usurping a *Frieseomelitta freiremaiai* colony (Kerr et al. 1962) or *Friesella schrottkyi* usurping a conspecific colony (Nogueira-Neto 1954) (see also Chap. 7). Usurpation as a method of swarming seems to be more common in *Tetragonisca angustula* and may be the only mode of swarming in *Lestrimelitta* robber bees (Sakagami et al. 1993; Grüter et al. 2016).

The time period from nest site selection to completion of the nest and oviposition by the new queen is variable. In *Tetragonisca angustula* or *Scaptotrigona postica*, it can take as little as 2 weeks from the beginning of swarming to oviposition (Kerr 1951; Kerr et al. 1962; Roubik 1989). *Frieseomelitta varia* in southern Brazil completed a nest in only 3 days (Terada 1972). Camargo (cited in Roubik 1989, p. 205), on the other hand, described a *Ptilotrigona* sp. nest built in hard sand substrate that apparently took 3 years before it was finished.

A progressive colony foundation could lead to higher survival chances of incipient colonies due to the continuous access to the food supplies from the mother colony. Indeed, Slaa (2006) found no difference in the survival chances of newly founded colonies and established colonies in several Costa Rican species. In the Asian *Tetragonula minangkabau,* survival chances were somewhat lower in incipient colonies (~40% chance to survive the first year, Inoue et al. 1993; see Chap. 1 for more information on colony mortality), which is still higher than the survival chances reported for newly founded honey bee colonies (~20%) (Seeley 1978, 2017). On the other hand, stingless bee colonies reproduce less often than honey bee colonies, often less than once a year (see also Chap. 1) (Roubik 1989; van Veen and Sommeijer 2000a). For example, *Tetragonula minangkabau* colonies reproduced with a probability of ~0.7 per year (Inoue et al. 1993), whereas *Scaptotrigona pectoralis* colonies in Costa Rica produced only ~0.06 swarms per year (Slaa 2006). Africanised honey bees in the Neotropics, on the other hand, often swarm several times per year and European honey bees swarm between ~1 and 3.6 times (Roubik 1989; Winston 1992). These estimates in combination with data on colony mortality (Chap. 1) suggest that stingless bees follow a life-history strategy of high colony survival and low colony reproduction (Slaa 2006).

What proportion of the work force accompanies the queen and moves to the new nest site? In the Asian *Tetragonula laeviceps,* about 800–1000 workers—about 30% of the total worker population—moved to the daughter nest during swarming (Inoue et al. 1984b). Swarm size in the American *Partamona bilineata* was similar, about 700–800 bees (Wille and Orozco 1975). In *Tetragonisca angustula*, the proportion of workers moving to the new nest was even lower, around 10% (500–1000 bees) (van Veen and Sommeijer 2000a). These preliminary data indicate that less than half of all workers leave the nest to start a new colony.[2] Based on the age-related colouration of workers (see Chap. 6), Darchen (1977) was able to determine that, initially, mainly older workers are involved in preparing the new nest in the African *Hypotrigona*, but that they are soon joined by younger workers. In *Tetragonula laeviceps,* about half of the workers that moved to the daughter nest were relatively young bees (Inoue et al. 1984b). We might expect a large proportion of young workers to undertake this journey because new colonies need a considerable amount of time before they can produce their own workers: the time from queen arrival to the emergence of the first workers will often exceed 50 days (see Table 5.4 for worker developmental times), which is longer than the average worker lifespan of many species (Table 1.1). Staying connected and receiving young workers from the mother nest could, therefore, be crucial for daughter colonies to avoid a shortage of workers just before the first generation of new workers emerges. Additionally, young workers are needed for the preparation and provisioning of larval food (Chaps. 5 and 6) in the new nest.

Arrival of the Queen

After the initial preparations have taken place and workers have started to build the nest-structures, an unmated queen accompanied by more workers arrives at the new nest site (Wille and Orozco 1975; Darchen 1977; Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995). Some authors have described this as a mass migration (Schwarz 1948; Darchen 1977; Inoue et al. 1984b; Engels and Imperatriz-Fonseca 1990). For example, *Tetragonisca angustula* queens were observed flying from the mother to the daughter nest followed (seconds to minutes later) by several hundred workers (van Veen and Sommeijer 2000a). How queens find the new nest is not yet known, but chemical signals are likely to play an important role. In other species, however, no mass migration of workers was observed during swarming (Nogueira-Neto 1954). In *Paratrigona subnuda*, *Tetragonisca angustula* and *Scaptotrigona postica*, more than one unmated queen

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[2] In *Apis mellifera*, about two thirds of all workers leave the colony in the prime swarm, leaving one third behind (Seeley 2010).

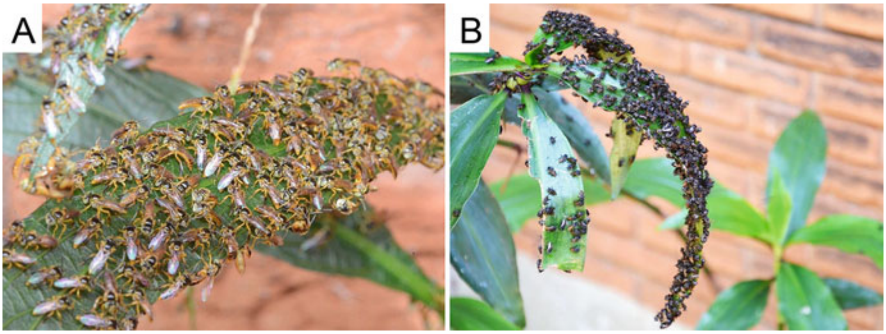

Fig. 4.2 Male aggregations in *Tetragonisca angustula* (**a**) and *Scaptotrigona depilis* (**b**). In both cases, several hundred males were resting on vegetation near a newly established colony (Photos: C. Grüter)

flies to the new nest (Kerr et al. 1962; Imperatriz-Fonseca 1977; Engels and Imperatriz-Fonseca 1990). Spare queens are either executed or kept in "imprisonment chambers" (see Chap. 3) (Kerr et al. 1962).

Drone Arrival

In most stingless bees, males (or drones) aggregate outside the newly established nest or, in the case of supersedure (see below), outside the nest receiving a new queen (Michener 1946; Nogueira-Neto 1954; Kerr et al. 1962; Engels and Engels 1984; Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995; Galindo López and Kraus 2009; Fierro et al. 2011). Males often arrive within a few days after swarming has started and often before nest structures such as food pots have been built (Michener 1946; Nogueira-Neto 1954; Kerr et al. 1962). In many species, they can be seen sitting or flying near the entrance (Fig. 4.2), without interacting much with each other (Roubik 1990; von Zuben 2017, but see below for exceptions).

Males often arrive in the morning and disappear in the evening (Michener 1946; Engels and Engels 1984; Roubik 1990; Cortopassi-Laurino 2007), with activity peaking in the early afternoon (Kerr et al. 1962). Male aggregations can build up rapidly or increase in size over a few days (Michener 1946; Kerr et al. 1962; Wille and Orozco 1975; Engels and Engels 1984; Roubik 1990). In *Partamona bilineata,* a swarm of about 800 males appeared nearly instantaneously 3 days after scouts inspected a new nest site (Wille and Orozco 1975). The number of males in this swarm diminished continuously over the next 4 days. Male aggregations can contain from a few dozens to several thousand males (up to 3000 in *Tetragonisca angustula*, Kerr et al. 1962; Fierro et al. 2011 and up to 7000 in *Tetragonula laeviceps*, Bänziger and Khamyotchai 2014). The same males can visit a particular aggregation for several days (Cortopassi-Laurino 2007; Koffler et al. 2016). The time a male spends in an aggregation was found to correlate negatively with its body size and was linked to sperm characteristics in *Scaptotrigona depilis* (Koffler et al. 2016). Koffler et al.

(2016), therefore, hypothesised that staying duration in an aggregation could predict male quality.

The males in an aggregation originate from many different colonies, often located several hundred metres away (Kerr et al. 1962; Paxton 2000). *Tetragonisca angustula* males in one study area in Brazil travelled on average ~600 m from their natal nest to the aggregation and one male originated from a nest ~1600 m from the aggregation (Santos et al. 2016), which is a remarkable distance for such a small bee.[3] In the Asian *Tetragonilla collina,* a large aggregation consisted of males from an estimated 132 colonies (Cameron et al. 2004). About 15–60 colonies contributed males to an aggregation of *Scaptotrigona mexicana* (Kraus et al. 2008; Müller et al. 2012; Sánchez et al. 2018). None of the males came from colonies in the immediate vicinity, which could indicate that males avoid aggregations in close proximity to their own nest (Müller et al. 2012). Similar results were found in studies showing that aggregations very rarely contain brothers of the unmated queen (Cameron et al. 2004; Santos et al. 2016) and that the males in aggregations are usually not related (see also Paxton 2000). The strategy to avoid nearby aggregations could reduce the risk of inbreeding in a tribe with relatively short swarming distances (see below). After mating, aggregations sometimes dissolve rapidly (Kerr et al. 1962), whereas in other cases, they persist for many days even after the queen has mated (Galindo López and Kraus 2009; Fierro et al. 2011). The signals that attract the males are highly species specific as only a very small portion (<1% in *Scaptotrigona postica* and 1.4% in *Tetragonula laeviceps*) of heterospecific males are found in male aggregations (Kerr et al. 1962; Bänziger and Khamyotchai 2014).

Males temporarily (e.g. in *Scaptotrigona postica*, *Melipona favosa* or *M. quadrifasciata*) leave the aggregation to feed on flowers (Kerr et al. 1962; Sakagami 1982; Sommeijer et al. 2004; Boongird and Michener 2010). *Nannotrigona* and *Scaptotrigona* males are apparently attracted to the flowers of the orchid *Mormolyca ringens* and, while attempting to copulate with pseudocopulatory flowers, pollinate the orchids (Singer et al. 2004). At night, males hide in the vegetation or on flowers, rather than returning to their natal colony (Roubik 1990; Santos et al. 2014). Other observations suggest that drones occasionally rest in groups ("sleeping roosts") on tree branches (Santos et al. 2014).

The identity and the origin of the signals that attract males to a nest have not yet been identified conclusively. Some have suggested that the unmated queen produces chemical signals that lead to the male aggregations (Engels and Engels 1984; Engels 1987; Fierro et al. 2011; Verdugo-Dardon et al. 2011), whereas others propose that workers carry a chemical signal into the environment, e.g. when foraging (Roubik 1990; von Zuben 2017). The observation that unmated queens are attractive to males in bioassays, such as when males and queens are joined in mating boxes, supports the view that queen odours cause drones to aggregate (de Camargo 1972; Engels and Engels 1988; Fierro et al. 2011; Veiga et al. 2017). Engels and co-workers (Engels et al. 1990, 1997) identified volatiles in cephalic secretions of *Scaptotrigona postica*

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[3] About ~4 mg, based on the weights of workers (Grüter et al. 2012).

queens that are attractive to drones. They found that different compounds produced by the queens were attractive over different distances: secondary alcohols were attractive over a distance of a few metres, whereas 2-ketones had an attractive effect over a distance of a few centimetres and induced copulatory attempts. In *S. mexicana*, attraction also seems to be caused by substances produced in the queens' head (Verdugo-Dardon et al. 2011). The secondary alcohol 2-nonanol, in particular, is likely to be important for attraction. In *Tetragonisca angustula*, on the other hand, the abdomen of the queen was the source of one attractive compound, isopropyl hexanoate (Fierro et al. 2011).

There are also observations that challenge the view that these signals are responsible for the long-distance attraction of males. Bioassays usually test queen attraction over relatively short distances (from a few cm to a few m) and Fierro et al. (2011) found that queen attraction rapidly decreases with increasing distance and does not reach further than 20 m. Furthermore, there are several reports of males starting to arrive at new nest sites before the queen has moved in (Kerr et al. 1962; Roubik 1990; Imperatriz-Fonseca and Zucchi 1995; Velthuis et al. 2005). Kerr et al. (1962), for instance, described a swarm of *Scaptotrigona postica* males that arrived 3 days after swarming started to a new nest site. The queen arrived 2 days later. It has, therefore, been suggested that workers produce the signal that is responsible for long-distance attraction and, once the first males have arrived, they themselves might attract more males (Roubik 1990; Imperatriz-Fonseca and Zucchi 1995). For instance, if forager activity is prevented in *Scaptotrigona depilis*, no males are attracted to orphaned colonies (von Zuben 2017). Foragers in queenless colonies might themselves produce a signal or they could pick-up a signal through contact with an attractive queen, which they then carry into the environment (Engels and Imperatriz-Fonseca 1990; von Zuben 2017). Males patrolling or foraging at flowers could then find the workers carrying the signal and follow them to the nest (Roubik 1990; von Zuben 2017). In *Melipona favosa*, males' behaviour towards workers further suggests that worker pheromones play an important role in the establishment of aggregations (Sommeijer et al. 2004). However, such a worker signal has not yet been identified and in *Scaptotrigona mexicana*, males were not attracted to workers,[4] but they showed attraction to other males (Galindo López and Kraus 2009). These male signals appear to be left on the aggregation surfaces and provide an additional positive feedback once an aggregation has started to form (Galindo López and Kraus 2009).

Some species of the genus *Melipona* are an exception to the rule that male aggregations are nest-based (Michener 1946; van Veen et al. 1997; Sommeijer et al. 2004; Cortopassi-Laurino 2007). In *M. favosa*, aggregations consisting of up to several hundred males did not appear to be associated with a particular nest (Sommeijer and De Bruijn 1995; Sommeijer et al. 2004). Several queens, apparently

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[4] It should be noted that the workers were not specifically taken from colonies receiving a new queen. Workers might only produce or carry odours that are attractive to males if their colony is receiving a new queen.

attracted by male-produced odours (and possibly by workers), visited aggregations on a given day (Sommeijer et al. 2004). This is similar to the situation in honey bees, where drones and queens meet at drone congregation areas that can be several km from the parent colonies of drones and queens (Winston 1987; Ruttner 1988). The males in these *Melipona* aggregations not only interacted aggressively with each other but also frequently engaged in trophallaxis. The attraction of flying males and queens and the behaviour of queens after landing suggest that the substrate on which the males rest is covered with an attractive pheromone (Sommeijer and De Bruijn 1995). Michener (1946) observed similar drone aggregation sites (DASs) in *Melipona phenax* in Panama, but how widespread DASs are in *Melipona* remains unknown. *Melipona costaricensis* and *M. flavolineata*,[5] for instance, follow the more common pattern of nest-based male aggregations (van Veen et al. 1997; Veiga et al. 2018). No matings have yet been observed in non-nest-based aggregations.

Mating Flight

Males in aggregations do not enter the colony, but wait outside until the queen emerges for her mating flight (Roubik 1990). Nogueira-Neto (1954) suggests that this is a general pattern in stingless bees. This could mean that unmated queens are not yet attractive to males during the initial migratory flight (Engels and Engels 1988; Veiga et al. 2017; but see van Veen and Sommeijer 2000b). Mating flights usually happen when queens are 1–14 days old (da Silva et al. 1972; Vollet-Neto et al. 2018).

Even though mating flights have rarely been observed, it is assumed that unmated stingless bee queens go on one mating flight and use the sperm acquired during this flight for the rest of their life (see also Sect. 4.6) (Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995; Vollet-Neto et al. 2018). Detailed observations of the events leading up to a mating flight were made by da Silva et al. (1972) during supersedure (Sect. 4.4) in *Melipona quadrifasciata*. Before the mating flight took place, queens started to enlarge their abdomen and workers were seen forming a royal court (see Fig. 4.3). The period before the mating flight coincides with a period of increased vitellogenin production, a precursor of egg-yolk protein[6] (Engles and Engels 1977; Engels 1987). One or two days before the mating flight, *M. quadrifasciata* queens were seen near the entrance or in the entrance tube or they performed short flights from one comb or pot to another inside the nest (da Silva et al. 1972). After leaving the nest, queens turned around at a distance of about half a metre from the nest to face the entrance. They then flew in circles of increasing size, presumably to learn the visual features of the nest entrance and the surrounding area. Similar orientation flights by unmated queens were

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[5] This species is unusual in that it produces two male morphs of different colour, light (~80% of emerging males) and dark (~20%) (Veiga et al. 2018).

[6] Little is known about the links between vitellogenin (vg) and reproduction in stingless bees. The function does not seem to be straightforward, as vg is also expressed in workers of sterile species (Dallacqua et al. 2007), but was not found in *Melipona quadrifasciata* workers, which frequently lay eggs (Paes de Oliveira et al. 2012).

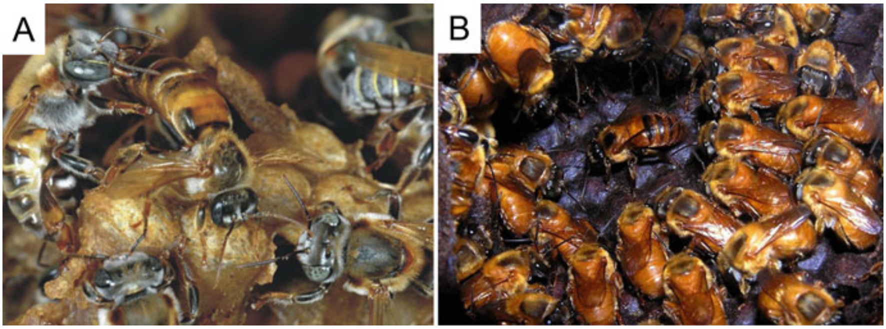

Fig. 4.3 Workers attacking a virgin queen in *Melipona beecheii* (from Jarau et al. 2009). Workers forming a royal court around an accepted virgin queen in *Melipona flavolineata* (Photo: Cristiano Menezes)

observed in *Tetragonisca angustula*, but not in *Melipona beecheii* (van Veen and Sommeijer 2000b). Mating flights lasted 30–100 min in *M. quadrifasciata*, (da Silva et al. 1972), ~50 min in *M. quinquefasciata* (de Camargo 1972), 5–40 min in *M. beecheii* (van Veen and Sommeijer 2000b), ~30 min in *Paratrigona subnuda* (Imperatriz-Fonseca 1977), 2–20 min in *Tetragonisca angustula* (van Veen and Sommeijer 2000b), ~15 min in *Plebeia droryana* (da Silva 1972) and just 2–8 min in *Melipona favosa* (Sommeijer et al. 2003b) (see Vollet-Neto et al. 2018 for more details on mating flight durations).

In *Tetragonisca angustula*, mating flights tend to occur on the same day or the day after the unmated queen arrived at the new nest (van Veen and Sommeijer 2000b). A queen of this species was observed leaving the colony and, after drones pounced on her, dropping to the ground where mating occurred (Imperatriz-Fonseca et al. 1998). In the Asian *Tetragonula* and *Heterotrigona* species studied by Boongird and Michener (2010), mating occurred after queens landed near the nest entrance. In their study, copulations occurred after dawn and queens frequently entered the nest during copulation, sometimes followed by a number of other males. These observations seem to differ from observations made in Neotropical species, but it is not clear whether they indicate different mating patterns in different clades.

Whether mate choice plays a role in stingless bee mating is not well understood. Queens could attempt to wrestle off males and, thereby, select against males in poor condition (Smith 2020) and males could be attracted more strongly to queens of higher quality, e.g. gauged via chemical signals. In *Scaptotrigona depilis*, for instance, males aggregate more in front of orphaned colonies with large biomass (von Zuben 2017), which could mean that the overall quality of a colony and/or a queen affects male attraction. Because male genitalia are torn off during mating, males are not able to mate more than once (Engels and Imperatriz-Fonseca 1990; Veiga et al. 2018; Smith 2020).

In *Melipona quadrifasciata*, queens return from mating flights with the male genitalia still attached (Kerr et al. 1962) and the queens themselves seem to be able to remove the male mating apparatus (“mating sign”) with their hind legs (da Silva et al. 1972), usually within an hour (Vollet-Neto et al. 2018). In other species, queens require the help of workers to remove the male mating sign (Colonello and Hartfelder 2005). The mating signs consist of the burst genital capsule of the male (Colonello and Hartfelder 2005). After mating, it can take 2–7 days in *Tetragonisca angustula,* ~6 days in *Melipona quadrifasciata*, ~16 days in *Melipona rufiventris*, up to ~30 days in *Melipona bicolor schencki* and up to ~40 days in *Plebeia droryana* before the queen starts to lay eggs (Kerr 1948, 1951; Kerr et al. 1962; Terada et al. 1975; da Silva 1972; van Veen and Sommeijer 2000a; Vollet-Neto et al. 2018). It should be noted, however, that there is considerable variation among queens of the same species in the time it takes from mating to egg laying. In *M. quadrifasciata*, for instance, the time between mating and the start of oviposition can last from 3 to 20 days (Kerr 1951; da Silva et al. 1972).

Queens are still attractive to males after mating in *Lestrimelitta ehrhardti* and *Schwarziana sp*., where males tried to copulate with physogastric queens when nests were opened (Sakagami and Laroca 1963; Sakagami 1982). Likewise, bioassays show that physogastric queens are as attractive as unmated queens in *Tetragonisca angustula* and the two types of queens had qualitatively similar chemical profiles (Fierro et al. 2011). In *Scaptotrigona mexicana*, on the other hand, drones were more strongly attracted to unmated queens than to physogastric queens (Verdugo-Dardon et al. 2011). In other social insects, queens often stop being attractive to males after mating (Oppelt and Heinze 2009). This suggests that queens of some stingless bees continue to produce sex pheromones, possibly because they have other functions in colony organisation.

4.2 Swarming Distances

Swarming distances have important implications for foraging competition, population genetics and the ability of stingless bees to respond to habitat change (see also Chap. 2). Swarming distances in stingless bees tend to be much shorter than in honey bees, where colonies frequently migrate >1000 m and sometimes several km to a new nest site (Lindauer 1955; Schmidt 1995; Seeley and Buhrman 1999). In *Tetragonisca angustula*, swarming distances ranged from 2 to 280 m (Kerr 1951; van Veen and Sommeijer 2000a). In *Tetragonula laeviceps,* colonies migrated ~20 m to a new nest site (Inoue et al. 1984b) and swarming distances of 100 m in *Partamona bilineata* (Wille and Orozco 1975), 75 m in *Melipona orbignyi* (Nogueira-Neto 1954) and 40 m in *Plebeia poecilochroa* (Drumond et al. 1995) were observed. The fact that several nests of the same species are sometimes found in the same tree or termite nest (Chap. 3) suggests that swarming distances of less than a couple of metres are not uncommon. The longest distance has been recorded in a *Plebeia* sp. colony that established a new nest ~365 m from the mother colony (Nogueira-Neto 1954). Greater migration distances might not be feasible due to energy costs (which accumulate over time because workers fly back and forth between the new and the old nest) or are not necessary because of an abundance of available nest sites. It could also be that swarming to more distant cavities is more difficult to observe and that, therefore, instances of long-distance swarming are under-reported in the literature (van Veen and Sommeijer 2000a).

4.3 Swarming Period

Tropical and sub-tropical environments experience seasonality in climate and in foraging conditions and one might expect a certain degree of seasonality in swarming too. Von Ihering (1903), for example, observed more stingless bee swarms in summer and autumn in São Paulo state, Brazil. These are also the seasons with more rain. In Panama and Costa Rica, countries with more pronounced wet and dry seasons, swarming was more frequent in the dry season than during the wet season (Michener 1946; Slaa 2006). Michener (1946) explained this by the tendency of colonies to swarm on sunny days, whereas Slaa (2006) argued that food availability might explain this trend. Interestingly, Peckolt (1894, cited in Schwarz 1948) observed swarming events in *Trigona spinipes*[7] during thunderstorms in late summer in the south of Brazil. In *Tetragonula iridipennis*, colonies in southern India were more likely to swarm towards the end of the rainy season (August–September) (Schwarz 1948). A clear seasonality was also found in *Hypotrigona* colonies in Gabon, equatorial Africa, where colonies swarmed from November to May, peaking in December and January, which corresponds to the end of the wet season and the beginning of the dry season (Darchen 1977). This time period coincides with the presence of large amounts of food stores in the colonies and good foraging conditions. *Apis mellifera* swarms at the same time in this area, suggesting that similar ecological conditions favour swarming in these two types of bees (Darchen 1977).

Nogueira-Neto (1954), on the other hand, observed no particular swarming period as different species swarmed in different months in São Paulo state. *Nannotrigona testaceicornis* and *Plebeia mosquito*, for instance, swarmed in all four seasons. Only during the coldest month (June) in winter, he observed no swarming. *Tetragonula minangkabau* colonies in Sumatra also do not seem to show strong seasonality with respect to swarming, but swarming was observed less during months with very dry or very rainy weather (Inoue et al. 1993).

The cues that promote swarming are not well studied, but van Veen and Sommeijer (2000a) observed that swarming *Tetragonisca angustula* colonies were stronger than non-swarming colonies. The amount of stored food could provide a cue that triggers swarming (Roubik 1982), but more research is needed to confirm this plausible prediction. In honey bees, reproductive swarming often occurs after a period of strong colony growth accompanied by a build-up of food reserves (Seeley and Visscher 1985; Winston 1992). Workers could perceive colony strength by an

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[7] As *Trigona ruficrus*, but uncertain species identity according to Camargo and Pedro (2013, Moure's Bee Catalogue).

Fig. 4.4 *Melipona flavolineata* colony with two physogastric queens (Photo: Cristiano Menezes). The photo also shows empty open cells and an open cell containing brood food

increase in worker density, lower amounts of queen signal or a direct detection of large food stores (Seeley 1979).

4.4 Supersedure (Queen Replacement)

When the old queen dies, produces diploid males (see below), runs out of sperm or is not very productive, she is usually replaced by a new queen. Unproductive queens are often killed by workers, sometimes with the help of unmated queens (Imperatriz-Fonseca 1978; Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995). In *Paratrigona subnuda*, for example, unmated queens were observed to initiate supersedure by discharging mandibular gland secretions of unknown content on the physogastric queen (Imperatriz-Fonseca 1977, 1978). This caused workers to lick the queen and, subsequently, eliminate either the unmated or the physogastric queen. Unmated queens in *Plebeia droryana*, *P. remota* and *Frieseomelitta languida* have been observed to contribute to supersedure by attacking the physogastric queen (da Silva 1972; Imperatriz-Fonseca and Zucchi 1995; van Benthem et al. 1995). The new queen is occasionally accepted by the workers before the old queen has died (da Silva et al. 1972; Imperatriz-Fonseca 1978) and, as a result, colonies can temporarily have more than one egg-laying queen (Fig. 4.4) (Engels and Imperatriz-Fonseca 1990; Alves et al. 2011). Imperatriz-Fonseca (1978) observed that queen supersedure is more common during the swarming process. This could be caused by increased competition between the physogastric queen and unmated queens during the swarming process or greater tolerance of workers towards unmated queens. How frequently supersedure occurs under natural conditions has not yet been quantified.[8]

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[8] The frequency by which male swarms appear in front of colonies could be used as an indirect way to estimate supersedure frequency.

4.5 Queen Production and Selection

One major difference between stingless bees and honey bees is that stingless bees produce queens throughout the year, whereas *Apis mellifera* colonies produce queens only when needed. The genus *Melipona,* in particular, is well known for producing a large number of queens, most of which are killed by the workers soon after they emerged (see below) (Kerr 1946; Kerr et al. 1962; Imperatriz-Fonseca and Zucchi 1995; Koedam et al. 1995; Wenseleers et al. 2004; Wenseleers and Ratnieks 2004; Ratnieks et al. 2006). Other stingless bee species keep spare unmated queens in the nest during most of the year (Fig. 3.14; Sect. 3.2.4) (Kerr et al. 1962; Inoue et al. 1984b; Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995). The number of queens that are reared depends on the species, colony size, foraging conditions or the reproductive status of the physogastric queen (see also Sect. 5.4.3) (Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995; Van Veen et al. 2004). In *Scaptotrigona postica*, up to 13 unmated queens were observed in a strong colony (Engels and Imperatriz-Fonseca 1990) and von Ihering (1903) reported that 24 unmated queens were present in a colony of an unidentified species. A *Trigona crassipes* colony reared 132 queens in royal cells (Camargo and Roubik 1991), but it is not known how many of these queens were kept alive after emergence.

One reason for the continued presence of spare queens might be that the production of new queens takes longer in stingless bees than in honey bees (Chap. 5; Table 5.4). Thus, having spare queens might be an insurance policy against queen loss (Engels and Imperatriz-Fonseca 1990). There are, however, reports that some species are able to produce emergency queens in a relatively short time period (Faustino et al. 2002; Nunes et al. 2015; Luz et al. 2017). In *Frieseomelitta varia*, a species that builds clustered brood cells (see Chap. 3), workers of an orphaned colony constructed auxiliary cells containing food next to cells with late-stage larvae. They then built a connection between the auxiliary cell and the cell containing the larvae, which allowed the larvae to consume an extra portion of food (Chap. 5) (Faustino et al. 2002; see also Luz et al. 2017). Another hypothesis for the presence of multiple queens posits that this allows workers to continuously compare different queens and guarantee the presence of a high-quality queen (Koedam et al. 1995).

The emergence of a new queen is a potentially significant event for workers. The emerging queen could become the reproductive queen and, thus, contribute to the evolutionary fitness of the workers (Sect. 4.5). On the other hand, she might be born at a time when no new queen is needed. So, how are newly emerged queens treated by workers? In some species (some *Melipona*), workers initially show little interest in them, but start to respond a few days after emergence (Imperatriz-Fonseca and Zucchi 1995; Souza et al. 2017). In other species, e.g. *Meliponula bocandei*, *Tetragonula laeviceps* or *Plebeia remota*, queens that have emerged cause an immediate worker response (friendly or aggressive) (Sakagami et al. 1977; Inoue et al. 1984b; van Benthem et al. 1995). In *Melipona beecheii*, a species that produces many queens, aggression against queens often starts when queens are still attempting to emerge from their cells (van Veen et al. 1999) and newly emerged *M. beecheii* queens were estimated to survive about a day on average (Wenseleers et al. 2004). In *Plebeia remota*, unwanted queens are usually killed by workers within a few hours after emergence (van Benthem et al. 1995). Workers probably recognise queens by a specific blend of hydrocarbons on their cuticle (Abdalla et al. 2003; Kerr et al. 2004; Nunes et al. 2009; Ferreira-Caliman et al. 2013; Souza et al. 2017). The difference in cuticular hydrocarbon profiles (CHC's, see Sect. 7.3.2) between queens and workers increases with time after emergence, e.g. in *Melipona bicolor* and *M. scutellaris* (Abdalla et al. 2003; Souza et al. 2017), which could explain the change in worker behaviour towards queens.

When colonies need a new queen, workers become more tolerant and the survival chances of emerging queens increase (Kärcher et al. 2013), even though orphaned colonies may continue to kill emerging queens for some time (da Silva et al. 1972; van Veen et al. 1999; Kärcher et al. 2013). Given that there are usually several unmated queens to choose from, how do workers select their next queen? Imperatriz-Fonseca (1977) describes that, in some species, emerging queens emit a strong smell that can be perceived even by human observers and that attractiveness measured as the number of workers around the queen varies greatly among queens. It is possible that these queen odours convey information about the quality of a queen, but the pheromones that are involved have not yet been identified (Jarau et al. 2009). Cruz-Landim et al. (1980) found that queen attraction to workers is correlated with secretional activity of tergal glands, particularly glands located bilaterally on the tergites. This could indicate that the chemicals produced by these glands have pheromonal function (Cruz-Landim et al. 2006). In line with this hypothesis are reports that young queens inflate their abdomen and expose their tergite pockets as they walk around, presumably to release a pheromone (Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995; van Veen et al. 1999; Jarau et al. 2009; Kärcher et al. 2013). In *Melipona beecheii*, queens that were accepted by the workers inflated their abdomens for longer time periods (van Veen et al. 1999). Further evidence supporting a role of chemicals produced by abdominal glands is the observation that workers often attack the abdomen of unmated queens that are not selected (e.g. Fig. 4.3) (Engels and Imperatriz-Fonseca 1990; van Veen et al. 1999; Jarau et al. 2009).

Cuticular hydrocarbons are also suspected to affect worker behaviour towards the queen (Abdalla et al. 2003; Souza et al. 2018). In *Friesella schrottki*, for instance, CHCs on the queens' body have pheromonal function and inhibit worker ovaries and, thus, regulate reproductive division of labour in this species (Nunes et al. 2014). Nunes et al. (2014) provide further evidence that abdominal glands (possibly the Dufour's gland) release queen pheromones. Abdominal movements by the queen seem to play an important role during ritualised interactions with workers. However, their data also indicate that a complex blend of chemical compounds emitted from different glands and body parts affects worker behaviour and physiology. Taken together, these studies suggest that pheromones produced by abdominal glands play important roles during the process of queen selection, but they also highlight that our understanding of the signals that mediate queen selection are poorly understood (see van Oystaeyen et al. 2014; Oi et al. 2015; Oliveira et al. 2015 for information about queen signals in other social insect groups).

In *Schwarziana quadripunctata*, a species that produces both large and miniature ("dwarf") queens (Chap. 5), workers seem to prefer the large queens: miniature queens, which represent more than 80% of all emerging queens are mostly killed by workers, whereas large queens are kept in "imprisonment chambers" (Engels and Imperatriz-Fonseca 1990; Wenseleers et al. 2005; Ribeiro et al. 2006). As a result, only about ~20% of colonies are headed by miniature queens (Chap. 5) (Wenseleers et al. 2005). Since miniature queens are less fecund, workers might respond to chemical, behavioural or morphological fertility indicators (Camargo 1974; Wenseleers et al. 2005).

Queen behaviour could also affect queen choice. Workers in *Melipona beecheii* did not attack freshly killed queens or queens "feigning death" (the strategy of lying motionless with their extremities held close to the body), suggesting that the agitated behaviour of a queen could trigger worker aggression (van Veen et al. 1999; Jarau et al. 2009). Unmated queens often try to avoid their execution by running and by performing rapid turns when attacked. Most importantly, they hide in the periphery of the nest or in empty storage pots (Engels and Imperatriz-Fonseca 1990; van Veen et al. 1999; Jarau et al. 2009). Kärcher et al. (2013) found that the behaviours that correlated most with queen survival and acceptance in *Melipona quadrifasciata* were the time queens spent hiding and their ability to avoid fights. These factors increase the chance that an unmated queen is alive during the critical time period when workers are receptive for a new queen, some hours to a few days after queen loss (da Silva et al. 1972; Imperatriz-Fonseca 1977; van Veen et al. 1999; Sommeijer et al. 2003b; Kärcher et al. 2013). Unmated queens have also been observed to form groups and establish dominance hierarchies (Engels and Imperatriz-Fonseca 1990).

Recently emerged *Melipona* queens have been observed to evade worker aggression by leaving the nest. In *M. favosa,* 57% of the emerged queens left the colony alive (Sommeijer et al. 2003b). These queens may try to sneak into conspecific colonies and take over the role of the egg-laying queen (Sommeijer et al. 2003a, b; Wenseleers et al. 2011; van Oystaeyen et al. 2013). Such a strategy could be beneficial from both the colony's and the queen's perspective if the chances of an unmated queen to replace the physogastric queen in the mother nest are very small. There is evidence that these dispersing queens preferentially invade queenless colonies (in *M. scutellaris*, 8.6% of all colonies were estimated to be queenless in one region, Wenseleers et al. 2011) and they attempt to enter other nests in the evening when entrance guarding is less intense (van Oystaeyen et al. 2013). This seems to be a relatively successful strategy as 25–37.5% of *M. scutellaris* queens were replaced by alien queens during supersedure in studies by Wenseleers et al. (2011) and van Oystaeyen et al. (2013). Sommeijer et al. (2003a, b) speculate that this option of social parasitism could partly explain why *Melipona* colonies produce so many queens (see Chap. 5 for alternative explanations). Unmated queens might be able to survive for long time periods by feeding from flowers (observed in *M. favosa*) (Sommeijer et al. 2003a), while searching for a queenless nest to invade.

Once a queen has been accepted, she may herself join the attacks on other queens (da Silva et al. 1972). The chosen queen will frequently receive food from workers and in several species, queens have been observed to offer a yellowish fluid of unknown content to workers, which in return increased the feeding of the newly accepted queen (da Silva et al. 1972; Engels and Imperatriz-Fonseca 1990). In *Melipona quadrifasciata*, workers gathered around the chosen queen to form a royal court after a choice became apparent (Kärcher et al. 2013). The queen then performed a dance-like behaviour (similar to the honey bee "round dance", see von Frisch 1967). During this dance, the queen shakes her inflated abdomen and touches workers with it, possibly signalling her presence to workers (Imperatriz-Fonseca and Zucchi 1995). Shortly after this "enthronement dance," both queen and workers calm down. In *Scaptotrigona postica*, selected queens performed this "enthronement dance" on top of the brood nest (Engels and Imperatriz-Fonseca 1990). The "enthronement dance" in *S. postica* was performed during 1–2 days and caused excitement among the workers. It has been speculated that queens also make their presence felt by pheromones in their faeces, which is eaten by workers (Sakagami et al. 1977; Engels and Imperatriz-Fonseca 1990; Kleinert 2005).

4.6 Single Mating in Stingless Bees

Stingless bee queens usually mate with only one male or they use the sperm of only one male (Kerr et al. 1962; da Silva et al. 1972; Peters et al. 1999; Strassmann 2001; Green and Oldroyd 2002; Palmer et al. 2002; Tóth et al. 2002, 2004; Jaffé et al. 2014; Vollet-Neto et al. 2018, 2019). As a result, estimates of worker-worker relatedness do not usually differ from the predicted $r = 0.75$ for full sisters in hymenopterans (Fig. 4.5) (Ratnieks et al. 2006).

Single mating was first suggested based on sperm counts: Kerr et al. (1962) found that *Melipona quadrifasciata* males had on average 1,156,850 sperm cells and a recently mated queen had about one million sperm cells in her oviducts and spermatheca.[9] Sperm counts on their own, however, are inconclusive and molecular tools have been used to show that effective paternity is close to 1 in more than 20 species of stingless bees (Peters et al. 1999; Strassmann 2001; Palmer et al. 2002; Tóth et al. 2002, 2004; Jaffé et al. 2014). As a result, females in a stingless bee colony are more closely related to each other than in honey bees, where queens mate with many males and relatedness among workers is about ~0.3 (Strassmann 2001; Ratnieks et al. 2006).

Occasionally, lower relatedness estimates have been reported (Fig. 4.5) (Peters et al. 1999; Paxton et al. 1999; Paxton 2000) and Imperatriz-Fonseca et al. (1998) have observed a *Tetragonisca angustula* queen mating with two males. Queens of the same species have also been observed to go on two mating flights (van Veen and Sommeijer 2000b). This suggests that multiple mating might occasionally occur

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[9] This is much lower than in *Apis mellifera* (~5 mio sperm in the spermatheca; Baer 2005).

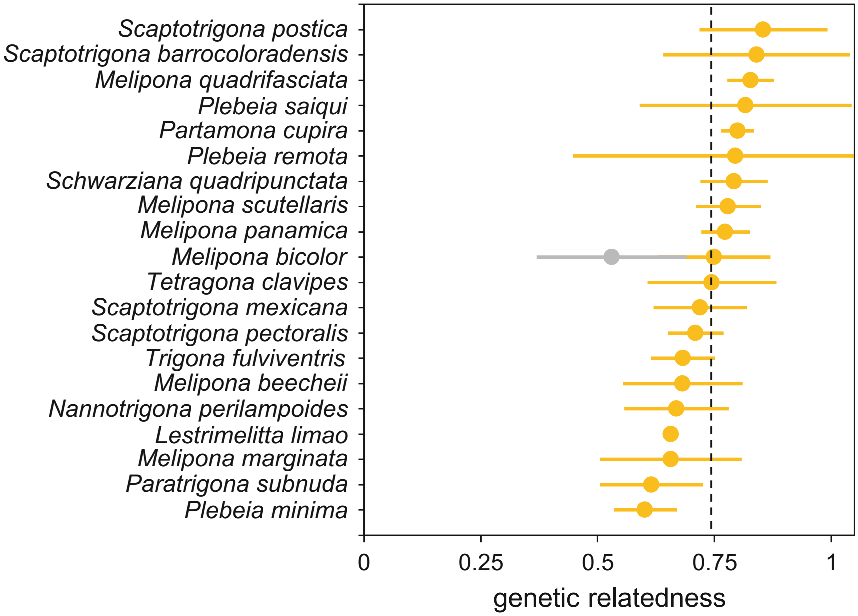

Fig. 4.5 Average relatedness and 95% confidence intervals for among nest-mates in 20 stingless bee species. Due to a small sample size, standard errors are not estimated for *Lestrimelitta limao*. The green dot represents polygynous *Melipona bicolor* colonies (data from Paxton et al. 1999; Peters et al. 1999; Palmer et al. 2002; Tóth et al. 2002; Reis et al. 2011)

(Engels and Engels 1988; Paxton et al. 1999; Paxton 2000; Francini et al. 2012; Viana et al. 2015). There are alternative explanations for lower relatedness estimates, however, such as worker drifting, queen replacement, null alleles or the accidental inclusion of males in the analysed samples (Paxton 2000; Palmer et al. 2002; Vollet-Neto et al. 2018). Further research is required to explore whether multiple mating might occur more commonly in certain species.

The high relatedness in stingless bees compared to honey bees is likely to have important consequences for the social organisation and overall performance of stingless bee colonies (Peters et al. 1999; Ratnieks et al. 2006). For example, the production of males by workers is favoured on relatedness grounds in stingless bees because workers are more closely related to the sons of their sisters ($r = 0.375$) than the sons of their mother, the queen ($r = 0.25$) (see Chap. 5 for more details) (reviewed in Ratnieks et al. 2006). This could help to explain why a substantial proportion of males are worker-produced in many stingless bee species (Tóth et al. 2004; Ratnieks et al. 2006; Grüter 2018). Higher relatedness could also increase the tolerance of workers towards unmated queens, thus explaining why some queens are kept in “imprisonment chambers” (Chap. 3) (Peters et al. 1999). On the other hand, mating multiply increases the genetic diversity among workers, which can improve colony health (Baer and Schmid-Hempel 1999), collective behavioural performance (Mattila and Seeley 2007) and colony resilience following environmental changes

(Jones et al. 2004; Oldroyd and Fewell 2007). So why is it that stingless bee queens do not mate with several males like honey bee queens do?

Mating multiply could be costly due to increased exposure to predators, risk of injury by aggressive males or disease transmission (Strassmann 2001). However, honey bee queens probably face similar costs, which does not prevent them from mating with multiple males. The "diploid-male hypothesis" argues that single mating in stingless bees is favoured by natural selection due to the system of sex determination in hymenopterans combined with a particular feature of stingless bee biology, the rearing of brood in mass-provisioned and sealed cells (Chap. 5) (Ratnieks 1990). Sex in bees is determined by complementary sex determination (CSD) (Ratnieks 1990; Cook and Crozier 1995; Heimpel and de Boer 2008; Zayed and Packer 2005; Hasselmann et al. 2008). Bees that are heterozygous at the complementary sex-determining locus are female, whereas bees that have either just one copy (hemizygous/haploid) or have identical copies (homozygous) become males. In the latter case, diploid males are produced, which are often sterile or non-viable in insects (Cook and Crozier 1995; Zayed and Packer 2005; Heimpel and de Boer 2008). At least in some stingless bees, e.g. *Melipona quadrifasciata, Tetragonula carbonaria* or *Tetragonisca angustula*, diploid males are viable and have been found in male aggregations (Santos et al. 2013; Vollet-Neto et al. 2015), but they appear to have a shorter lifespan and are probably infertile (de Camargo 1982; Green and Oldroyd 2002; Tavares et al. 2003). As a result, diploid males are a lost investment for a colony.

If diploid males can be recognised and killed by workers early in their development, like in the progressively feeding honey bees, then the costs are low (Ratnieks 1990). In stingless bees, however, cells are mass-provisioned and sealed immediately after oviposition (Chap. 5) (Michener 1974; Sakagami 1982; Wille 1983), which means that diploid males are reared until they emerge (Carvalho 2001; Francini et al. 2012). This imposes substantial costs as diploid male production by singly mated queens results in the loss of 50% of potential worker brood[10] (de Camargo 1979, 1982; Ratnieks 1990; Green and Oldroyd 2002; Cameron et al. 2004; Vollet-Neto et al. 2018, 2019), which is likely to have a considerable effect on colony fitness due to a slower colony growth and a reduced colony size (Ratnieks 1990). Even if they represent a smaller proportion of the emerging brood, diploid males are likely to have a negative impact on colony growth due to the loss of workers and space for worker rearing (Ratnieks 1990; Vollet-Neto et al. 2017). Therefore, queens producing diploid males have a lower fitness and are hypothesised to be killed by the workers (de Camargo 1979; Ratnieks 1990; Carvalho 2001; Alves et al. 2011; Francini et al. 2012; Vollet-Neto et al. 2017, 2019).

The probability of a queen mating with a male with the same sex allele, which would lead to the production of diploid males (known as a matched-mating), is estimated to be 5–20% in different stingless bees (Vollet-Neto et al. 2017). With

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[10] If the queen has mated with a male that has a matching sex allele, 50% of all fertilised eggs (which would usually develop into workers) develop into diploid males.

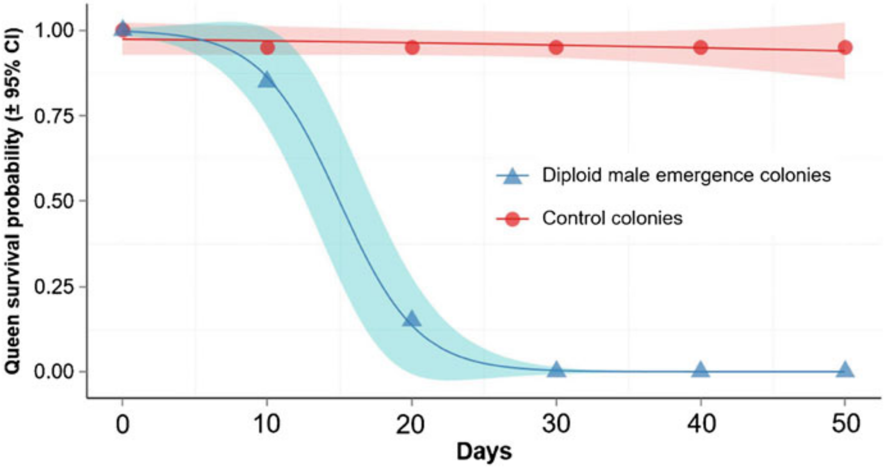

Fig. 4.6 Survival of *Scaptotrigona depilis* queens that were introduced into colonies where excess diploid males were produced versus that of queens introduced to colonies where no diploid males were produced (from Vollet-Neto et al. 2017)

multiple mating, the risk of a matched mating increases and, according to the "diploid-male hypothesis", also the risk that the queen is killed by workers. Support for a link between mating frequency, diploid male production and queen execution was recently found in *Scaptotrigona depilis*. *Scaptotrigona depilis* queens that produce large proportions of diploid males (25% or more) or queens that are introduced at a time when diploid males emerge are executed (Fig. 4.6) (Vollet-Neto et al. 2017, 2019). The timing of the executions also coincided with the development of a distinct cuticular hydrocarbon profile in diploid males, suggesting that workers can chemically recognise diploid males (Vollet-Neto et al. 2017). Queen execution was rare if diploid males represented 12.5% or less of the emerging brood (Vollet-Neto et al. 2019). This suggests that queens would not be executed if they would mate with four or more males, if just one of these matings was a matched mating. However, the high risk of execution for doubly mated queens is hypothesised to prevent the evolution of multiple mating in stingless bees (Vollet-Neto et al. 2019).

4.7 Monogyny in Stingless Bees

Stingless bees are predominantly monogynous, which means that colonies have only one egg-laying queen (Sakagami 1982; Nogueira-Neto 1997). As mentioned above, transient polygyny can occur and has been observed in several species (Vollet-Neto et al. 2018). Eight physogastric full-sister queens temporarily coexisted in one *Melipona quadrifasciata* colony (Alves et al. 2011). However, there is only one known species where stable polygyny is common, *Melipona bicolor*. Up to five queens share reproduction in *M. bicolor* colonies and, as a result, worker-worker relatedness is lower than in monogynous colonies (Fig. 4.5) (Michener 1974; Bego 1983; Velthuis et al. 2001, 2006; Reis et al. 2011). The queens are likely to be related, either a mother with daughters or a group of sisters (Velthuis et al. 2006), but invasion by alien queens seems to occur (Reis et al. 2011). Queens do not show aggression towards each other (Engels and Imperatriz-Fonseca 1990), but the reproductive output of queens shows considerable skew, possibly because queens that are more attractive to workers receive more food, e.g. in the form of trophic eggs (Velthuis et al. 2001, 2006). Artificial feeding appears to favour polygyny in laboratory colonies (Imperatriz-Fonseca and Zucchi 1995), but polygynous colonies do not seem to be more productive than monogynous colonies. Thus, the benefits of polygyny in *M. bicolor* are not yet clear (Velthuis et al. 2006).

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Brood Rearing

5

Stingless bee brood rearing is a complex process that involves workers performing a number of different jobs and requires coordination with the queen. After a new brood cell is finished, several workers regurgitate larval food into this cell, a process called mass provisioning. Shortly afterwards, the queen lays her egg on top of the larval food, immediately followed by the sealing of the cell (Sakagami 1982; Engels and Imperatriz-Fonseca 1990; Zucchi et al. 1999). Mass provisioning followed by oviposition and cell sealing is often considered an ancestral trait because it is also found in most solitary wasps and bees (Sakagami and Zucchi 1974; Sommeijer 1985; Roubik 1989). In stingless bees, however, cell building, provisioning, oviposition and cell sealing represent different stages of a highly integrated social process, with the queen acting as a pacemaker. This process differs considerably from brood rearing in honeybees where queens lay eggs into re-usable empty cells, which are then provisioned progressively for several days before the cell is sealed. This peculiar way of rearing brood is likely to affect colony health, reproductive conflicts (Sect. 5.6) and the mating system (Chap. 4).

5.1 Provisioning and Oviposition Process

After an empty brood cell has been selected by the queen and workers,[1] the latter fill the cell with larval food, followed by queen oviposition. Immediately after oviposition, young workers seal the cell. This seemingly simple provisioning and oviposition process (POP) has attracted considerable interest from researchers, partly due to the conspicuous behaviours performed by the workers and the queen and the behavioural differences found among species (e.g. Sakagami and Oniki 1963; Sakagami et al. 1973, 1977; Sakagami and Zucchi 1974; Sakagami 1982; Bego 1990; Bego et al. 1999; Zucchi et al. 1999; Pereira et al. 2009). The behaviour of

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[1] This selection of a cell was often called “fixation” in early descriptions of the POP.

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both workers and the queen is often described as agitated or excited and the interactions as ritualised. There is an element of subjectivity to these descriptions, but a consensus among researchers exists that interactions among the queen and workers appear ritualised in many species (e.g. *Melipona compressipes*, *Plebeia droryana*, *Scaptotrigona postica* or *Trigonisca duckei*, less so in *Tetragona clavipes* or *Friesella schrottkyi*) and that participants behave in an agitated and even overtly aggressive manner (Sakagami and Oniki 1963; Sakagami and Zucchi 1967, 1974; Sakagami et al. 1977; Sakagami 1982; Bego 1990; Drumond et al. 1999; Drumond et al. 1996; Bego et al. 1999; but see Chinh et al. 2003).

5.1.1 Pre-provisioning Phase

The arrival of the queen on the brood comb often causes workers to move around rapidly and frequently inspect open, empty cells. The queen appears equally excited and often vibrates her wings (Sakagami and Oniki 1963; Sakagami and Zucchi 1967; Bego 1990; Drumond et al. 1996; Bego et al. 1999), which may help disperse queen pheromone (Sommeijer 1985). In *Friesella schrottkyi* and *Mourella caerulea*, queens dart towards the workers that are inspecting the empty cells (Sakagami et al. 1973; Wittmann et al. 1991), whereas *Melipona quinquefasciata* and *Trigonisca duckei* queens aggressively beat workers waiting near the cell with their antennae and forelegs (Sakagami and Zucchi 1974; Sakagami 1982). A droplet of a yellowish liquid of unknown origin and composition is regurgitated by queens of some species (see also Sect. 4.5) (Sakagami 1982). Sakagami (1982) proposed that this liquid functions as a releaser stimulus for workers that are ready to discharge larval food into empty cells. In most species, but not in the African *Meliponula bocandei* and the Asian *Lepidotrigona hoozana*, workers only start to provision a cell if the queen is waiting next to this cell (Sakagami et al. 1977; Sakagami and Yamane 1987). In *Mourella caerulea*, the initiation of provisioning may require queen aggression towards workers near the cell (Wittmann et al. 1991). The pre-provisioning phase tends to be longer for the first cell to be provisioned after the queen visits the brood comb area (i.e. the first cell of a “batch”, see below) (Sakagami and Zucchi 1967; Sakagami et al. 1973).

5.1.2 Provisioning and Oviposition Phase

The behavioural pattern of the food discharges is similar in several studied species: a worker enters the cell head-first before performing one rapid abdominal contraction (Sakagami and Oniki 1963; Sakagami et al. 1973). Thus, each worker is responsible for 1 discharge. Individual cells are provisioned by 2–19 bees, depending on the species (Table 5.1) and until the cell is filled to about 2/3 of its capacity (Fig. 5.1a). In *Leurotrigona muelleri*, cells are almost always provisioned by just two workers, which is the lowest recorded number of food discharges (Sakagami and Zucchi 1974). After a discharge, the worker often rapidly retreats from the cell

Table 5.1 The number of discharges per cell, estimated daily egg-laying rate of the queen (corresponds also to the cell-building rate) and colony size

Species	Larval food discharges	Queen egg-laying rate	Colony size	References
Austroplebeia australis		40–50	1000–5000	Drumond et al. (1999)
Austroplebeia cassiae		40–50	1000–5000	Drumond et al. (1999)
Friesella schrottkyi	3–5	40–65	300–2500	Sakagami et al. (1973)
Frieseomelitta nigra	2–5			Sommeijer et al. (1984)
Hypotrigona gribodoi	4–6	20–80	100–750	Bassindale (1955), Darchen (1972)
Lestrimelitta limao		600–740	2000–7000	Wittmann et al. (1991)
Leurotrigona muelleri	2	Up to 100	500–1000	Sakagami and Zucchi (1974)
Melipona favosa		Up to 50	150–3000	Sakagami and Oniki (1963), Chinh et al. (2003)
Melipona beecheii	6–19			van Veen (2000)
Melipona compressipes	6–15			Sakagami and Oniki (1963)
Meliponula bocandei	4–9			Sakagami et al. (1977)
Mourella caerulea	5–7			Wittmann et al. (1991)
Plebeia droryana	4–7	~80	2000–3000	Drumond et al. (1996)
Plebeia julianii	4–12	13–30	300	Drumond et al. (1998)
Plebeia remota	4–12	Up to 220	2000–5000	van Benthem et al. (1995)
Tetragona clavipes		150–500	7000–29,000	Sakagami and Zucchi (1967)
Tetragonisca angustula	4–14			Segers et al. (2015)
Tetragonula carbonaria		~250	10,000	Yamane et al. (1995)
Trigona recursa		~720		Yamane et al. (1995)
Trigonisca duckei	4–6			Sakagami and Zucchi (1974)

("post-discharge retreat") (Sakagami and Zucchi 1974; Sakagami et al. 1977; Sommeijer et al. 1984; Yamane et al. 1995; Drumond et al. 1999). At least in some species, the discharging workers receive the larval food from other workers

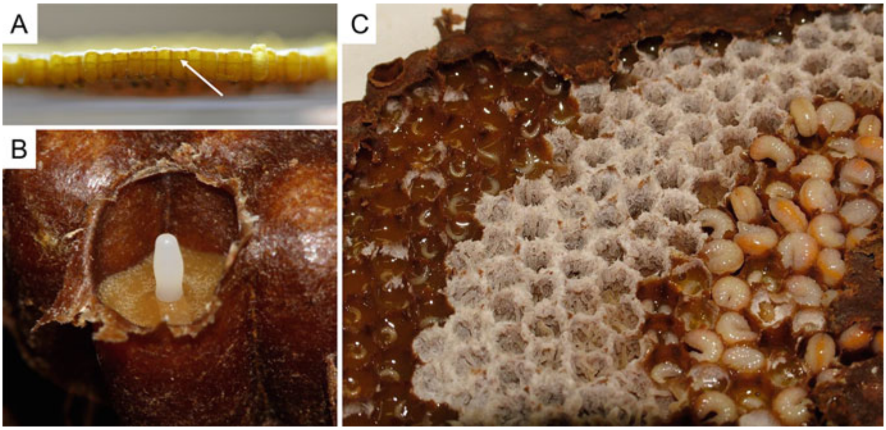

Fig. 5.1 (**a**) A *Tetragonisca angustula* comb showing cells filled to about 2/3 with larval food (white arrow). One cell is still open, showing the typical rim of wax, called collar (photo by C. Grüter). (**b**) Reproductive egg on larval food in *Melipona fasciculata* (photo by Cristiano Menezes). (**c**) Fungus growing in *Scaptotrigona depilis* cells. This fungus is eaten by the larvae. The age and size of the larvae increase from left to right. The oldest larvae have already eaten all the fungus (Photo by Cristiano Menezes)

through trophallaxis rather than preparing the larval food themselves (Sommeijer et al. 1984; Cristiano Menezes, personal communication).

In *Tetragonisca angustula*, the cells in the centre of the brood comb receive a larger number of discharges than cells in the periphery, most likely because central cells are slightly larger (Segers et al. 2015). The larger soldiers emerge from these cells (see Chap. 6). In *Geotrigona mumbuca*, more discharges per cell were observed in weak colonies compared to strong colonies even though cells in weak colonies were smaller and smaller workers emerged from the cells (Lacerda et al. 1991). It is, thus, likely that individual workers of weak colonies discharged smaller amounts of food into cells.[2]

Before oviposition, the queen shortly inspects the cell by inserting her antennae (Sakagami and Oniki 1963; Sakagami et al. 1973; Sakagami and Zucchi 1974; Drumond et al. 1996).[3] In many species, the queen eats from the larval food before oviposition (Sakagami and Oniki 1963; Sakagami et al. 1977; Sommeijer 1985; Wittmann et al. 1991; Drumond et al. 1996). These meals in combination with trophic eggs (see below) are probably the main sources of energy for queens since direct food delivery by workers through trophallaxis is rare in most stingless bees (with the exception of *Lepidotrigona hoozana* where queens are frequently fed by

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[2] The positive relationship between colony size and worker size in stingless bees (Segers et al. 2016) suggests that worker population is an important factor in determining the size of cells, amount of provisioned larval food and worker size.

[3] *Plebeia remota* and *P. julianii* queens are exceptions in that the queen does not usually inspect the cell before oviposition (van Benthem et al. 1995; Drumond et al. 1998).

workers) (Sakagami et al. 1977; Sakagami and Yamane 1987; Wittmann et al. 1991; Yamane et al. 1995; Drumond et al. 1998). Oviposition is immediately followed by cell capping (Sakagami et al. 1973).

A distinction is often made depending on whether provisioning and oviposition occur in batches or in a more continuous fashion (Sakagami and Zucchi 1974). A batch refers to a group of cells that are provisioned and then oviposited before the queen leaves the brood area. In *Scaptotrigona postica*, for instance, up to 30 cells are provisioned and then oviposited by the queen in a relatively short time period (Bego 1990, for batch sizes in other species, see Sakagami and Zucchi 1967, 1974; Sakagami et al. 1973; Sakagami and Yamane 1987; Bego et al. 1999; Segers et al. 2015). In some species, multiple cells are filled simultaneously (e.g. *Frieseomelitta nigra*, *Plebeia droryana*, *P. remota*, *Scaptotrigona postica, Tetragonisca angustula* or *Tetragonula carbonaria*), whereas in others, the cells of a batch are filled one after the other (e.g. *Tetragona clavipes* or *Cephalotrigona*) (Sakagami and Zucchi 1967; Sommeijer et al. 1984; Sommeijer 1985; Yamane et al. 1995; Drumond et al. 1996; Zucchi et al. 1999; Segers et al. 2015). There is evidence that batch size and the time interval between batches are linked to colony size and food availability (Sakagami and Zucchi 1967). For example, van Benthem et al. (1995) found that batch size is larger in stronger colonies. In *Melipona*, a genus with predominantly small colony sizes (Table 1.3) (Michener 1974; Roubik 1983) and *Meliponula bocandei*, POP does not occur in batches, but each cell is provisioned and oviposited sequentially (Sakagami and Oniki 1963; Sakagami and Zucchi 1967; Sakagami et al. 1977; Sommeijer 1985). The distinction between batched POP and successive POP is not always clear (e.g. *Melipona quadrifasciata*) (Sakagami and Zucchi 1974).

Overall, queens lay from just a few eggs per day in species with small colonies to several hundred eggs in species with larger colonies (Table 5.1). As was the case with oviposition, cell building can either be successive (one after the other, e.g. in *Hypotrigona gribodoi* or *Schwarziana quadripunctata*) or simultaneous (e.g. in *Lepidotrigona ventralis* or *Plebeia remota*) (Sakagami 1982). When conditions are not favourable, e.g. during periods of food shortages, workers may open cells and consume the content (Sakagami and Zucchi 1974; Roubik 1982).

Queen-Worker Interactions

In several species (e.g. *Austroplebeia* spp. *Melipona* spp., *Plebeia droryana, Scaptotrigona postica* or *Tetragonisca angustula*), the workers surrounding a cell that is to be provisioned or just has been provisioned perform “darting” or “rocking” behaviours towards the queen, which may lead to further queen agitation, escape or aggression (Sakagami and Oniki 1963; Bego 1990; Bego et al. 1999; Drumond et al. 1996, 1999, 2000). Darting at the queen is occasionally also observed outside the POP context, e.g. in *Meliponula bocandei* (Sakagami et al. 1977). In *Trigonisca duckei*, workers bend their body to present their thorax to the queen, a behaviour interpreted as a ritualised “barricading” of the cell against the approaching queen (Sakagami and Zucchi 1974). Likewise, *Plebeia remota* workers appear to “defend” the cell against the queen before oviposition (van Benthem et al. 1995). *Plebeia droryana* workers perform a behaviour that has been called “hypnotic turning”

(Sakagami 1982; Drumond et al. 1996). In *Lepidotrigona hoozana*, it is the queen that performs a distinctive turning behaviour during the POP (Sakagami and Yamane 1987).

Several authors have proposed that the ritualised and seemingly aggressive queen-worker interactions during the POP are behavioural expressions of a conflict between an ancestral instinct of workers to prevent the queen from laying an egg in an empty cell and a more derived queen-avoidance behaviour, which is also often observed when workers encounter a roaming queen or following a food discharge (Sakagami et al. 1973, 1977; Sakagami and Zucchi 1974; Sakagami 1982; Sommeijer 1985; Drumond et al. 1996, 2000).[4] In other words, the interactions may be the manifestations of an ancient (and ongoing) conflict over who produces the males (see also Sect. 5.8) (Hamilton 1972; Crespi 1992; Drumond et al. 1999; Grüter 2018). Likewise, Crespi (1992) and Oldroyd and Pratt (2015) argued that aggression and ritualised interactions between workers and the queen are an ancestral trait found in species where workers can reproduce. It should be noted, however, that several species with substantial worker reproduction, e.g. *Melipona favosa* (see below), show little aggression during interactions (Chinh et al. 2003; Velthuis et al. 2005). In *Frieseomelitta paupera*, on the other hand, workers perform ritualised rocking and darting towards the queen even though workers are sterile (Sommeijer et al. 1984). Thus, there does not seem to be a straightforward link between worker reproduction and the presence of conspicuous queen-worker interactions, but further comparative analyses are needed to explore associations between reproductive conflicts (Sect. 5.8) and ritualised interactions.

In queenless colonies, the POP still occurs but is often more irregular and uncoordinated than in the presence of the queen, which highlights that the queen plays an important role as pacemaker during the POP (Sakagami 1982; Engels and Imperatriz-Fonseca 1990).

Larval Food Composition

The semi-liquid larval food mainly consists of honey, pollen, hypopharyngeal and labial gland secretions (Quezada-Euán et al. 2011). In some species, glandular secretions do not seem to be added in large quantities (Quezada-Euán et al. 2011), which is different from honey bees, where glandular secretions make up a large part of the larval food (Winston 1987). Another difference is a lower water content (40–60%) in stingless bee larval food compared to honeybees (60–70%), while the quantities of sugars (5–20%) and free amino-acids (0.2–1.3%) are similar (Hartfelder and Engels 1989; Velthuis et al. 2003; Wang et al. 2016). Pollen grains represent the main source of protein (Velthuis et al. 2003; Menezes et al. 2007). The low water content of stingless bee larval food could be explained by the need to produce a highly viscous liquid that prevents eggs and larvae from submerging and allows them to float on top of the larval food (Fig. 5.1b) (Hartfelder and Engels 1989;

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[4] A royal court is often formed when the queen stands still for some time or when she arrives at a cell before oviposition (e.g. Sakagami 1982; Yamane et al. 1995).

Velthuis and Velthuis 1998).[5] The composition of larval food can change with the season, especially with respect to the protein content (Quezada-Euán et al. 2011), which affects the size of the emerging workers (Quezada-Euán et al. 2011).

In *Scaptotrigona depilis*, larvae also consume a fungus (genus *Zygosaccharomyces*) that grows on the internal walls of the brood cells and on the surface of the larval food (Fig. 5.1c) (Menezes et al. 2015; Paludo et al. 2018). Larvae reared without the fungus die. The fungus seems to provide steroid precursors that are required for metamorphosis and which the bees of this species cannot synthesise de novo (Paludo et al. 2018). The larvae do not depend on the nutrients provided by the fungus. It is not clear how this bee-fungus relationship evolved, but it is possible that, initially, the fungus may have functioned as a nutritional or hormonal supplement. Fungus growth inside brood cells was also observed in *Tetragona clavipes* and *Melipona flavolineata* (Menezes et al. 2013), two phylogenetically distant species. These observations suggest that the bee-fungus mutualism might be more common in stingless bees than is currently known.

5.2 Worker-Laid Eggs

Active ovaries and frequent oviposition by workers are a notable difference between many stingless bees and other social insects where queen presence induces sterility in workers (Hoover et al. 2003; Matsuura et al. 2010; van Oystaeyen et al. 2014; Oi et al. 2015; Grüter and Keller 2016; Grüter 2018).[6] At the end of larval food provisioning, a worker can occasionally be seen laying an egg on the inside of the collar of the provisioned cell or directly on the larval food (Bassindale 1955; Sakagami and Oniki 1963; Beig 1972; Sakagami and Yamane 1987; Wittmann et al. 1991; Bego et al. 1999; Koedam et al. 1996; Segers et al. 2015). In some *Plebeia* species, workers may lay eggs anywhere on the comb and often before provisioning, but stimulated by the proximity of the queen (Drumond et al. 1996, 1998, 2000). The timing and the different locations of worker oviposition raise the question whether these worker-laid eggs are little packages of energy to feed the queen or whether they represent attempts by workers to produce males.[7]

In a pioneering study, Beig (1972) observed that in *Scaptotrigona postica*, many cells contained more than one egg in a period when males were abundant in the colony. Young bees often had developed ovaries and were seen laying two different

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[5] Additionally, the biophysical properties of the egg surface help eggs to stay in position (Velthuis and Velthuis 1998).

[6] Silva-Matos et al. (2006) found that in a *Trigona cilipes* colony, workers had developed ovaries and mimicked oviposition behaviour, but never laid eggs. Whether this a common condition in this species, e.g. with some communicative function or as a sign of an ongoing conflict between the queen and the workers, is not known.

[7] Due to the haplodiploid system of sex determination in the Hymenoptera, stingless bee workers can produce haploid male eggs, but are unable to produce diploid females because they cannot mate with a male (see Chap. 1).

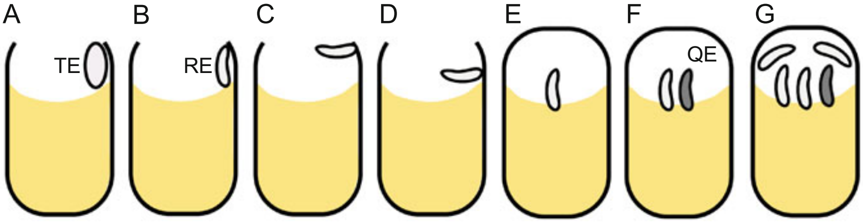

Fig. 5.2 Different types of worker oviposition in *Scaptotrigona postica*. (**a**) Normal position of trophic eggs (TE). (**b**) Position of reproductive eggs (RE) when laid to feed queen. (**c**, **d**) Alternative positions of worker laid eggs. (**e**) Brood cell being closed before or (**f**) after queen oviposition (QE = queen egg). (**g**) Several reproductive eggs laid by workers after queen oviposition (modified from Bego 1990)

types of eggs, both larger than female eggs: (1) elongated male producing eggs were laid on the larval food shortly after queen oviposition and before cell capping and, more commonly, (2) more spherical eggs were laid on the inner upper edge of the cell and served as food for the queen, i.e. trophic eggs (Fig. 5.2). Reproductive eggs were mainly produced by young workers, whereas trophic eggs were also laid by middle-aged bees (Bego et al. 1983; Bego 1990). Dissection showed that workers had fully developed ovaries from day ~10 to ~25. Later in life, i.e. during the foraging phase, ovaries degenerate (Bego et al. 1983). In *Melipona subnitida*, workers laid trophic and reproductive eggs at the same average age (~15 days), but they started to lay reproductive eggs at a younger age (Koedam et al. 1999).[8]

Workers of several other species lay both reproductive and trophic eggs (Table 5.2) and queens (and occasionally workers) may eat both types of eggs (Beig 1972; Sommeijer 1985; Bego 1990; van Benthem et al. 1995; Koedam et al. 1999, 2001; Velthuis et al. 2002; Tanaka et al. 2009). In other species, e.g. the American *Geotrigona mombuca*, *Tetragonisca angustula* and the Australian *Austroplebeia cassiae* or *A. australis*, workers only lay trophic eggs as long as the queen is present (Table 5.2) (Drumond et al. 1999; Koedam et al. 1996; Bego et al. 1999; Lacerda and Zucchi 1999; Grosso et al. 2000). Queen signals are likely to induce worker sterility in these cases (Nunes et al. 2014). In the absence of a queen, however, workers start to lay reproductive eggs (Sakagami et al. 1973; Koedam et al. 1996; Drumond et al. 1999; Nunes et al. 2014). *Geotrigona mombuca* is an exception as workers continue to lay only trophic eggs even when the queen is lost (Lacerda and Zucchi 1999).

Finally, there are species like the American *Duckeola ghilianii*, *Frieseomelitta* spp., *Leurotrigona muelleri, Trigonisca duckei* or the Asian *Tetragonula minangkabau*, where workers do not lay any type of egg when an egg-laying

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[8] In *Melipona bicolor*, 30–40% of all workers were laying eggs and workers laying reproductive eggs were more involved in the POP than non-reproductive workers or workers laying trophic eggs (Cepeda 2006; Koedam and Imperatriz-Fonseca 2012). A similar proportion (~35%) of workers involved in the POP had activated ovaries in *Tetragonisca angustula*, a species where workers only lay trophic eggs under queenright conditions (Koedam et al. 1997).

Table 5.2 List of species with and without worker reproduction under queenright conditions

Condition	References
Workers never lay eggs	
Duckeola ghilianii	Sakagami (1982)
Frieseomelitta languida	Engels and Imperatriz-Fonseca (1990)
Frieseomelitta paupera	Sommeijer et al. (1984)
Frieseomelitta silvestrii	Cruz-Landim (2000)
Frieseomelitta varia	Boleli et al. (1999)
Tetragonula carbonaria	Gloag et al. (2007)
Tetragonula minangkabau	Suka and Inoue (1993)
Workers mainly lay reproductive eggs when queenless	
Leurotrigona muelleri	Sakagami and Zucchi (1974)
Friesella schrottkyi[a]	Nunes et al. (2014)
Plebeia lucii	Nunes et al. (2017)
Workers commonly lay eggs under queenright conditions	
Workers lay both trophic and reproductive eggs	
Melipona asilvai	Pereira et al. (2006)
Melipona bicolor	Koedam et al. (2001)
Melipona fasciculata	Pereira et al. (2006)
Melipona quadrifasciata	Cruz-Landim (2000)
Melipona subnitida	Hartfelder et al. (2006)
Melipona favosa	Sommeijer et al. (1999)
Melipona scutellaris	Tóth et al. (2002a), Pereira et al. (2006)
Paratrigona subnuda	Tóth et al. (2002a)
Plebeia droryana	Drumond et al. (1996)
Plebeia remota	van Benthem et al. (1995)
Scaptotrigona postica	Beig (1972)
Scaura sp.	Sakagami (1982)
Workers mainly lay reproductive eggs	
Friesella schrottkyi[a]	Imperatriz-Fonseca and Kleinert (1998)
Workers mainly lay trophic eggs	
Austroplebeia australis	Drumond et al. (1999)
Austroplebeia cassiae	Drumond et al. (1999)
Geotrigona mombuca[b]	Lacerda and Zucchi (1999)
Hypotrigona gribodoi	Sakagami (1982)
Lestrimelitta sp.	Sakagami (1982)
Plebeia minima	Drumond et al. (2000)
Plebeia saiqui	Tóth et al. (2004), Drumond et al. (2000)
Tetragonisca angustula	Grosso et al. (2000)
Schwarziana quadripunctata	Tóth et al. (2003)

[a] Low rate of reproductive egg laying under queenright conditions
[b] This species also only lays trophic eggs when queenless

queen is present in the colony (Table 5.2) (Sakagami et al. 1973; Sakagami and Zucchi 1974; Sommeijer et al. 1984; Suka and Inoue 1993), but some workers may activate their ovaries when the colony has become queenless (e.g. *Friesella schrottkyi*, *Leurotrigona muelleri*) (Tóth et al. 2004; Nunes et al. 2014). Workers in other species are incapable of laying eggs (Table 5.2) (Sakagami et al. 1964, 1973; Sakagami and Zucchi 1974; Suka and Inoue 1993; Cruz-Landim 2000). In *Frieseomelitta varia* and *F. silvestrii*, for instance, ovaries are reabsorbed during the larval stage (Boleli et al. 1999; Cruz-Landim 2000; but see Luna-Lucena et al. 2018).

Reproductive eggs laid either after or before queen oviposition are often produced by the worker that also seals the cell (Beig 1972; Koedam 1999; Koedam et al. 2001, 2005; Velthuis et al. 2005; Koedam and Imperatriz-Fonseca 2012). In *Melipona bicolor*, several reproductive workers may aggressively compete to gain access to an empty brood cell (Velthuis et al. 2002). Multiple workers laying one egg each in a cell has also been observed in some species (*Hypotrigona gribodoi* or *Melipona compressipes*) (Bassindale 1955; Sakagami and Oniki 1963) and Nogueira-Neto (1963) observed cells with more than one larva in *Melipona quadrifasciata*, resulting in competition among larvae. Beig (1972) found that in cells with both male and female larvae, the male larva is usually larger, more mobile and eventually kills his companion.

Workers sometimes reopen cells after the POP to eat the queen-laid egg and lay their own egg on top of the larval food (Imperatriz-Fonseca and Kleinert 1998; Tóth et al. 2002a; Velthuis et al. 2002, 2005; Koedam et al. 2007; Koedam and Imperatriz-Fonseca 2012). Queens encountering such workers may try to push them away from the cell, but this does not seem to stop workers from returning to the cell later to lay their eggs (Tóth et al. 2002a). In *Melipona trinitatis*, on the other hand, a queen was observed that opened a cell with her mandibles and ate a worker-laid egg (Sommeijer et al. 1984), but this kind of post-POP queen policing does not seem to be common. Indeed, the immediate cell capping probably makes it difficult for the queen or other workers to recognise and eat worker-laid reproductive eggs (i.e. queen or worker policing, which is common in many other social insects; see Sect. 5.6). Nonetheless, these behaviours show that there are conflicts between the queen and workers and among workers when it comes to egg laying (see Sect. 5.6).

Trophic and reproductive eggs usually differ in size, shape and in the surface pattern (trophic eggs lack a reticulate chorion) (Fig. 5.3) (Sakagami 1982; Sommeijer 1985; Bego 1990; Wittmann et al. 1991; Koedam et al. 1996, 1997, 2001; Pereira et al. 2006; Tanaka et al. 2009).[9] Furthermore, they lack a nucleus (Koedam et al. 1996) and either lack or have a highly reduced micropyle, an area of small openings of channels crossing the chorion through which sperm enters the egg (Fig. 5.3e, f) (Koedam et al. 1996).

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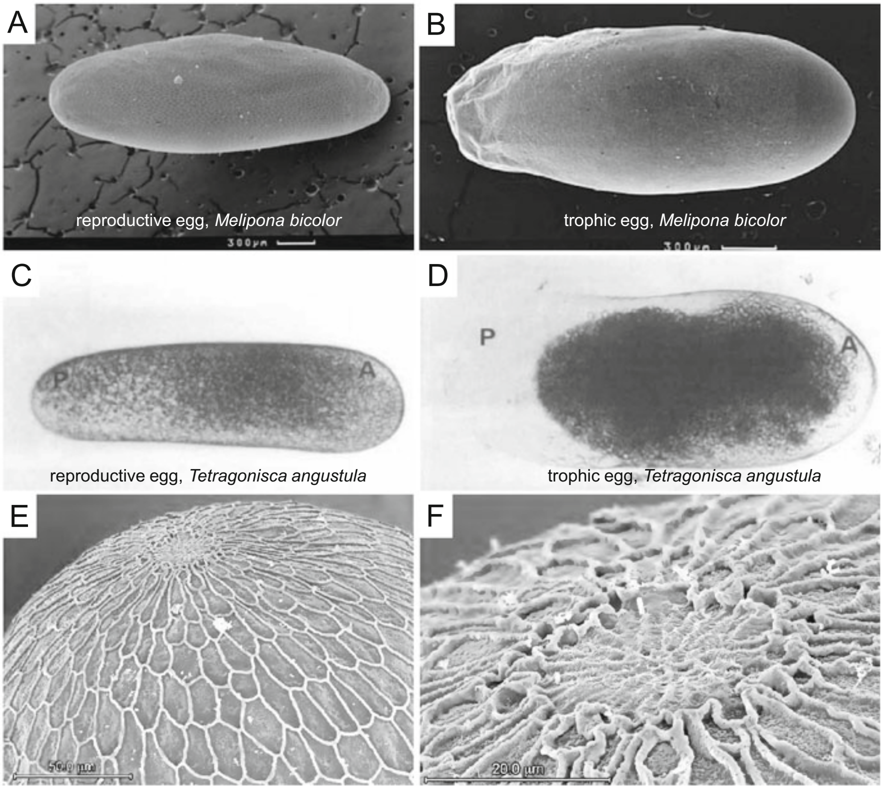
[9] Depending on the species, trophic eggs can be larger (*Scaptotrigona*) or smaller (some *Melipona*) than reproductive eggs (Beig 1972; Koedam et al. 2001; Pereira et al. 2006). In *Melipona*, worker-laid reproductive eggs are smaller than queen-laid reproductive eggs (Pereira et al. 2006).



reproductive egg, *Melipona bicolor*

trophic egg, *Melipona bicolor*

reproductive egg, *Tetragonisca angustula*

trophic egg, *Tetragonisca angustula*

Fig. 5.3 Scanning electron microscope (**a**, **b**) and light microscope (**c**, **d**) images of reproductive (**a**, **c**) and trophic (**b**, **d**) eggs of *Melipona bicolor* and *Tetragonisca angustula*. *P* posterior, *A* anterior. Image (**a**) shows the reticulate patterned chorion, which transitions into a smooth basal apex (from Koedam et al. 1996, 2001). (**e**) SEM image of the reticulate chorion (outermost membrane) of reproductive eggs, here *Scaptotrigona pectoralis* (from Rozen et al. 2019). (**f**) Close-up SEM image of the micropyle (from Rozen et al. 2019)

Trophic eggs are rich in lipids and proteins, which makes them a nutritious food source for the queen (Hartfelder et al. 2006; Tanaka et al. 2009; Luna-Lucena et al. 2018). An alternative or additional function of trophic eggs could be that they keep worker ovaries functional, which could be beneficial for workers that are capable of laying reproductive eggs should the queen die (West-Eberhard 1981). It is also noteworthy that trophic eggs are usually laid into open cells and during the POP. Were they simply food for the queen or had the purpose of maintaining ovarian function, we might expect workers to lay them outside the context of the POP and in other locations whenever workers encounter the queen. It has been argued that trophic eggs are a leftover of an ancient conflict about who lays the eggs, which was won by the queen in the ancestors of some species (Peters et al. 1999). However, a recent ancestral state estimation found that trophic eggs are more likely to represent the original condition in stingless bees, whereas the laying of reproductive eggs in

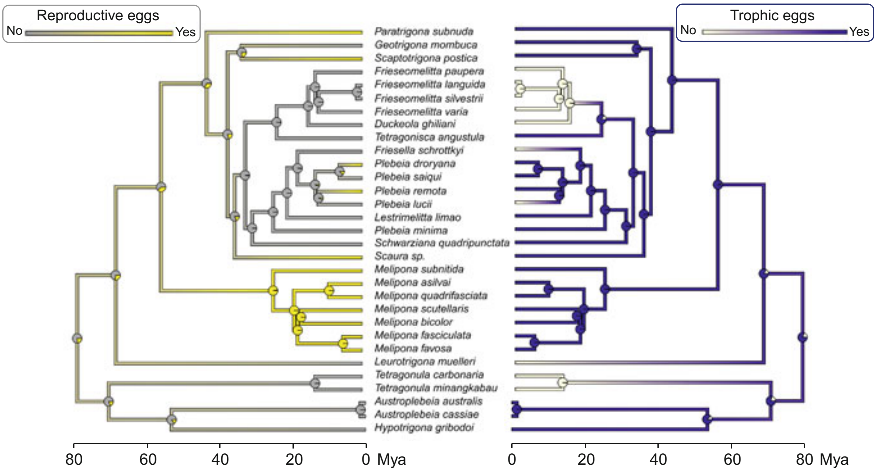
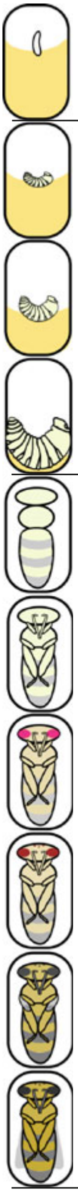


Fig. 5.4 Ancestral state estimation for the laying of reproductive eggs and trophic eggs in queenright conditions. The colour of branches is based on an MCMC approach to estimate the ancestral condition. Pie charts provide estimates based on continuous-time Markov chain models (see Grüter 2018 for details)

queenright condition appeared later in time (Fig. 5.4) (Grüter 2018). There seems to be a strong link between trophic and reproductive eggs in that the laying of reproductive eggs in queen presence is mostly found in species that also lay trophic eggs (Table 5.2). Thus, worker oviposition may have had an altruistic function in stingless bee ancestors, but this condition may then have facilitated the evolution of selfish worker reproduction in some species (Grüter 2018). This would not exclude the possibility that trophic eggs derived from reproductive eggs in a bee species that predated stingless bees, perhaps in a common ancestor of the Meliponini and the Melikertini, the extinct eusocial relatives of the stingless bees (see Chap. 2).

5.3 Developmental Stages of Brood

Brood development is usually divided into ten stages: egg, three larval stages, a prepupal stage and five pupal stages (Fig. 5.5) (Nates-Parra et al. 1989; Salmah et al. 1996; Moo-Valle et al. 2004; Amaral et al. 2010; Cardoso-Júnior et al. 2017). The three larval stages differ in size and colouration and correspond to three different instars (Nates-Parra et al. 1989; Salmah et al. 1987, 1996; Amaral et al. 2010). The final larval stage is characterised by the presence of dorsolateral pairs of conical tubercles on the thoracic segments and some abdominal segments (Rozen et al. 2019). Amaral et al. (2010) have further divided the third instar into five phases that are characterised by the consistency of the larval food and the presence of faeces. During the prepupal stage, the head, thorax and abdomen become visible, but without visible legs (Salmah et al. 1987, 1996). This is followed by the white-eyed



Stage	M	Q	W
Egg	8	8	8 (5.5)
Larva 1	5	5	5 (1)
Larva 2	4	4	4 (2)
Larva 3	10	8	9 (7)
Prepupa	2	2	2 (1)
Pupa1	3	3	3 (5.3)
Pupa 2	8	6	8 (15.7)
Pupa 3	6	4	6 (4)
Pupa 4	3	4	3 (3)
Pupa 5	6	7	5 (2)
Total duration:	53.5	50.8	52.7 (46.5)

Fig. 5.5 Developmental stages of stingless bee brood. The durations in days are given for males (M), queens (Q) and workers (W) in the American species *Melipona beecheii* (Moo-Valle et al. 2004). Values in brackets are for workers in the Asian *Sundatrigona moorei* (Salmah et al. 1987). See also Salmah et al. (1996) for data on other species

pupa with visible legs and antennae (pupa 1), the pink-eyed pupa (pupa 2), the brown-eyed pupa with pigmented ocelli (pupa 3), the brown-eyed pupa with pigmented wing pads and folded wings (pupa 4) and, finally, the brown-eyed pupa with a more pigmented body and extended wings (pupa 5). The duration of the different stages has only been measured for a small number of species (Fig. 5.5) (Nates-Parra et al. 1989; Salmah et al. 1987, 1996; Moo-Valle et al. 2004).

5.4 Brood Production

There is often a degree of seasonality in brood production, which is linked to the availability of food sources or the amount of stored food (Terada et al. 1975; Roubik 1982; Bego 1990; van Benthem et al. 1995; Ribeiro et al. 2003a; van Veen et al. 2004; Chinh and Sommeijer 2005; Alves et al. 2009a; Halcroft et al. 2013; Prato and Soares 2013; Maia-Silva et al. 2016). Species that experience cool winters or extreme seasonality in rainfall may stop rearing brood altogether during the coldest or wettest/driest periods of the year (Santos et al. 2014, 2015). This reproductive diapause occurs in several *Plebeia* and *Melipona* species in the Neotropics and in *Lepidotrigona hoozana* in Taiwan (Terada et al. 1975; van Benthem et al. 1995; Ribeiro et al. 2003a; Borges and Blochtein 2006; Sung et al. 2008; Alves et al. 2009a; Nascimento and Nascimento 2012; Santos et al. 2014; Ferreira-Caliman et al. 2017). Reproductive diapause usually lasts 1–4 months, but can last up to 5–-6 months (e.g. in *Plebeia saiqui* and *Melipona marginata*) (Pick and Blochtein 2002; Borges and Blochtein 2006). Before diapause starts, workers often reduce their cell-building rate and increase the strength of the involucrum by adding additional layers (see also Chap. 3) (Borges and Blochtein 2006; Santos et al. 2014). Foraging for pollen is reduced (Pick and Blochtein 2002), workers have an increased lifespan (van Benthem et al. 1995), and, in *P. remota*, queens were observed to lose weight and show a lesser degree of physogastry during reproductive diapause (Ribeiro et al. 2003a).

5.4.1 Worker Production

Between 65% and 99% of all reared brood is worker brood (Table 5.3). Worker rearing correlates positively with food stores, pollen in particular (Roubik 1982; Chinh and Sommeijer 2005; Maia-Silva et al. 2016), colony size and foraging conditions (Santos-Filho et al. 2006; Prato and Soares 2013; Maia-Silva et al. 2015).[10] Worker brood requires from ~35 to ~55 days from oviposition to emergence of the adult bee (Fig. 5.5, Table 5.4). This duration does not seem to depend on the size of the bee (Fig. 5.6a). Thus, worker production in stingless bees requires, on average, about twice as much time as in temperate honeybees (~21 days) (Winston 1987). One reason could be that honeybees are better at maintaining high and constant temperatures in the brood area, at least compared to some stingless bee

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[10] Workers occasionally cannibalise existing young brood and their provisions if food stores are depleted (Roubik 1982).

Table 5.3 The proportion of queens, workers and males reared in stingless bee species

Species	Queens (%)	Workers (%)	Males (%)	Queen cell?	References
Apotrigona nebulata	~0	89.5	10.5		Darchen (1969)
Lepidotrigona ventralis	0.08	96.8	3.1	Yes	Chinh and Sommeijer (2005)
Lisotrigona carpenteri	7.90			Yes	Chinh et al. (2005)
Melipona asilvai	6.2	76.2	17.6	No	Santos-Filho et al. (2006)
Melipona beecheii	5.3–11.3	65.8–89	5.7–22.9	No	Moo-Valle et al. (2001), van Veen et al. (2004)
Melipona bicolor	6.2	87.6	6.2	No	Santos-Filho et al. (2006)
Melipona fasciata[a]	10.30	79.30	10.40	No	Kerr (1948)
Melipona favosa	4.2–7.7	71–78.5	17	No	Koedam (1999), Sommeijer et al. (2003)
Melipona flavipennis[b]	5.10	75.50	19.40	No	Kerr (1951)
Melipona interrupta[b]	13.70	86.30	0	No	Kerr (1951)
Melipona marginata	19.1	68.20	12.70	No	Kerr (1948)
Melipona melanoventer[b]	6.30	62.10	31.60	No	Kerr (1951)
Melipona quadrifasciata	10.30	78.2	11.50	No	Kerr (1951)
Melipona subnitida	7.8	85.1	7.1	No	Santos-Filho et al. (2006)
Melipona trinitatis	5.20	87.10	7.70	No	Sommeijer et al. (2003)
Nannotrigona testaceicornis	0.01	98.3	1.7	Yes[c]	Eterovic et al. (2009)
Plebeia remota	0.1	73.4	26.5	Yes[c]	Alves et al. (2009a)
Schwarziana quadripunctata	0.60	81.20	18.2	Yes[c]	Wenseleers et al. (2005), Santos-Filho et al. (2006)
Tetragonisca angustula	0.16	86.7	13.1	Yes	Prato and Soares (2013)
Trichotrigona extranea			0–18		Camargo and Pedro (2007)

[a] Uncertain identity, possibly *Melipona rufiventris* according to Camargo and Pedro (2013)
[b] Low sample size
[c] Species with miniature queens

Table 5.4 Developmental times (i.e. the duration from oviposition to emergence) of workers, males and queens in different stingless bee species

Species	Developmental time (days)			Average worker lifespan (days)	References
	Workers	Males	Queens		
Austroplebeia australis	~55				Halcroft et al. (2013)
Friesella schrottkyi	35–36		39–48	30.1	Sakagami (1982), Giannini (1997)
Frieseomelitta varia			25		Faustino et al. (2002)
Geotrigona mombuca	52 ± 1.5				Lacerda et al. (1991)
Heterotrigona itama	46.5				Salmah et al. (1996)
Hypotrigona gribodoi	~35	~32			Bassindale (1955)
Melipona beecheii	52 ± 1.3	53.4 ± 1.12	50.8 ± 1.5	51	Biesmeijer and Tóth (1998), Moo-Valle et al. (2004)
Melipona quadrifasciata	34–37		27–31		Salmah et al. (1987), da Silva et al. (1972)
Melipona scutellaris	49			43.8	Moo-Valle et al. (2004), Santos (2013)
Nannotrigona perilampoides	36–38				Quezada-Euán et al. (2011)
Plebeia remota	40–45	40–45	48–54	67.7	van Benthem et al. (1995), Grosso and Bego (2002)
Scaptotrigona depilis	~35				Vollet-Neto et al. (2017)
Sundatrigona moorei	46.5				Salmah et al. (1987)
Tetragonisca angustula	33.5–40.3			27	Nates-Parra et al. (1989), Hammel et al. (2016)
Tetragonula laeviceps	~50			45	Inoue et al. (1984)
Tetragonula minangkabau	~42			37	Salmah et al. (1987), Inoue et al. (1996)

Average worker lifespan is provided in days, see Table 1.1

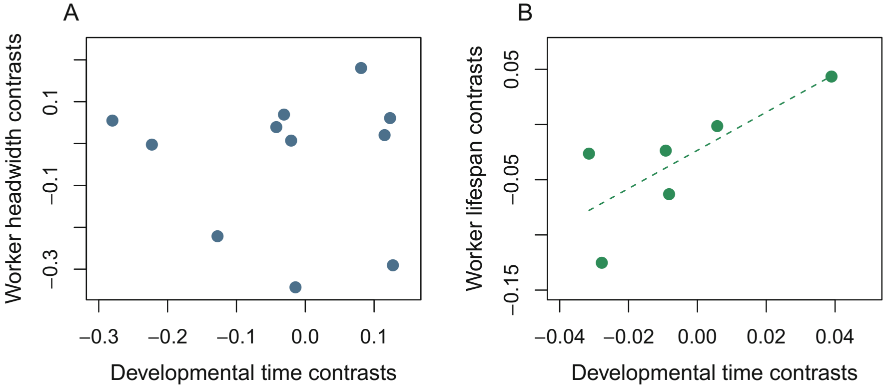

Fig. 5.6 Relationship between the developmental time (from oviposition to eclosion) of workers and average worker size in 12 species (**a**) (size data mainly from Grüter et al. 2017) and average worker lifespan of 7 species (**b**) (data from Table 5.4) is shown. Phylogenetically independent contrasts and linear regression through the origin were used (Felsenstein 1985; Garland et al. 1992). Phylogenetic relationships and branch lengths were based on a published phylogenetic tree (Rasmussen and Cameron 2010). There was no relationship between developmental time and worker size (*t*-value = 0.02; $p = 0.98$), but longer developmental times were associated with longer worker lifespans (*t*-value = 2.83, $p = 0.048$) (data and tree can be found here: www.socialinsect-research.com/book.php). The latter relationship could be driven by various ecological factors, e.g. climate

species (Chap. 3). A reduced capacity to maintain constant nest temperatures could explain why the developmental times are also more variable in stingless bees than in honeybees. For example, van Veen (2000) observed that in Costa Rica, *Melipona beecheii* workers emerged after 43 days, whereas bees required more than 50 days for development in a Mexico population (Moo-Valle et al. 2004). Another reason for the longer developmental times could be that stingless bee larval food is more difficult to digest for larvae due to the higher pollen content and a lower proportion of glandular secretions (Moo-Valle et al. 2004). This, however, might not explain why also the pupal stages take longer in stingless bees compared to honeybees.

Stingless bee workers tend to live longer than honeybee workers (Tables 1.1 and 5.4), and a longer developmental time might be required to produce longer-lived worker bees. There is some support for this based on data from seven species that shows a positive correlation between developmental time and worker lifespan (Fig. 5.6b). In *Tetragonisca angustula*, development time also depends on the distance from the centre of the comb (Nates-Parra et al. 1989), which could be related to the fact that food amount and bee size decrease with increasing distance from the comb centre (Segers et al. 2015).

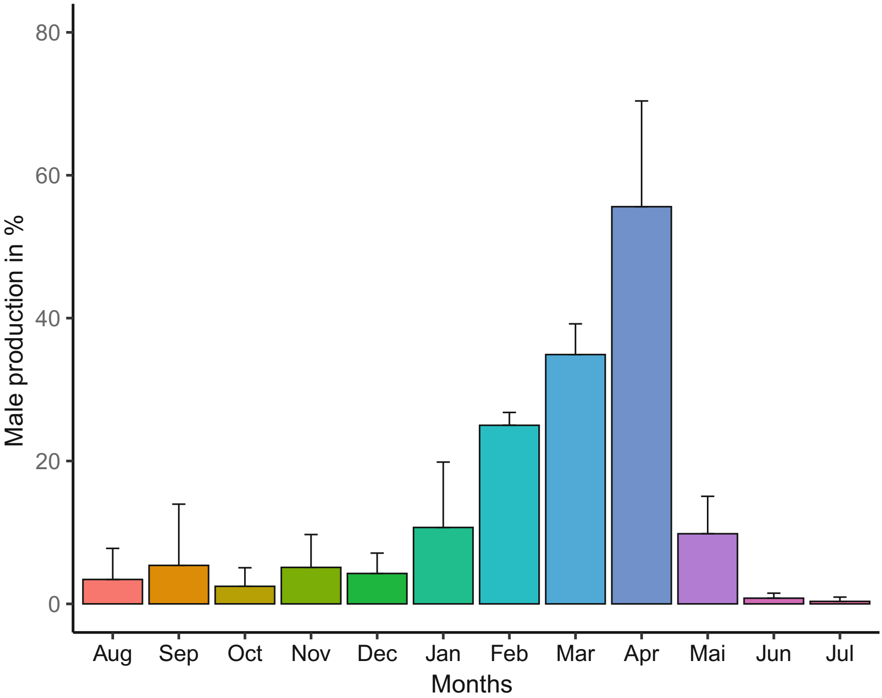

Fig. 5.7 Male production in three queenright colonies of *Tetragonisca angustula* in São Paulo state between August 2008 and July 2009 (mean ± SD; data from Prato and Soares 2013)

5.4.2 Male Production

Between ~1% and ~30% of all brood is male (Table 5.3). The developmental time of males is similar to that of workers (Table 5.4), which is to be expected since males and workers are reared in identical cells and with a similar amount of food.[11]

There is seasonal variation in male production, which is related to extrinsic factors such as rainfall, temperature and food availability and intrinsic factors such as colony size and food storage (see Chap. 4) (Kerr 1948; Bego 1990; van Veen et al. 2004; Chinh and Sommeijer 2005; Koedam et al. 2005; Velthuis et al. 2005; Prato and Soares 2013; Becker et al. 2018). In *Tetragonisca angustula* colonies in southern Brazil, male production is seasonal, with most males emerging in late summer, towards the end of the wettest and hottest period in this area (Fig. 5.7) (Prato and Soares 2013). In *Lepidotrigona ventralis*, more males were produced during the rainy season when colonies had more food stores (Chinh and Sommeijer 2005), and in *Scaptotrigona postica*, male production coincided with periods of

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[11] *Scaptotrigona postica* cells containing worker laid male eggs contained less larval food than cells containing queen-laid male eggs (37.3 μl vs. 38.9 μl) (Beig et al. 1982). The difference was small, but statistically significant.

Fig. 5.8 The distribution of castes and sexes in a comb of *Melipona fulva* (from Kerr et al. 1967)

greatest colony productivity (Bego 1990). The idea that food reserves affect male production is further supported by the finding that experimentally manipulating food reserves affects the proportion of males reared in *Melipona beecheii* (Moo-Valle et al. 2001). Adding pollen stimulated the laying of male eggs by both workers and the queen in *M. subnitida* (Koedam et al. 2005). Since also swarming is likely to be promoted by food reserves and colony size (see Chap. 4) (Darchen 1977), we would predict a degree of temporal correlation between male production and the probability of swarming (but see Moo-Valle et al. 2001).

In some species, male production occurs in temporal clusters (male producing periods; MPPs) that are not synchronised among colonies of the same species and area (Chinh et al. 2003; Chinh and Sommeijer 2005; Velthuis et al. 2005; Lacerda et al. 2010). One explanation for these MPPs could be a periodic production of males by workers (Chinh et al. 2003; Koedam et al. 2005). Male brood is distributed throughout the comb in some species (Fig. 5.8) (*Melipona beecheii, Scaptotrigona postica and Tetragonisca angustula*) (Beig 1972; Moo-Valle et al. 2004; Segers et al. 2015), but appears clustered in others, often in the centre of the brood combs (*M. quadrifasciata*, *M. favosa* and *Nannotrigona testaceicornis*) (Sakagami et al. 1965; Koedam 1999; Eterovic et al. 2009). Whether males are clustered or not might depend on whether they are predominantly produced by the queen or by workers. If workers are mainly responsible for producing males, a clustered appearance of male brood can result from workers laying eggs in certain time periods, e.g. in *Melipona favosa*, or because workers prefer to lay eggs in cells of the newest combs (thus, they cluster in the centre of combs), e.g. in *M. subnitida* (Koedam et al. 1999, 2005; Chinh et al. 2003; Moo-Valle et al. 2004).

Colonies that produce more males have less resources and space to produce worker bees, which leads to an energy allocation trade-off (Velthuis et al. 2005). As a result, a negative correlation between male and worker production is often observed (Moo-Valle et al. 2004; Santos-Filho et al. 2006; Prato and Soares 2013). Colonies might shift their brood production more towards males when food sources are plentiful (see above), possibly because under these conditions colonies can afford to have fewer workers and because swarming is likely to be more common. Furthermore, selfish worker reproduction might be less costly for the colony when foraging conditions are favourable (see Sect. 5.8).

Giant Males

Occasionally, males are reared in queen cells.[12] Such giant males have been found in *Friesella schrottkyi* (Camillo 1971), *Geotrigona mombuca*, *Nannotrigona testaceicornis*, *Paratrigona subnuda* (Engels and Imperatriz-Fonseca 1990), *S. postica* (Bego and de Camargo 1984), *Schwarziana quadripunctata* (Santos-Filho et al. 2006) and several *Plebeia* species (Engels and Imperatriz-Fonseca 1990; Alves et al. 2009a). Giant males have longer developmental times and produce more sperm cells (Camillo 1971), but whether they are functional and can fertilise queens is not known.

5.4.3 Queen Production

Even though queens are often produced throughout the year (Chap. 4), some species show seasonality in queen production too. In *Melipona beecheii* colonies in Costa Rica, for example, queens represented ~15% of all brood in June, whereas only ~3% of all produced bees were queens in August (van Veen et al. 2004). Queen production correlated positively with drone production and pollen stores (van Veen et al. 2004; see also Moo-Valle et al. 2001). A similar seasonality was observed in Brazil by Kerr (1948) in different *Melipona* species. In this latter study, queen production was highest between September and April, which coincided with favourable foraging conditions in the study area. More generally, since favourable conditions do seem to favour both the production of males and queens, a positive correlation between male and queen production is expected (e.g. Kerr 1948; Moo-Valle et al. 2001; van Veen et al. 2004).

In most species, queens represent a small proportion of the brood (Table 5.3). As a result, the sex ratio of reproductive individuals is heavily male-biased, as would be expected in species that reproduce by swarming because each swarm (containing only one or a few queens) represents a large investment for the mother colony (Cronin et al. 2013). The major exception is the genus *Melipona*, where about 10% ± 4.5% (mean ± SD, $N = 12$ species) of all brood emerge as queens (Table 5.3). This means that the queen-male ratio is nearly 1 (~13% are males). In non-*Melipona* species, however, only ~0.2%[13] of all bees emerge as queens. The

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[12] Very rarely, queen cells may give rise to giant workers, which is puzzling given that female larvae provided with large amounts of food should become queens (Imperatriz-Fonseca and Zucchi 1995). These workers probably result from developmental mistakes, but they may perform normal worker tasks (Engels and Imperatriz-Fonseca 1990).

[13] *Lisotrigona carpenteri* seems to be an interesting outlier (Chinh et al. 2005), but was not used in this calculation because the proportion of worker brood is not known.

killing of most of the emerging queens by workers (Chap. 4) means that *Melipona* colonies lose ~10% (up to ~25%) of their investment in brood.

Excess queen production in *Melipona* seems highly wasteful, and several explanations for this phenomenon have been proposed. For example, unmated *Melipona* queens sometimes attempt to enter other colonies to take over reproduction, which could increase the reproductive success of mother colonies that produce a larger number of queens (see Chap. 4). However, since most emerging queens are killed by workers, colonies still face a considerable loss of investment (e.g. Wenseleers et al. 2004b; Jarau et al. 2009; Kärcher et al. 2013). It seems more likely that *Melipona* produce excess queens for other reasons and that reproductive parasitism evolved secondarily as an alternative strategy that allows some *Melipona* queens to obtain direct fitness. *Melipona* colonies might produce many queens because this allows workers to constantly compare unmated queens with the physogastric queen and make sure that only high quality queens head the colony (Chap. 4) (Roubik 1989; Imperatriz-Fonseca and Zucchi 1995). This could be particularly important in this genus because *Melipona* colonies only produce "miniature" queens (see below), which might be lower in fertility. We might then expect a negative relationship between queen rearing and the productivity of the current queen as workers should invest less in the rearing of alternative queens if the present queen is particularly productive. However, the correlation between colony strength and queen rearing does not seem to be negative (see also below) and it is unlikely that the benefits of a constant comparison can outweigh the costs of a continuous queen culling.

Excess queens could also be an insurance policy in case the mother queen suddenly dies (Michener 1974). However, other species also keep virgin queens present in their colonies at all times (e.g. in "imprisonment chambers", see Chap. 4), without producing excess queens. A fourth explanation is based on a caste fate conflict: many *Melipona* larvae might become queens because this is in their individual interest and the particular system of queen determination in *Melipona* means that workers cannot prevent them from doing so (see Sect. 5.6 for a more detailed discussion of this hypothesis).

5.5 Queen Determination

Queen determination in social insects usually falls into two categories, environmental and genetic queen determination. In some species, it is a combination of both (Schwander et al. 2010). The major environmental factor is the amount and/or the quality of food, but other factors, such as temperature, are known to be important for queen development. In stingless bees, trophic and genetic factors are also key for queen development (reviewed in Velthuis 1976; Hartfelder et al. 2006). In most species, some larvae receive more food, which triggers a response of the endocrine system that leads to queen development (Hartfelder et al. 2006). In the genus *Melipona*, queen determination most likely depends on a combination of genetic and nutritional factors.

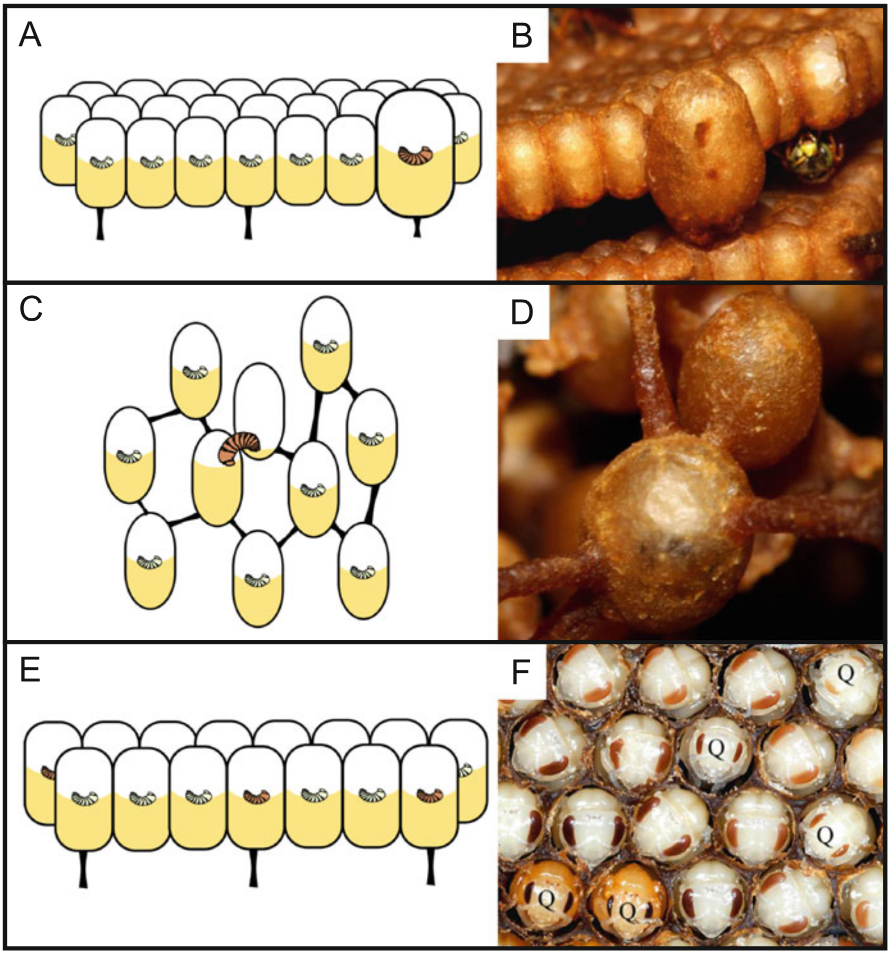

Fig. 5.9 Different modes of queen rearing. In most species, queens are reared in larger queen cells, containing more food (**a**, **b**). These cells are often at the edge of brood combs. In cluster-building species, some larvae eat the cell content of an adjacent auxiliary cell after finishing their own larval food (**c**, **d**). In *Melipona*, queens, workers and males are reared in cells of the same size (**e**, **f**). This can also be found in species that produce both large and miniature queens. (**b** shows *Tetragonisca angustula*, **c** shows *Frieseomelitta longipes*, photos by Cristiano Menezes; F shows *Melipona subnitida*, from Wenseleers and Ratnieks 2004)

5.5.1 Trophic Queen Determination

In species with trophic queen determination, two different modes exist. In the first situation, found in most genera, queens are reared in large queen cells that contain more food than the worker and male cells (Fig. 5.9a, b). Actual measurements are rare, but in *Scaptotrigona depilis* and *S. postica*, queen cells contain about 4 times as much larval food as worker cells, and a *Tetragonisca angustula* queen is made with

6–7 times more larval food than a worker (Engels and Imperatriz-Fonseca 1990; Menezes 2010; Cham et al. 2019). As a result, larvae in queen cells can eat for longer and grow larger (see also Fig. 1.6). This delays the onset of metamorphosis and results in a longer developmental times of queens compared to workers in species with this mode of queen determination (see *Friesella schrottkyi* and *Plebeia remota* in Table 5.3).[14] Queen cells are usually located in the periphery of brood combs (Fig. 5.9a, b), but can occasionally be found more centrally (Sakagami 1982).

The quantity of food seems to be the main caste determining factor (Velthuis 1976; Hartfelder and Engels 1989). For example, when de Camargo (1972b) reared *Scaptotrigona postica* larvae in vitro on different amounts of food, queens were produced when larger amounts were added, whereas only workers were produced when larvae received a quantity corresponding to the average food amount found in a worker cell (see also Velthuis 1976). Darchen and Delage-Darchen (1971) were even able to rear queens from heterospecific larval food. This differs from the situation in the honeybees, where workers are produced after a switch from the feeding of royal jelly to the feeding of worker jelly after the third larval instar (Haydak 1970; Mao et al. 2015). The stimuli that cause stingless bee workers to build a new queen cell remain to be studied.

In the second type, which is found in some (and possibly most) cluster-building species (e.g. *Austroplebeia cassia, Celetrigona longicornis*, *Frieseomelitta* spp., *Leurotrigona muelleri, Plebeia minima* and *P. lucii*), all larvae are reared in regular cells but some gain access to the larval food of an adjacent cell to get a second portion, leading to the development of a queen (Fig. 5.9c, d) (Sakagami 1982; Engels and Imperatriz-Fonseca 1990; Faustino et al. 2002; Ribeiro et al. 2006b; Luz et al. 2017). In *Leurotrigona muelleri*, for instance, some last instar larvae bite through their cell wall after consuming all food in their cell to eat the larval food of a cell that was built later (Engels and Imperatriz-Fonseca 1990). Workers often combine two cells to build one large one for the developing queen. This mode of queen rearing could facilitate the production of emergency queens: if new queens are suddenly needed, workers build and provision an "auxiliary" cell in close contact to a cell containing a late-stage female larva (Faustino et al. 2002; Nunes et al. 2015; Luz et al. 2017).[15] These auxiliary cells do not contain eggs and are usually built next to cells containing larvae that have consumed all their larval food (Luz et al. 2017). As a result, queens consume about twice as much larval food as workers (~27 μl for workers and ~50–65 μl for queens in *Frieseomelitta varia*, Baptistella et al. 2012).

It is not yet known why some larvae bite through their cell wall to consume the cell content of an adjacent cell and others not, but it could be that the workers trigger the process by building a connection between the two cells (Faustino et al. 2002).

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[14] In *Trigona hypogea*, queen larvae were observed to stay in a quiescent state in their cells for up to 11 months (Imperatriz-Fonseca and Zucchi 1995).

[15] *Tetragonula carbonaria* combines the two modes of queen rearing when rearing emergency queens: queens are normally reared in larger cells, but if a new queen is suddenly needed due to queen loss, two worker cells are fused to create a larger queen cell (Nunes et al. 2015).

5.5.2 Genetic Queen Determination

In *Melipona*, a Neotropical genus that contains more than 70 species, queens and workers are reared in cells that are indistinguishable in size and the amount of larval food they receive (Fig. 5.9e, f). This makes it unlikely that food quantity is the key factor determining caste fate (Kerr 1946, 1948; Sakagami 1982). Instead, Kerr (1946, 1948, 1950a, b) proposed a genetic basis for caste determination. He proposed a situation where two genes with two alleles acting in a Mendelian fashion are responsible for queen determination. Only females that are heterozygous at both loci (*AaBb*) can develop into queens, leading to a 3:1 proportion of workers to queens in colonies with a singly mated mother queen.[16] This hypothesis was based on the observation that under natural conditions up to 25% of the female brood develops into queens (Kerr 1948, 1951; de Camargo et al. 1976; Velthuis 1976). Even though this hypothesis is widely accepted, it still lacks conclusive evidence, such as the identification of the caste determining loci.[17] The fact that in most species less than 25% of the female brood develop into queens (on average ~10%, Table 5.3) (Kerr 1948; Koedam 1999; Moo-Valle et al. 2001; Sommeijer et al. 2003; van Veen et al. 2004; Wenseleers and Ratnieks 2004; Santos-Filho et al. 2006) suggests that other factors play an important role. It is currently assumed that under conditions with suboptimal food quality and/or quantity, some larvae with queen genotype develop into workers (Kerr 1948; Kerr and Nielsen 1966; Darchen and Delage-Darchen 1975; Koedam 1999; van Veen 2000; Hartfelder et al. 2006). There are suggestions that worker phenotypes with queen genotype may still be recognisable by the queen-like fusion of abdominal ganglia during metamorphosis (Kerr and Nielsen 1966; this observation was not confirmed in *Melipona beecheii* by Darchen and Delage-Darchen 1975) and the number of tergal glands (Cruz-Landim et al. 1980), but this requires further study. Thus, fewer queens would be produced if conditions are not favourable, such as with low-food reserves. However, the evidence that food stores indeed play a crucial role is mixed. Moo-Valle et al. (2001) either added or removed food to colonies, but found that high-food colonies produced only slightly more queens (13.5 vs. 10.5%), whereas male production ceased almost completely in low-food colonies. In support of a role of food amount in individual cells, Maciel-Silva and Kerr (1991) found that in *Melipona fasciculata* queen development is unlikely if cells are underfed, but queen production approaches a 3:1 worker-queen

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[16] Originally, Kerr (1946, 1948, 1950a) suggested also a three gene loci model for some *Melipona* species to explain why they produce a lower percentage of queens than the predicted 25%. The three loci model would predict a 12.5% proportion of queens, but he abandoned this explanation in favour of a larger role of nutrition in determining queen fate.

[17] Evidence could also be provided by breeding experiments. For example, the application of juvenile hormone leads to queen development of most treated larvae. This means that queens with worker genotype can be produced. Artificial mating (de Camargo 1972a) of such queens would, in theory, allow us to test whether colonies headed by "worker" queens produce different queen/worker ratios. However, such experiments have so far failed due to the high mortality of queens that are produced in this way (Engels and Imperatriz-Fonseca 1990).

ratio if cells received an above-average food quantity. Above this threshold, food quantity did not affect the probability of queen development. Their data also indicate that queen-destined larvae were heavier than worker-destined larvae, which suggests that queen larvae eat their food quicker. This could explain why queen development is ~2–10 days shorter than worker development in the *Melipona* species studied so far (Table 5.3). Similar effects of food supplementation to individual cells have been found in four other *Melipona* species (de Camargo et al. 1976). Interestingly, Darchen and Delage-Darchen (1975) found that the addition of food to cells of *Melipona beecheii* larvae increased the proportion of queens among the female brood to ~40–60% (and up to ~70%),[18] which challenges the two-gene/two-allele mechanism proposed by Kerr that predicts an upper limit of 25% of queens.[19] It should be noted that queens and workers are normally reared on the same food quantity and have the same weight at emergence (Wenseleers et al. 2004b). An intriguing observation reported by both Kerr and Nielsen (1966) and van Veen (2000) is that queen-producing cells seem to receive a lower number of food discharges from workers, suggesting that individual bees discharge larger quantities into cells that produce queens. One might speculate that these larger-than-average food discharges differ in the composition of larval food, e.g. in the glandular secretions to pollen to honey ratio.

Glandular secretions have also been suspected to determine caste fate (Darchen and Delage-Darchen 1975; Jarau et al. 2010). Jarau et al. (2010) suggested that geraniol, produced in the labial glands of nurse bees, is a key trigger of queen development. Geraniol might be involved in the pathway that leads to the biosynthesis of juvenile hormone (JH), which triggers queen development in stingless bees: treatment with JH increases the probability that larvae develop into queens in several species, sometimes to nearly 100% (Velthuis 1976; Campos and Coelho 1993; Bonetti et al. 1995; Pinto et al. 2002; Hartfelder et al. 2006; Cardoso-Júnior et al. 2017). Furthermore, the size of the *corpora allata* (where JH is synthesised) predicts queen development in *Melipona quadrifasciata* (Kerr et al. 1975). The JH-sensitive period for caste determination is the so-called spinning phase of the last larval instar (Pinto et al. 2002; Hartfelder et al. 2006; Luna-Lucena et al. 2018).

Jarau et al. (2010) hypothesised that nurse workers control the caste fate of female larvae by adding sufficient quantities of geraniol only to some cells. This hypothesis, however, raises the question why workers would rear so many queens and then kill most of them immediately after emergence (Chap. 4 and Sect. 5.6). Observations by Maciel-Silva and Kerr (1991) suggest there is no straightforward relationship between food composition and larval fate in *Melipona fasciculata*. They homogenised larval food to make sure that all larvae receive the same larval food.

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[18] Adding or removing food to cells affected the size of the emerging bees and researchers have found both tiny queens, giant workers and intercastes (Darchen and Delage-Darchen 1975; Sakagami 1982; Engels and Imperatriz-Fonseca 1990).

[19] This study (Darchen and Delage-Darchen 1975) has been criticised for not providing actual sample sizes for these experiments (see Velthuis 1976), which makes it difficult to get an idea of the statistical power of their data.

In their study, ~18% of female larvae developed into queens. Since differences in food composition can be ruled out, we are again left wondering which factors determined queen development in *Melipona fasciculata*. Despite these open questions, the most parsimonious explanation for queen determination in *Melipona* remains a combination of genetic and trophic factors that jointly affect JH production in the *corpora allata*. JH then induces changes in ecdysteroid levels and the expression of genes that drive caste development (Judice et al. 2004, 2006; Hartfelder et al. 2006). One such gene that is affected by JH was recently identified by Brito et al. (2015). They found that the *feminizer* gene, which is highly expressed at the embryonic stage, shows higher expression in *Melipona interrupta* queens than in workers and thus could play an important role for queen development.

5.5.3 Miniature Queens in Non-*Melipona* Species

Several species not only produce large queens in royal cells, but also miniature (or "dwarf") queens reared in worker cells. Miniature queens have been found in *Cephalotrigona capitata*, *C. femorata*, *Nannotrigona testaceicornis*, *Plebeia droryana*, *P. emerina*, *P. julianii*, *P. mosquito*, *P. pugnax*, *P. remota* and *Schwarziana quadripunctata* (Camargo 1974; Engels and Imperatriz-Fonseca 1990; Imperatriz-Fonseca and Zucchi 1995; Imperatriz-Fonseca et al. 1997; Bourke and Ratnieks 1999; Wenseleers et al. 2005; Ribeiro et al. 2006b; Alves et al. 2009a). In *Plebeia mosquito*, *Schwarziana quadripunctata* and *Nannotrigona testaceicornis,* the majority of emerging queens were miniature queens, whereas in *Plebeia remota,* most emerging queens seem to be of large size (Imperatriz-Fonseca et al. 1997; Ribeiro and Alves 2001; Wenseleers et al. 2005; Ribeiro et al. 2006b; Santos-Filho et al. 2006). However, miniature queen production can be highly variable within species and it is not clear how queen development is initiated. For instance, it is not known whether geraniol is linked to the production of miniature queens in these species, as has been suggested for *Melipona* (Jarau et al. 2010).

About 20% of examined *S. quadripunctata* colonies and ~14% of *P. remota* colonies were headed by miniature queens (Ribeiro and Alves 2001; Wenseleers et al. 2005; reviewed in Ribeiro et al. 2006b), which shows that they are functional. There is evidence, however, that they have reduced ovary weight, fewer ovarioles, lower egg-laying rates and they may lay smaller eggs (Imperatriz-Fonseca et al. 1997; Cruz-Landim 2000; Ribeiro and Alves 2001; Wenseleers et al. 2005; Ribeiro et al. 2006b; but see Ribeiro et al. 2006a). Ribeiro et al. (2003b) hypothesised that already a small increase in the amount of larval food might be enough to increase the likelihood that a *Plebeia remota* larva develops into a queen. This is consistent with the finding that miniature queens were intermediate in weight between workers and normal queens in some colonies of this species (Ribeiro et al. 2006b). Thus, miniature queens could be the results of an accidental overfeeding of cells. However, in *Nannotrigona testaceicornis* and *S. quadripunctata* (and in *P. remota* colonies that had a particularly high miniature queen production), the weight of miniature queens did not differ significantly from worker weight (Imperatriz-Fonseca et al.

1997; Wenseleers et al. 2005; Ribeiro et al. 2006b), suggesting that in many cases miniature queens are not the result of overfeeding (Bourke and Ratnieks 1999; Wenseleers et al. 2005; Ribeiro et al. 2006b). An alternative explanation is that miniature queens represent developmental mistakes (Camargo 1974; reviewed in Ribeiro et al. 2006b). However, the fact that miniature queens can head colonies suggests that they represent an alternative reproductive strategy. Some larvae seem to be able to evade the social control of workers (exerted by controlling larval food amount) and increase their fitness by becoming the reproductive queen of a colony (Wenseleers et al. 2005; Ribeiro et al. 2006b). Workers, on the other hand, are likely to prefer large-sized queens due to their higher fecundity and they are more likely to kill miniature queens (see also Chap. 4) (Engels and Imperatriz-Fonseca 1990; Ribeiro et al. 2006b). This could be one reason why egg-laying miniature queens are underrepresented given the rate at which they are produced (Ribeiro et al. 2006b). In *Schwarziana quadripunctata,* for example, ~20% of colonies are headed by a miniature queen even though 80–90% of all emerging queens are of this size (Wenseleers et al. 2005; Ribeiro et al. 2006b). An alternative explanation for this underrepresentation of miniature queens could be that they are less good at flying, which might lower their survival chances during swarming or mating flights, or they are less attractive to males (Ribeiro et al. 2006b).

There are parallels between these miniature queens and the queens produced in *Melipona*. In both cases, queens are reared in regular, "worker" cells, often in large numbers. Furthermore, excess queens are usually killed by workers (Ribeiro et al. 2006b). Queen rearing in larger cells most likely represents the ancestral condition, but was lost in the ancestors of *Melipona*. It has been proposed that a *Melipona* ancestor may also have reared both normal-sized queens and miniature queens, but that the large phenotype was lost because the large number of miniature queens meant that queens with the large phenotype had a low probability of heading colonies (Ribeiro et al. 2006b). As a result, workers would have stopped producing large queen cells altogether. This would have required miniature queens in the *Melipona* ancestor species to be relatively fecund compared large-sized queens. Ribeiro et al. (2006b) also discuss the possibility that the mechanism determining queen fate is similar in *Melipona* and in species producing miniature queens. Specifically, they suggest that genetic factors might determine the development into a miniature queen.

5.6 Reproductive Conflicts

Social insects are well-known for their cooperation and altruism (e.g. Wilson 1971; Hölldobler and Wilson 2009; Bourke 2011), but conflicts inside a colony exist and are particularly conspicuous in stingless bees. Especially when it comes to reproduction, the members of a colony do not always pull in the same direction (Hamilton 1972; Ratnieks 1988; Ratnieks and Reeve 1992; Queller and Strassmann 1998; Bourke and Franks 1995; Bourke 2011; Ratnieks et al. 2006; Ratnieks and Helanterä 2009).

Kin selection theory can explain both altruism and conflicts in family groups. Kin selection means that an organism can increase its fitness by helping a relative to increase theirs, even if this reduces the helper's survival or reproductive success. This is because the helper and the recipient of help share genes (reviews in Hamilton 1972; West-Eberhard 1975; Dawkins 1976; Bourke and Franks 1995; Queller and Strassmann 1998; Ratnieks et al. 2006; Bourke 2011). As relatedness (r) among individuals increases, so do the potential fitness benefits for the helper. However, kin selection can also lead to conflicts. Because colonies are headed by a singly mated queen (see Chap. 4) and due to haplodiploidy (females come from fertilised diploid eggs, males from unfertilised haploid eggs) (Wilson 1971), stingless bee workers are three times more related to their sisters ($r \sim 0.75$, see also Fig. 4.5) than to their brothers (i.e. the sons of the queen) ($r = 0.25$). The queen is equally related to her sons and daughters ($r = 0.5$). As a result, workers are more closely related to the sons of other workers, i.e. their nephews ($r = 0.375$), than to their own brothers.

These relatedness asymmetries lead to several potential areas of conflict (reviewed in Ratnieks et al. 2006; Bourke 2011). For example, workers would benefit more from rearing their own sons ($r = 0.5$) and the sons of their sisters ($r = 0.375$) than from rearing the sons of the mother queen ($r = 0.25$). The queen, however, benefits more from the rearing of her sons than from the rearing of worker sons. Thus, queens should attempt to prevent workers from reproducing. Another potential conflict is caste fate: even though sterile workers gain indirect fitness by helping the queen reproduce more successfully, they would have higher fitness if they themselves were the reproductive queen since they would be more closely related to the brood (Bourke and Ratnieks 1999; Ratnieks 2001). Stingless bee workers cannot become the reproductive queen because they are unable to mate (reviewed in Luna-Lucena et al. 2018), but female larvae could attempt to develop into a queen rather than a worker. There are other potential areas of conflict (reviewed in Ratnieks et al. 2006), but I will focus on these two conflicts, male rearing and female caste fate because they are the most pertinent conflicts in stingless bee colonies and are likely to be connected (Ratnieks 2001; Wenseleers and Ratnieks 2004; Tóth et al. 2004; Wenseleers et al. 2013).

5.6.1 Conflicts over Male Production

Since stingless bee workers are more closely related to their own sons ($r = 0.5$) and the sons of their sisters ($r = 0.375$) than to the sons of the queen ($r = 0.25$), worker reproduction in stingless bees should be widespread. Indeed, in several species large proportions of males have a worker as their mother. For example, in *Melipona favosa*, *M. mondury*, *M. quadrifasciata*, *Paratrigona subnuda* or *Tetragona clavipes*, more than 50% of all males were commonly produced by workers (Sommeijer et al. 1999; Tóth et al. 2002a,b; Viana et al. 2015), and in about half of all species, workers are responsible for at least 10% of all males (Tóth et al. 2004). This is different from honeybees and other groups of social insects where worker reproduction is very rare and worker-laid eggs are usually eaten by other workers (or the queen) (Ratnieks and Wenseleers 2005; Ratnieks et al. 2006; Wenseleers and Ratnieks 2006; Zanette et al. 2012). This so-called worker policing is favoured in honeybees on relatedness grounds because multiple mating by the queen lowers worker-worker relatedness, resulting in a situation where workers are more closely related to the sons of the queen than to the sons of their sisters (which are now likely to be half-sisters). Thus, workers should favour rearing their brothers over their nephews in colonies with multiply-mated queens (Ratnieks 1988; Ratnieks and Visscher 1989).

In stingless bees, workers also occasionally eat worker-laid eggs (both trophic and reproductive, see above), but whether this significantly reduces the proportion of males produced by workers is not currently known. Furthermore, studies that compare the proportion of worker-laid and queen-laid male eggs eaten by workers or the queen (as done by Ratnieks and Visscher 1989 in honeybees) have not yet been performed. There is some evidence that workers are able to recognise worker-laid eggs. Koedam et al. (2007) found that reproducing *Melipona bicolor* workers usually laid their eggs in cells that contained another worker-laid egg and ate the latter, rather than replacing queen-laid eggs, which were most likely female eggs. In other social insects, workers are able to recognise whether eggs are queen or worker-laid, most likely based on chemical cues (Ratnieks and Visscher 1989; Endler et al. 2004).

Queen policing has been observed in stingless bees (see above), but is considered less efficient than worker policing (Tóth et al. 2004; Ratnieks et al. 2006) because it is harder for a single queen to control worker oviposition in large colonies. Even in species with small colonies, queen policing does not seem to be common. For example, *Melipona* tend to have relatively small colonies (Table 1.3), but worker reproduction is common. In *Melipona favosa*, queens do not show any aggression towards reproducing workers (Chinh et al. 2003).

Relatedness among colony members changes if the resident queen is replaced by a new queen (e.g. due to supersedure, Chap. 4). The new queen will produce workers that are less related to the old workers. This should increase the incentive for worker reproduction among the old workers because the costs are now carried more by individuals with whom they are less related (their nieces) (Hamilton 1964; Ratnieks et al. 2006). In accordance with this prediction, Alves et al. (2009b) found that ~80% of worker-laid male eggs in *Melipona scutellaris* originated from workers that were the daughters of the previous queen. Their data suggest that these old workers represented only a small proportion of the workforce, but they were extremely long-lived (up to 110 days) and probably used their energy for reproduction instead of performing the riskier tasks that are usually performed by older workers, mainly foraging and nest guarding (Chap. 6).

In about half of all studied species, workers do not reproduce as long as an egg-laying queen is present. In species belonging to four genera, *Duckeola*, *Frieseomelitta, Geotrigona* and *Tetragonula* (Table 5.2), workers never reproduce. In these species, workers either do not have functional ovaries or they only lay trophic eggs even under queenless conditions. This cannot easily be explained on relatedness grounds, so why are these workers sterile and allow the queen to lay all the male eggs? One explanation could be that queens are somehow able to prevent workers from reproducing. The queen is larger and could physically control the workers involved in the POP. However, queens have relatively weak mouth parts (Schwarz 1948), which makes biting less likely (Tóth et al. 2003), and present-day stingless bee queens do not usually use overt dominance behaviour to monopolise reproduction (Sakagami et al. 1977).[20] Alternatively, the queen could chemically inhibit worker reproduction, but this is unlikely to be stable over evolutionary time and not supported by empirical evidence (Keller and Nonacs 1993; Oi et al. 2015; Grüter and Keller 2016; Van Oystaeyen et al. 2014). In line with this, Nunes et al. (2017) provide evidence that worker sterility in stingless bees is not enforced chemically by the queen, but most likely evolved because it is of some benefit to workers. In other words, it pays workers to refrain from reproduction as long as a fertile queen is present.

One explanation for self-restraint (and worker policing) is that worker reproduction is costly for the colony (Ratnieks and Reeve 1992; Tóth et al. 2003; Ratnieks et al. 2006). As indicated above, reproducing workers might have less time for other tasks or use up more resources (Tóth et al. 2003; Ratnieks et al. 2006; Alves et al. 2009b). Secondly, producing males reduces the number of workers that can be reared, which is likely to reduce the productivity of the entire colony (Koedam et al. 1999; Tóth et al. 2002a; Wenseleers et al. 2013). Thirdly, overt conflict over male production could lead to injuries to the queen, which is a risk for the entire colony (Ratnieks and Reeve 1992). This highlights that a worker's strategies should reflect a balance between the individual benefits of its own reproduction and the collective costs that result from worker reproduction. If the colony-level costs of worker reproduction are substantial, worker policing or worker self-restraint should evolve (i.e. worker sterility) (Ratnieks et al. 2006). Self-restraint can also evolve indirectly if a very efficient worker policing removes the incentives for worker reproduction (Wenseleers et al. 2004a; Wenseleers and Ratnieks 2006). The fact that stingless bee species differ so much in the degree of worker reproduction—despite having similar kin structure—suggests that worker reproduction imposes different colony-level costs in different species (Wenseleers et al. 2013). For example, costs of worker reproduction might be lower in *Melipona* because many of the replaced queen-laid eggs would become excess queens (see also Tóth et al. 2004 for a discussion). This could explain why worker reproduction is common in this genus.

5.6.2 Conflicts over Female Caste Fate

Female larvae could potentially benefit from becoming a queen rather than a worker even if this has some costs for the colony (Bourke and Ratnieks 1999; Ratnieks

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[20] However, in some species, food solicitation by the queen appears so violent, with the queen holding the worker down, that it has the appearance of an "extortion" (Sakagami et al. 1977; Sakagami and Yamane 1987).

2001; Wenseleers et al. 2003). This can lead to a “tragedy of the commons” (Hardin 1968; Ratnieks et al. 2006), i.e. a situation where many or most female larvae develop into queens for individual gains and, thereby, harm the success of the colony as a whole (Ratnieks et al. 2006). Such a situation would not be in the interest of the adult workers and the queen. The result is a potential conflict between female larvae and adult females. However, actual conflict is often prevented in stingless bees because trophic queen determination prevents most female larvae from becoming a queen (see Sect. 5.5) (Bourke and Ratnieks 1999; Ratnieks et al. 2006). In *Melipona,* however, queen development does not depend on food amount, which provides opportunities for larvae to develop into queens. Thus, it is expected that many more female larvae develop into queens in *Melipona* than in other species, and as we have seen earlier, this is indeed what is found (see Sect. 5.5, Table 5.3). The average proportion of queens among female brood (~10%) is somewhat lower than predicted by Ratnieks (2001) and Wenseleers et al. (2003) (their models predicted 14–20%). This could indicate that colony productivity is more strongly affected by excess queen production than assumed in the models.

The observation that in *Melipona* nearly all emerging queens are killed by workers supports the hypothesis that there is a conflict between workers and larvae over caste fate. The proportion of larvae that become a queen is also likely to depend on whether workers can reproduce. If worker reproduction is common, fewer larvae should decide to become a queen because worker reproduction increases the individual benefits of developing into a worker (Ratnieks 2001). We would, therefore, expect a negative correlation between worker reproduction and queen production in *Melipona*. This is not easy to test because both queen and male production are variable even within a colony, but Wenseleers and Ratnieks (2004) provide some tentative evidence for such as negative correlation in four species of *Melipona*.

It should be noted that this fitness-based explanation for excess queen production in *Melipona* is not necessarily an alternative to the genetic queen determination hypothesis or the hypothesis that compounds in larval food trigger queen development in *Melipona* (discussed in Sect. 5.5). Instead, caste fate conflict and individual-level selection could explain why queen determination mechanisms have evolved that allow large numbers of larvae to develop into queens (see also Bourke and Ratnieks 1999).

In cluster-building species that use cell-fusion for queen rearing, larvae could become a queen by consuming the content of an adjacent cell. In reality, however, larvae have little control over caste fate because they need another cell close to their own cell and this adjacent cell needs to contain enough food during a critical time window. Only then does a late-stage larva have access to a full cell content of another cell. This means that cell-building workers are likely to be in control of queen production in these species.

The examples described above suggest that stingless bee colonies can experience intense reproductive conflicts among its members. In some instances, conflict is suppressed because one party is able to force another party into certain roles, whereas in others, conflicts and their costs cannot be prevented. This highlights that altruism in stingless bees is partly voluntary and partly enforced (Ratnieks et al. 2006; Ratnieks and Helanterä 2009).

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Colony Organisation and Division of Labour

6

In his famous "pin factory" example, Adam Smith (1776) calculated that a group of labourers with division of labour could produce at least 240 times more pins per day than a group of labourers without division of labour. He attributed this massive increase in productivity to several factors. Firstly, division of labour results in workers becoming more skilled at specific tasks through repetition. Secondly, workers avoid time costs caused by frequent switching between tasks and, thirdly, workers can focus on tasks for which they show greater capability or "talent". All three factors are likely to apply to workers of social insect colonies as well, and there is empirical and theoretical support for the importance of these factors for the benefits of division of labour[1] (Julian and Cahan 1999; Duarte et al. 2012; Goldsby et al. 2012; Leighton et al. 2017; but also see Dornhaus 2008). It is, thus, not surprising that almost all social insect species studied so far show at least some degree of division of labour (Hölldobler and Wilson 1990, 2009). The level of behavioural specialisation for tasks varies among species, workers and tasks (Wilson 1984; Robinson and Page 1995; Sempo and Detrain 2004; Mertl and Traniello 2009). It is commonly assumed that division of labour is an important reason for the ecological success of social insects (Wilson 1971; Oster and Wilson 1978; Hölldobler and Wilson 1990). While it is challenging to experimentally quantify the benefits of division of labour (but see Ulrich et al. 2018), there can be little doubt that division of labour substantially improves the performance of an insect colony.

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[1] I use a broad definition of "division of labour" that refers to non-random variation among workers in the tasks they perform (Jeanne 2016). Long-term individual specialisation (Gordon 2016) is not a requirement for division of labour as used here and represents a special and extreme case of division of labour.

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6.1 Temporal Castes

The first to study division of labour in stingless bees were Bassindale (1955) and Kerr and Santos Neto (1956). Both studies found that workers perform different tasks as they age, a pattern that has since been found in more than a dozen other species of stingless bees (Table 6.1). Bassindale (1955), for example, found that in the African *Hypotrigona gribodoi*, young workers were responsible for the building and provisioning of brood cells, whereas older workers guarded the nest entrance or collected pollen, nectar, resin and other building materials (see Chaps. 3 and 8). This, however, does not mean that all workers of a colony go through the same sequence of tasks at the same age (Inoue et al. 1996; Hammel et al. 2016), but rather that workers show a tendency to perform certain tasks at certain ages. This temporal polyethism (or age polyethism) is also common in ants, bumble bees, honey bees and wasps (Rösch 1925; Lindauer 1952; Wilson 1971; Seeley 1982; Cameron 1989; Hölldobler and Wilson 1990; Johnson 2005; Yerushalmi et al. 2006; Shorter and Tibbetts 2009). As a general rule, younger workers show a greater tendency to care for the brood, whereas older workers defend the colony or forage for food. This sequence is thought to be adaptive at the colony level because it ensures that the workers that are closest to the end of their lives perform the riskiest tasks, whereas workers with greater life expectancy perform safer tasks and are, thus, more likely to be available to the colony for longer (Tofts and Franks 1992; Moroń et al. 2012).[2] A non-adaptive explanation for temporal polyethism is that workers emerge in the brood area and, therefore, are more likely to encounter brood stimuli. In other words, they are born into the first task (Tofts and Franks 1992) and, as a consequence, they are more likely to start with nurse duties (Tofts and Franks 1992; Beshers and Fewell 2001). As new workers emerge, older workers in the brood area find it harder to find work and move away from the brood area to find new tasks. This "foraging-for-work" model was inspired by observations on ants and honey bees, but seems less consistent with division of labour in stingless bees where building activities outside the brood comb area precede participation in cell building and the provisioning and oviposition process (Table 6.1).

The social, endocrine, neurochemical and molecular basis of age-related changes in behaviour has been studied extensively in honey bees (Winston 1987; Robinson 1992, 2002; Ben-Shahar et al. 2002; Schulz et al. 2002; Whitfield et al. 2003; Leoncini et al. 2004; Herb et al. 2012), but little is known about the proximate causes of division of labour and behavioural maturation in stingless bees. For example, juvenile hormone (JH) plays an important role in the behavioural maturation of honey bees (Fahrbach 1997; Sullivan et al. 2000; Schulz et al. 2002), but much less is known about the role of JH for division of labour in stingless bees. Interestingly, a recent study found that *Melipona scutellaris* foragers had lower JH

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[2] Ravaiano et al. (2018) also found that in *Melipona quadrifasciata*, forager bees have a lower number of hemocytes than nurse bees. Hemocytes suspended in the hemolymph play an important role in immune defence. Thus, foragers might also invest less in cellular immune response than younger nurse bees.

Table 6.1 Sequence of tasks performed by workers of 17 stingless bee species

	Phase II					Phase III				Phase IV		
Species	Building with cerumen and wax	Building brood cells	Worker oviposition	Feeding queen	POP	Waste manipulation	Nectar dehydration	Unloading foragers	Remove waste	Guarding	Foraging	References
Apotrigona nebulata	1	2			2				3	3	3	Darchen (1969)
Frieseomelitta varia	1	2		2	2	3				4	4	Cardoso (2010)
Hypotrigona gribodoi	1	1	1	1		2	2		3	4	4	Bassindale (1955)
Meliplebeia beccarii	1	2				3				4	4	Njoya and Wittmann (2013)
Melipona eburnea	1	1	1	1	1	2	2			3	4	Bustamante (2006)
Melipona fasciculata	1	2			3		4	4			5	Giannini (1997)
Melipona favosa	1	1	2		1	3				4	4	Sommeijer (1984)
Melipona lateralis	1	1	1	1	1	2	3			3	4	Bustamante (2006)
Melipona quadrifasciata	1	2		3	4	4	5	5	6	6	7	Kerr and Santos Neto (1956)
Melipona scutellaris	2	1	1		1	3	3	3			4	Santos (2013)
Melipona seminigra	1	1	2		1	3	3			3	4	Bustamante (2006)
Plebeia droryana	2	1			2		3				3	Terada et al. (1975)
Plebeia remota	1	2	2		2		3	4			5	van Benthem et al. (1995)

(continued)

Table 6.1 (continued)

Species	Phase II					Phase III				Phase IV		References
	Building with cerumen and wax	Building brood cells	Worker oviposition	Feeding queen	POP	Waste manipulation	Nectar dehydration	Unloading foragers	Remove waste	Guarding	Foraging	
Tetragonisca angustula	1	1		1	1	2			3	4	4	Grosso and Bego (2002), Hammel et al. (2016)
Trigona pallens	1	2			2	3					4	Silva (2008)
Scaptotrigona postica	1	1			1	2	3			4	5	Simões and Bego (1979)
Scaptotrigona xanthotricha	1	1		1	1	2	2		2	3	4	Hebling et al. (1964)

See text for description of phases. The numbers indicate the temporal sequence of tasks observed in these studies. Not all behaviours were studied in all studies. Phase I is not shown as it is characterised by self-grooming and inactivity titres than nurses, which is the opposite of what is found in honey bees (Cardoso-Júnior et al. 2017). Another open question is how flexibly colonies can reallocate workers to different tasks if conditions suddenly change (i.e. whether young bees can become foragers when more foragers are needed or whether foragers can revert to nursing when there is a shortage of young bees feeding the brood; see Robinson 1992 for honey bees). There is some evidence of behavioural flexibility in *Melipona quadrifasciata* (Waldschmidt et al. 1998), as colonies consisting of only young workers show normal foraging activity. On the other hand, in *Tetragonisca angustula* colonies that had their guards removed it took several days before the number of entrance guards reached pre-removal levels (Baudier et al. 2019). If and how social factors accelerate or slow down behavioural maturation in stingless bees remains to be studied.

6.2 Sequence and Description of the Main Tasks

Several studies have found a relatively consistent pattern of temporal polyethism in stingless bees. Below I divided the different behaviours into four phases (Tables 6.1 and 6.2). In many species these phases are accompanied by an increase in pigmentation (Fig. 6.1) (e.g. Bassindale 1955; Salmah et al. 1987, 1996) and, in *Melipona quadrifasciata*, in the volume of the mushroom bodies and the antennal lobes, two brain tissues that are important for sensory perception and cognition (Tomé et al. 2014). Compared to honey bees, stingless bee workers require a longer time to reach full pigmentation.

6.2.1 Phase I

The first few days after emergence workers can be seen grooming themselves and receiving food. Activity is low and workers are of light colour (Hebling et al. 1964; Bassindale 1955; Kerr and Santos Neto 1956; Bustamante 2006; Hammel et al. 2016).

6.2.2 Phase II

The next phase is dominated by building activities, followed by activities related to the provisioning and oviposition process (POP) (see Chap. 5) (Fig. 6.2). Bees mainly work inside or on the involucrum (the cerumen sheets surrounding the brood area, Chap. 3). Bees working with cerumen constantly smooth and shape their building material with their mandibles (Bassindale 1955). The provisioning of a brood cell requires several bees to discharge larval food into the empty cell before oviposition (Chap. 5) (Bassindale 1955; van Benthem et al. 1995; Segers et al. 2015). After oviposition, cells are immediately closed by *phase II* workers. Workers occasionally feed the queen, either by donating liquid food via trophallaxis or by laying trophic

Table 6.2 List of the tasks and behaviours that are frequently observed in studies of division of labour in stingless bees

Phase	Behavioural context	Behaviours
I		Self-grooming
		Receiving food
II	Building	Manipulating involucrum
		Producing wax
		Building storage pots
		Building brood cells
		Building entrance
	POP and brood care	Entering food pots and eat pollen or honey
		Inspecting empty brood cells
		Regurgitating food into brood cells
		Feeding queen
		Laying trophic egg
		Closing brood cell
		Opening brood cells to help workers emerge
	Regulation of nest climate	Ventilation
III	Handling food	Unloading nectar forager
		Ripening nectar
	Handling non-food material	Depositing wax on wax piles
		Handling resin or mud
		Closing gaps with resin or mud
		Transporting waste material to waste piles
		Making waste balls and drop waste outside
IV	Guarding	Patrolling entrance tube
		Guarding at nest entrance
		Inspecting incoming bees
		Fighting intruder
		Opening and closing nest entrance
	Foraging	Collecting nectar
		Collecting pollen
		Collecting resin or mud
		Collecting water
		Storing pollen in pollen pot
		Storing mud or resin on piles
		Unloading nectar to food processor
		Recruiting nestmates to resources
Various phases	Various contexts	Trophallaxis
		Walking
		Inactive
		Grooming nestmates
		Antennation

Behaviours taken from Bassindale (1955), Inoue et al. (1996), Cardoso (2010) and Hammel et al. (2016)

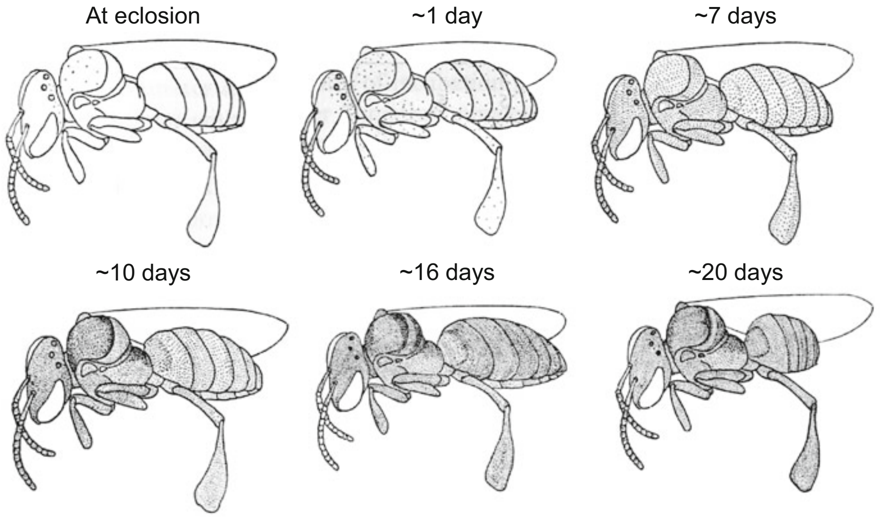

Fig. 6.1 Pigmentation of workers in the Indo-Malayan species *Sundatrigona moorei*. Foraging in this species starts at about 30 days after emergence (Salmah et al. 1987)

eggs (Chap. 5) (e.g. Sommeijer 1984), and some workers form a royal court around the queen when she is not moving around (Sakagami and Zucchi 1967; Imperatriz-Fonseca 1977). At about the same age (approx. week 1–3), workers of some species also lay reproductive eggs from which males emerge (Chap. 5) (Bego 1990; Koedam et al. 1999). This time period coincides with the peak of vitellogenin production in workers, a precursor of egg-yolk protein (Engles and Engels 1977; Engels 1987; Hartfelder et al. 2006). Closed cells receive little attention until, after some days (as soon as the larvae in the cells construct cocoons made of silk), *phase II* workers remove some of the cerumen on the cells and reuse it at other building sites (Michener 1974). Workers can also be seen starting to work outside the involucrum, for example building storage pots (Bassindale 1955; Hammel et al. 2016). Storage pots are also built by older workers (*phase III*) (Bassindale 1955; Mateus et al. 2019). *Phase II* workers start to secrete wax from wax glands located in tergites IV to VII (Cruz-Landim 1963), III to VI or III to V (Justino et al. 2018). Species differ in the exact position of the wax glands and in whether wax glands can be found in three or four consecutive tergites. Wax production is highest during this period, but can continue until workers reach foraging age (Kerr and Santos Neto 1956; Terada et al. 1975; Sakagami 1982; Bego et al. 1983; Ferreira-Caliman et al. 2010; Justino et al. 2018). For example, in *Melipona marginata*, workers start to secrete wax from day 5 to day 24, which is about the time when the first workers start to forage (Ferreira-Caliman et al. 2010). In *Friesella schrottkyi*, wax production peaked at around day 13 and then declined rapidly (Justino et al. 2018). Not all workers produce wax: in *F. schrottkyi*, about 30% of the studied workers did not seem to secrete wax at all, which could indicate the existence of different physiological and behavioural castes, but this requires further study (Justino et al. 2018). Workers use the distal end of the

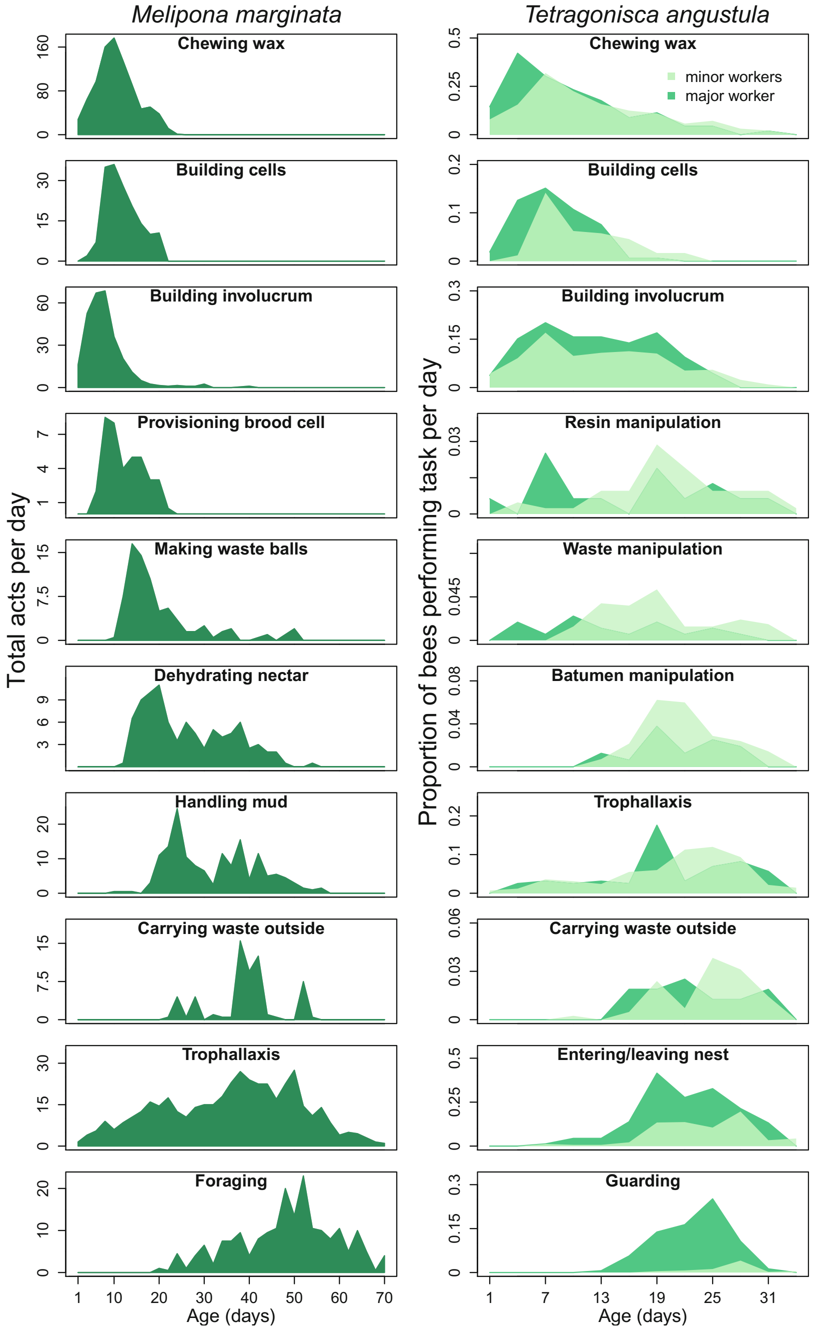

Fig. 6.2 Temporal polyethism in *Melipona marginata* and *Tetragonisca angustula* (Hammel et al. 2016; Mateus et al. 2019). Data is shown for both small workers and large workers (majors) in *T. angustula*. Note that activity per day is measured in different ways in the two studies (*y*-axis)

basitarsus to pull the wax scale off their abdomen (Fig. 3.7) (Cruz-Landim 1963). The wax is sometimes stored in piles located near active building areas (Bassindale 1955; Hebling et al. 1964).

6.2.3 Phase III

Workers relocate and start to perform many of their activities outside the involucrum. They unload foragers returning with liquid food (e.g. nectar, honeydew or fruit sap) and dehydrate the liquids to process it into honey (Chap. 1). Dehydrating workers stand quietly, rhythmically moving their proboscis to expand and decrease the size of the small droplet on the proboscis (so-called "tongue lashing") (Sakagami and Oniki 1963). Another common activity at this age is the manipulation of waste material at well-defined waste piles. This waste mainly consists of faeces, body parts of dead workers and old brood cells (Bassindale 1955; Medina et al. 2014). Unlike honey bees, stingless bee workers defecate inside the nest on these waste piles (with the exception of the queen whose faeces is eaten by workers) (da Silva et al. 1972; Sakagami et al. 1977). Waste balls are then formed using the material from the piles and later transported outside to be dropped in the vicinity of the nest (Fig. 6.3). Some *Melipona* fly nearly 50 m before dropping the waste ball, whereas *Tetragonisca angustula* workers fly just a short distance before dropping the waste[3] (Darchen 1969; Kerr and Kerr 1999; Medina et al. 2014). In some species, e.g. the American *Leurotrigona muelleri* or *Lestrimelitta limao*, the waste carrying bees walk to the edge of the nest entrance and drop the balls to the floor, leading to small piles of waste just below the nest entrance (Sakagami and Zucchi 1974). The bees that

Fig. 6.3 A *Scaptotrigona bipunctata* worker carrying a waste ball outside, São Paulo state, Brazil (Photo by C. Grüter)

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[3] According to Roubik and Patiño (2009), *Cephalotrigona* is the only genus known to accumulate the waste material at the base of the cavity, rather than removing it from the nest. The authors speculate that this habit may ultimately cause the death of the colony as space runs out.

deposit waste on the waste pile are a few days younger than the bees that carry it outside (Fig. 6.2) (Bassindale 1955; Hammel et al. 2016). In *T. angustula*, for example, the average age of bees working at waste piles was 19 days, which was 6 days younger than the bees carrying the waste outside. Bees that worked at waste piles were also ~4 times more likely to carry waste outside later in life compared to bees that were never seen working at waste piles,[4] suggesting that some bees have an affinity for handling waste. *Phase III* workers are also heavily involved in the handling of other materials, like resinous substances or mud (Fig. 6.2).

Phase III workers can be seen opening cells to help their sisters emerge (Bassindale 1955; Hammel et al. 2016). Workers of this age group are also responsible for fanning inside the entrance tube to create an air current (see also Chap. 3) (Bassindale 1955). In preparation for foraging, bees start to perform short orientation flights (5–10 min) to learn the surroundings of the nest (Biesmeijer and Tóth 1998).

6.2.4 Phase IV

These older workers start to perform tasks outside their nest, mainly guarding and foraging. Pollen foragers deposit their load directly into a pollen pot, sometimes after lingering for a while near the entrance to beg for some food (e.g. Sommeijer et al. 1985). Other bees touch the loaded pollen packages with their antenna or they nibble on it while the pollen is still attached to the pollen baskets of the forager (Bassindale 1955; Sommeijer et al. 1983). Nectar foragers unload their food to receiver bees (*Phase III*), a behaviour called trophallaxis. Sometimes, they transfer food to multiple receivers at the same time (see also Chaps. 8 and 10 for further discussions on the role of trophallaxis during foraging and recruitment) (Sommeijer et al. 1983; Hart and Ratnieks 2002). Thus, in many stingless bees, nectar collection is a partitioned task, whereas pollen collection and storage are performed by the same forager, as is the case in honey bees (von Frisch 1967; Ratnieks and Anderson 1999). This does not seem to be a universal rule, however: Sommeijer et al. (1983) describe that in *Melipona favosa*, returning foragers often keep a large quantity of nectar in their crop after having offered nectar to other bees. These foragers then regurgitate this remaining nectar load in an open storage pot in one abdominal contraction. Similarly, foragers of the African *Plebeina armata* were seen to occasionally discharge liquid food in storage pots without transferring it to another bee (Krausa et al. 2017, there as *P. hildebrandti*) and Darchen (1969) reports that in another African species, *Apotrigona nebulata*, the foragers themselves discharge the food in the storage pots. Thus, the extent to which nectar collection is a partitioned task in stingless seems to vary between species. More generally, the importance and role of trophallaxis in colony organisation (and the substances it contains, see LeBoeuf et al. 2016) in stingless bees remains poorly understood.

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[4] 44.4% or 24 of 54 bees vs. 12.3% or 17 of 138 bees; Chi-square Test: $\chi^2 = 23.8$, df $= 1$, $p < 0.001$ (recovered from supplementary information in Hammel et al. 2016).

Resin and other sticky plant materials are carried in the pollen baskets and are often (but not always) unloaded by the forager without the aid of other bees and, in some species, stored on specific resin piles (Fig. 3.7) (dos Santos et al. 2010). The unloading of resin takes only a few seconds as the bee presses her load directly against a resin pile or against a wall. Resin has many different functions (see Chaps. 3 and 7), e.g. to seal crevices and openings in the nest cavity, and this task is also performed by *phase IV* workers (Bassindale 1955). It is not clear, however, whether these resin manipulations are predominantly performed by the bees that also collect the resin. In species that collect mud as a nest-building material, mud is deposited on mud storage areas inside the nest by the collecting foragers (Sidnei Mateus, pers. comm.).

Foragers often specialise in the collection of one type of resource (Chap. 8): in *Melipona beecheii*, *M. marginata*, *M. favosa* and *Plebeia tobagoensis*, a large proportion of foragers showed a tendency to collect either nectar, pollen, water, resin or mud (in *M. marginata*) (Sommeijer et al. 1983; Biesmeijer and Tóth 1998; Hofstede and Sommeijer 2006; Mateus et al. 2019). Hofstede and Sommeijer (2006) also observed that foragers that show a higher degree of specialisation are more efficient than flexible foragers, which supports the idea that behavioural specialisation is associated with increased efficiency (see above). On the other hand, Biesmeijer and Tóth (1998) found no difference in efficiency between *Melipona beecheii* specialist foragers and foragers that switched between resources. In honey bees, foragers show a similar tendency to collect either pollen, nectar, water or resin and these preferences can be predicted by assessing the sucrose sensitivity (Pankiw and Page 2000; Scheiner et al. 2004; Simone-Finstrom et al. 2010). The relationship between sucrose sensitivity and foraging preferences remains largely unexplored in stingless bees, but Balbuena and Farina (2020) recently reported that *Tetragonisca angustula* pollen foragers have a higher sucrose sensitivity than non-pollen foragers (presumably nectar foragers), similar to what is found in honey bees (Pankiw and Page 2000; Scheiner et al. 2004). We also know little about how many bees are dedicated to foraging at any one moment, but Wille (1966) found that in the Neotropical *Tetragonisca buchwaldi*, about 10% of the hive population was actively foraging during a 3-day period.

Nest guarding is a transitional occupation in most species (Table 6.1), performed by a relatively small number of workers (Couvillon et al. 2008; Grüter et al. 2012; Segers et al. 2015), often before they become foragers. Guards can be seen in front of, on or inside the nest entrance (see Fig. 3.8). A notable exception is *Tetragonisca angustula* where guarding is performed by the larger soldiers that perform this task until their death (see below) (Hammel et al. 2016). Some soldiers perform guarding for up to 3 weeks, with an average guarding duration of approx. 5 days (Grüter et al. 2011; Hammel et al. 2016). Bees often start by hovering near the entrance tube while facing the air corridor leading to the entrance hole (hovering guards), before switching to standing on or near the entrance tubes (standing guards) after a few days (Fig. 6.4, see below) (Baudier et al. 2019).

The onset of guarding and foraging coincides with an increase in mortality risk for an individual bee (Fig. 6.5; see also Sect. 1.2) (Giannini 1997; Grosso and Bego

Fig. 6.4 Two types of guards in *Tetragonisca angustula*. Hovering guards fly near the entrance tube while facing the flight corridor leading to the entrance hole. Standing guards stand on or inside the entrance tube (Photo by C. Grüter)

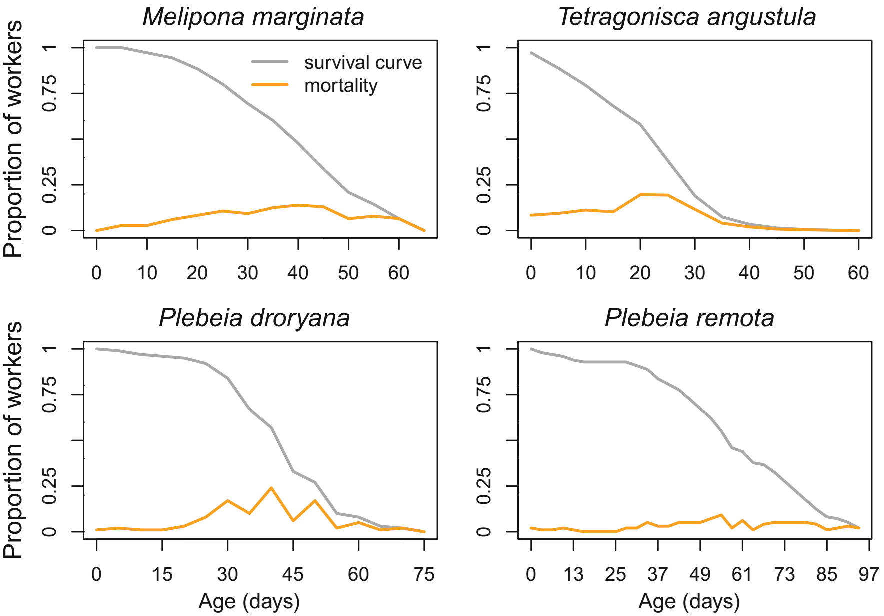

Fig. 6.5 Age-dependent survival and daily mortality in four Neotropical species (data from Terada et al. 1975; van Benthem et al. 1995; Grosso and Bego 2002; Mateus et al. 2019). The *x*-axis shows days after emergence, i.e. age of adult bees

2002; Njoya and Wittmann 2013; Lopes et al. 2020), most likely because these activities are metabolically more demanding and more dangerous than in-nest activities (Wolf and Schmid-Hempel 1989; Visscher and Dukas 1997; Page and Peng 2001). The negative impact of foraging activity on longevity is highlighted by the observation that *Melipona fasciculata* foragers live longer if they did not forage every day (Gomes et al. 2015) and *M. fulva* and *M. favosa* workers live almost twice as long during time periods with low foraging activity (Roubik 1982). In

M. beecheii, pollen foragers performed fewer trips per day than nectar foragers and lived substantially longer (Biesmeijer and Tóth 1998).

Honey bees and stingless bees show many similarities in temporal polyethism (Kerr and Santos Neto 1956), but there are also noteworthy differences (see also Sakagami 1982): (1) In stingless bees, many building activities are performed by very young bees and precede brood provisioning duties (Table 6.1), whereas in honey bees brood feeding is performed by young bees and comb building by middle-aged bees (Rösch 1927; Seeley 1982; Johnson and Frost 2012). (2) Stingless bees tend to collect larger amounts of resin and other building materials than honey bees. (3) Stingless bees start to leave the nest at a more advanced age than honey bees. One reason is that stingless bee workers defecate inside the nest and, therefore, do not need to leave the nests for toilet flights. In *Melipona quadrifasciata*, for example, the first excursions were performed at the age of 27–30 days by workers removing waste material, shortly before bees started guarding and foraging (Kerr and Santos Neto 1956). A similar pattern was found in other *Melipona* species (Bustamante 2006). (4) The provisioning and oviposition process (POP) in stingless bees is very different from provisioning and brood rearing in honey bees (Chap. 5). (5) The secretion of wax starts at a young age in stingless bees and continues until bees reach foraging age (e.g. from 5th to 36th day in *Melipona seminigra*) (Bustamante 2006; Sakagami 1982), whereas in honey bees wax is mainly secreted in middle-aged bees (Rösch 1925; but see Lindauer 1952).

Not all stingless bees seem to organise labour based on temporal polyethism. Inoue et al. (1996) studied the lifetime work profiles of individually marked bees in the Asian *Tetragonula minangkabau* and noted substantial individual differences in task repertoire. Using a cluster analysis they identified four behaviourally distinct groups of workers, which they called (1) core brood carers, (2) subsidiary brood carers, (3) foragers and (4) short-lived, largely inactive workers. Brood carers, for example, performed little outside work, but lived considerably longer than foragers. In *Melipona marginata*, different tasks were performed by workers that differed in age (Fig. 6.2), but Mateus et al. (2019) also found consistent differences among workers that were not related to age. A striking number of workers, ~60%, were never seen foraging, while others were not observed to be involved in the POP (Mateus et al. 2019). This suggests that different stingless bee species show a range of patterns of division of labour, which only become apparent if workers are marked individually and followed during their entire life.

6.3 Physical Worker Sub-castes

In many ant and termite species, division of labour is based on morphological differences between different worker sub-castes (Wilson 1953; Oster and Wilson 1978; Hölldobler and Wilson 1990, 2009; Korb and Hartfelder 2008). Species with physical sub-castes are often characterised by (a) allometric size relationships between different body parts, i.e. workers of different size also differ in shape and (b) a multimodal worker size-frequency distribution (Wilson 1953; Oster and Wilson

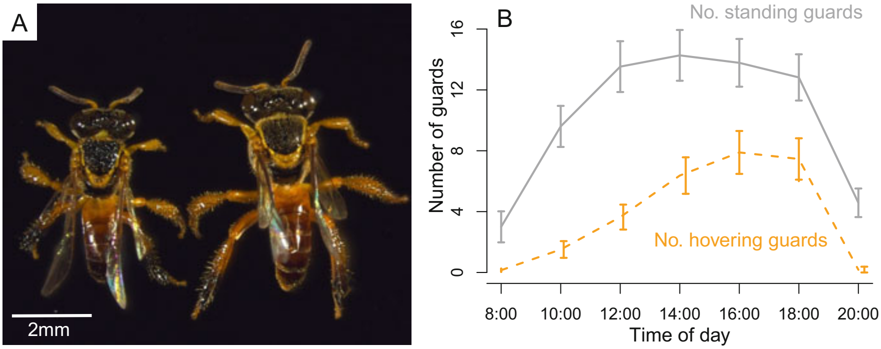

Fig. 6.6 (**a**) *Tetragonisca angustula* forager (left) and soldier (right). The thorax of foragers is covered with a thin layer of resin (as shown), the function of which is unknown. Guards have resin on their legs, but not on their thorax. Guards are 30% heavier than foragers (from Grüter et al. 2012). (**b**) The number of hovering and standing guards at the nest entrance depending on the time of day (from Grüter et al. 2011)

1978; Wheeler 1991). Physical castes are assumed to further increase the efficiency of division of labour because workers are morphologically adapted for particular tasks, e.g. soldiers have strong mandibles for defence.

While extreme cases of physical sub-castes as found in ants (e.g. in *Atta*, *Pheidole* or *Eciton*) have not yet been found in stingless bees, several species have moderately differentiated physical castes (Grüter et al. 2012, 2017a; Wittwer and Elgar 2018). The best studied example is the small *Tetragonisca angustula* (Fig. 6.6a) (Grüter et al. 2012; Segers et al. 2015, 2016; Hammel et al. 2016; Baudier et al. 2019), one of the most common species in the Neotropics (Freitas 2001; Slaa 2006; Velez-Ruiz et al. 2013). In *T. angustula*, two different groups of entrance guards are stationed at the nest entrance tube: ~10–30 standing guards stand in, on or near the wax entrance tube, whereas ~5–15 hovering guards monitor the flight corridor leading to the entrance tube (Fig. 6.4 and 6.6b) (Wittmann 1985; Wittmann et al. 1990; Bowden et al. 1994; Grüter et al. 2011; Kärcher and Ratnieks 2009; van Zweden et al. 2011; Baudier et al. 2019). Guard numbers are highest in the afternoon (Grüter et al. 2011). Hovering guards[5] mainly attack heterospecific intruders based on visual cues, whereas standing guards recognise conspecific non-nestmates that look the same, but smell different (Wittmann 1985; Wittmann et al. 1990; Bowden et al. 1994; Kärcher and Ratnieks 2009; Jones et al. 2012; Couvillon et al. 2013). Both types of guards are ca. 30% larger than foragers (Fig. 6.7a). Due to a small size variation within each task group, there is little size overlap between guards and foragers. Guards (or soldiers) represent about 1–6% of the workforce (Grüter et al. 2012; Segers et al. 2015). Waste removing workers are of intermediate size (Grüter et al.

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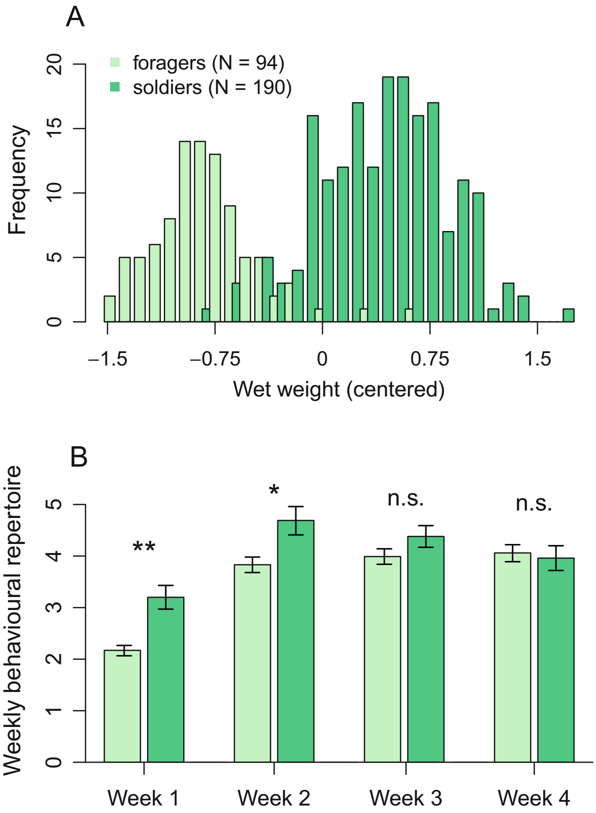
[5] This unusual feature of having a group of hovering bees next to the entrance could be the reason why in some areas this species is called “angelitas” (“little angels” in Spanish) and “us-kaab” (“fly-bee” in a Mayan language in Guatemala, Źrałka et al. 2018).

Fig. 6.7 (**a**) Weight distribution of foragers and soldiers from 20 different *Tetragonisca angustula* colonies (c. 5 foragers, 5 hovering and 5 standing soldiers per colony) from Fazenda Aretuzina, São Simão, Brazil. Hovering and standing soldiers were pooled for the figure. However, hovering guards weighed slightly (+5%), but significantly more than standing guards (linear mixed-effects model: hovering vs. standing guards, *z*-value = 3.9, $p < 0.001$) (data taken from Segers et al. 2016). (**b**) The number of different behaviours performed by small *T. angustula* workers (minors) and soldier-sized workers during the first 4 weeks. **$p < 0.01$, *$p < 0.05$, *n.s.* not significant; from Hammel et al. (2016)



2012) and Hammel et al. (2016) observed that some waste removing bees also performed guard duties.[6] It is possible that the larger waste removers become guards, while the smaller ones become foragers. On the other hand, since waste removal is likely to expose workers to pathogens, waste removers could represent another specialist group of workers that show a high tendency to persist in this task until they die. Waste removal in stingless bees remains a hardly studied activity.

Tetragonisca angustula soldiers and foragers also differ in their body shape. Guards have overall relatively smaller heads (but larger heads in absolute terms) and relatively larger hind legs. One might speculate that these differences in shape affect the workers abilities to perform their respective tasks, but this requires further study. Such a negative allometry between body size and head width seems to be a more

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[6] A role of body size in division of labour in *Tetragonisca angustula* (and *Scaptotrigona mexicana*) has also been shown by Goulson et al. (2005), but unlike more recent studies they suggested that foragers were larger than in-nest workers. However, this apparent contradiction is most likely explained by their collection method. Outside workers were caught using a net and it was assumed that these bees were foragers. Given that *T. angustula* entrances are defended by the larger hovering guards, it is likely that Goulson et al. (2005) captured guards rather than foragers.

common phenomenon in stingless bees. Grüter et al. (2017a) studied 28 Neotropical species and found nine species where the relative head size decreases as body size increases (negative allometry does not seem to be linked to larger guard size or the presence of soldiers).

A bimodal size distribution of guards and foragers has also been found in the closely related species *Tetragonisca fiebrigi*[7] and *Frieseomelitta longipes*[8] (Grüter et al. 2017a), indicating that these species also have a soldier sub-caste for colony defence. Species of the genus *Frieseomelitta* show another intriguing difference between guards and foragers. Guards are often darker (Fig. 6.8). The colour differences are particularly pronounced in the facial area, but many other body parts tend to be darker in guards as well (Fig. 6.8c). The reasons for this darker colouration remain unknown, but it has been argued that the darker guards might be better camouflaged when guarding the nest entrance (Fig. 6.8) (Grüter et al. 2017a), which could make it harder for predators that rely on visual cues, e.g. spiders, to locate the nest entrance. Increased melanisation could also increase resistance to pathogens and physical damage (Dubovskiy et al. 2013), which could be particularly beneficial for entrance guards.

In several other species, guards are significantly larger than foragers, but the size-frequency distribution is unimodal (Grüter et al. 2017a), a pattern that is similar to what has been found in some bumble bee species (Goulson et al. 2002). There are several reasons why it could be beneficial for guards to be larger. For example, larger *Tetragonisca angustula* guards were able to persist longer when fighting against *Lestrimelitta limao* robber bees (Grüter et al. 2012). This could give colonies more time to recruit additional defenders. Furthermore, guard size was positively associated with the ability to recognise non-nestmate *T. angustula* workers (Grüter et al. 2017b). In the latter study, the recognition accuracies of guards from 10 colonies were compared. The colony with the smallest guards accepted about 20% of non-nestmates, i.e. these non-nestmates were not aggressed, whereas the colony with the largest guards accepted only ~2% of non-nestmates. Larger guards had more sensory sensilla on their antennae, which has been linked to increased antennal perception (Spaethe et al. 2007; Gill et al. 2013). The density of sensilla on the antennae did not depend on size, but a larger antennal surface in larger guards resulted in a positive correlation between guard size and sensilla number (Grüter et al. 2017b). In *Tetragonula carbonaria*, another species with larger guards, sensilla density itself is higher in guards than in foragers (Fig. 6.9) (Wittwer and Elgar 2018). Month-Juris et al. (2020) analysed the antennae of two *Tetragonisca fiebrigi* soldiers

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[7] Some authors consider *T. angustula* and *T. fiebrigi* to be two sub-species (*T. angustula angustula* and *T. angustula fiebrigi*) that are in the process of speciation (Francisco et al. 2014). Visible differences include a distinct colouration (e.g. a darker mesepisternum in *T. angustula*), the morphology of male genitalia and the architecture of the brood combs (larger brood combs in *T. fiebrigi*, see Fig. 3.9a).

[8] The sample size used to assess worker size distribution in *F. longipes* was relatively low. A larger sample should be measured to confirm or refute whether the workers of this species follow a bimodal body size distribution.

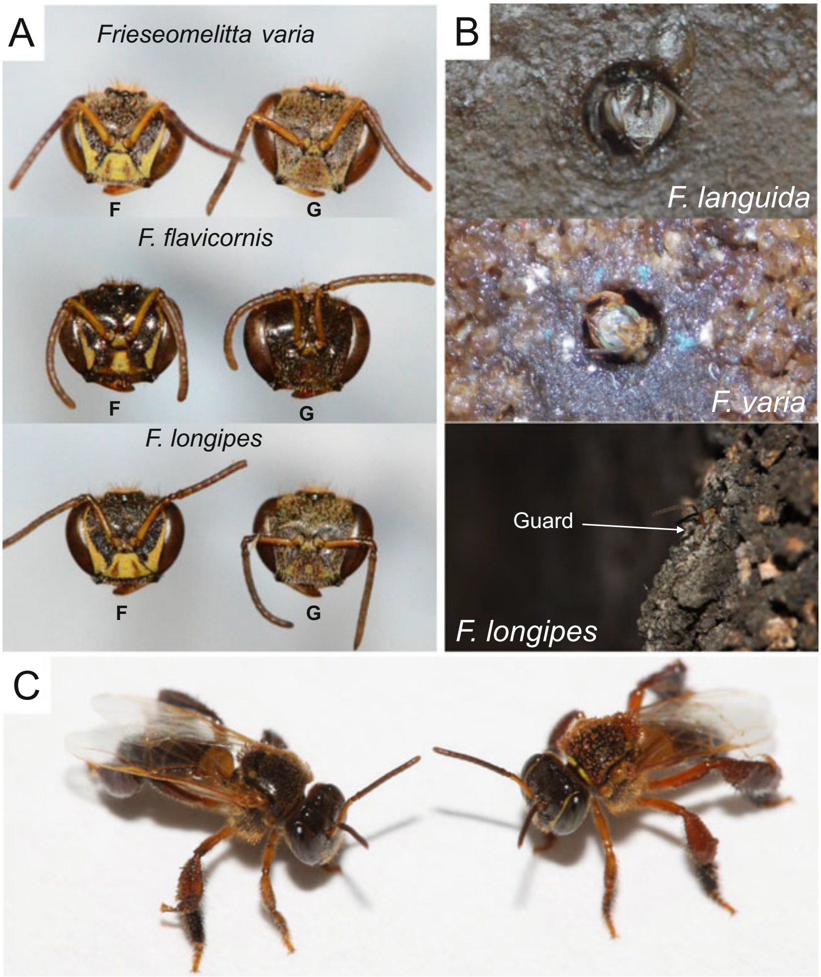
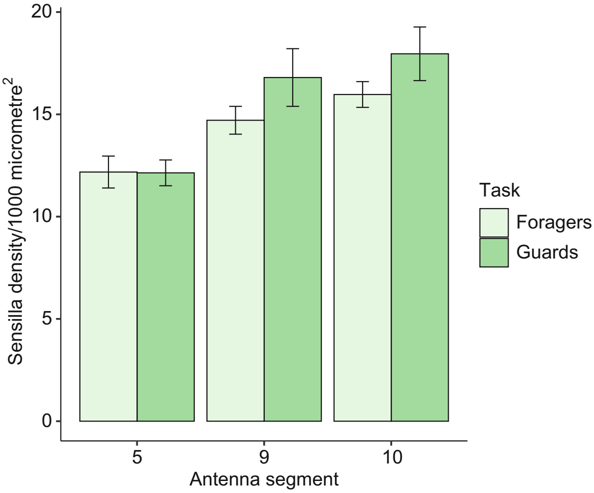


Fig. 6.8 (**a**) Heads of guards and foragers in three different *Frieseomelitta* species (Photos: C. Grüter). (**b**) *Frieseomelitta languida*, *F. varia* and *F. longipes* entrance holes. Entrance holes in *Frieseomelitta* are amongst the smallest in stingless bees (Couvillon et al. 2008). The darker species *F. languida* (and *F. silvestrii*) have darker entrances than species with lighter guards (*F. varia* or *F. longipes*). The workers cover the surface surrounding their nest entrance with sticky substances (Photos: first two by C. Grüter, third by Cristiano Menezes). (**c**) shows a guard (left) and a forager (right) of *F. flavicornis* (Photo: Cristiano Menezes)

and found indications that the length and number of some types of sensilla may also differ between soldiers and other workers in this species (see also Balbuena and Farina 2020).

The potential benefits of having larger guards for colony defence raise the question why not all stingless bee species have soldiers. A disadvantage of having specialised worker sub-castes could be an impaired ability of colonies to respond to

Fig. 6.9 Sensilla density (mean ± standard error) on antenna segments five, nine and ten of *Tetragonula carbonaria* foragers and guards. Density increases towards the tip of the antenna and is overall higher in guards than in foragers ($p = 0.037$, $N = 10$) (from Wittwer and Elgar 2018)



sudden environmental changes or changes in internal needs (Oster and Wilson 1978; Wheeler 1991). Workers that are very specialised might not be flexible in their behaviour (Oster and Wilson 1978; but see Mertl and Traniello 2009). Baudier et al. (2019) simulated a catastrophic predation event in *Tetragonisca angustula* by removing all guards. While colonies were able to partially compensate for the loss by reallocating smaller workers to guarding tasks, colonies needed several days to fully replace the lost guards. This slow collective response to the loss of a small, but vital group of workers (~1% of all workers are soldiers in *T. angustula*) could be costly for colonies.

Grüter et al. (2017a) hypothesised that the presence of larger guards is linked to the risk of robber bee attacks. *Lestrimelitta* robber bees diversified approx. 20–25 million years ago, which coincides with the estimated appearance of morphologically distinct guards (Grüter et al. 2017a). The idea that robbing by *Lestrimelitta* is an important driver of increased guard size in Neotropical stingless bees is supported by the observation that *Lestrimelitta* victim species are ~4 times more likely to have larger guards than species that are not known to be victim species: ~70% of victim species have guards of larger size, whereas only ~17% of non-victim species have larger guards. Whether the benefits of an increased specialisation of guards outweigh the potential costs of a less flexible workforce might, thus, depend on predation and parasitism risk.

Physical and temporal castes are not mutually exclusive strategies of division of labour, but are often found in combination (in ants: Wilson 1980; Seid and Traniello 2006; Camargo et al. 2007). In *Tetragonisca angustula*, small and large workers perform similar tasks when young, but large workers (soldier-sized) transition faster from task to task (Fig. 6.2) (Hammel et al. 2016). The behavioural repertoire seems to expand in both size classes as workers age (ants: Seid and Traniello 2006). Overall, soldier-sized workers had a larger task repertoire and were seen performing more work during the first 2 weeks after emergence (Fig. 6.7b) (Hammel et al. 2016). This contrasts with observations in ants where soldiers have a smaller behavioural repertoire than minors, i.e. they are more specialised and tend to work less (Wilson 1984; Beshers and Fewell 2001; Sempo and Detrain 2004).

6.4 Activity Distribution

Colony performance and resilience are not only likely to depend on the types of tasks that are performed and the number of workers performing them, but also on the intensity by which each individual performs the different tasks. Several studies in ants, honey bees and wasps have found highly skewed, non-random activity distributions, with few individuals performing most of the work and many others performing little work (Robson and Traniello 1999; Hurd et al. 2003; Pinter-Wollman et al. 2012; Tenczar et al. 2014). The same pattern has been found in *Melipona marginata* and *Tetragonisca angustula* (Fig. 6.10) (Hammel et al. 2016; Mateus et al. 2019). However, if we consider the lifetime work performance, rather than focusing on individual tasks, activity is less skewed (Fig. 6.10) and the remaining skew is mostly caused by early mortality rather than by consistent inactivity of workers (Hammel et al. 2016). The total number of behaviours that workers perform during their lifetime seems to follow an almost Gaussian distribution in three Neotropical species (Fig. 6.11).

6.5 Working Males and Queens

Males (or drones) do not usually perform altruistic work in eusocial hymenopterans and if working males are observed it is often not clear whether the behaviour is indeed altruistic or whether it has direct benefits for the males (Lucas and Field 2011). Several factors could explain the absence of male work: behavioural and morphological adaptations for brood care in solitary female ancestors might have predisposed females to perform offspring care (West-Eberhard 1975) and the lack of a sting could have precluded males from contributing to colony defence (Starr 1985). Furthermore, because of haplodiploidy, full sisters in Hymenoptera are more related to each other ($r = 0.75$) than are full brothers ($r = 0.5$), thereby causing an initial bias towards female altruism (Reeve 1993). Finally, the possibility to mate could incentivise males to allocate most of their resources to obtaining direct fitness benefits by mating, rather than indirect fitness benefits by helping others. Nonetheless, some examples of potentially altruistic work by males in some wasp and bumble bee species exist and males would be expected to contribute to work for which they have the required abilities.

Stingless bee males require 1–2 weeks to reach sexual maturity, after which they leave the nest (~7–12 days post-emergence in *Tetragonisca angustula*, Santos 2018; ~10–20 days of age in *Melipona*; van Veen et al. 1997; Velthuis et al. 2005; Bustamante 2006; Veiga et al. 2018). Drones that have left the nest do not seem to

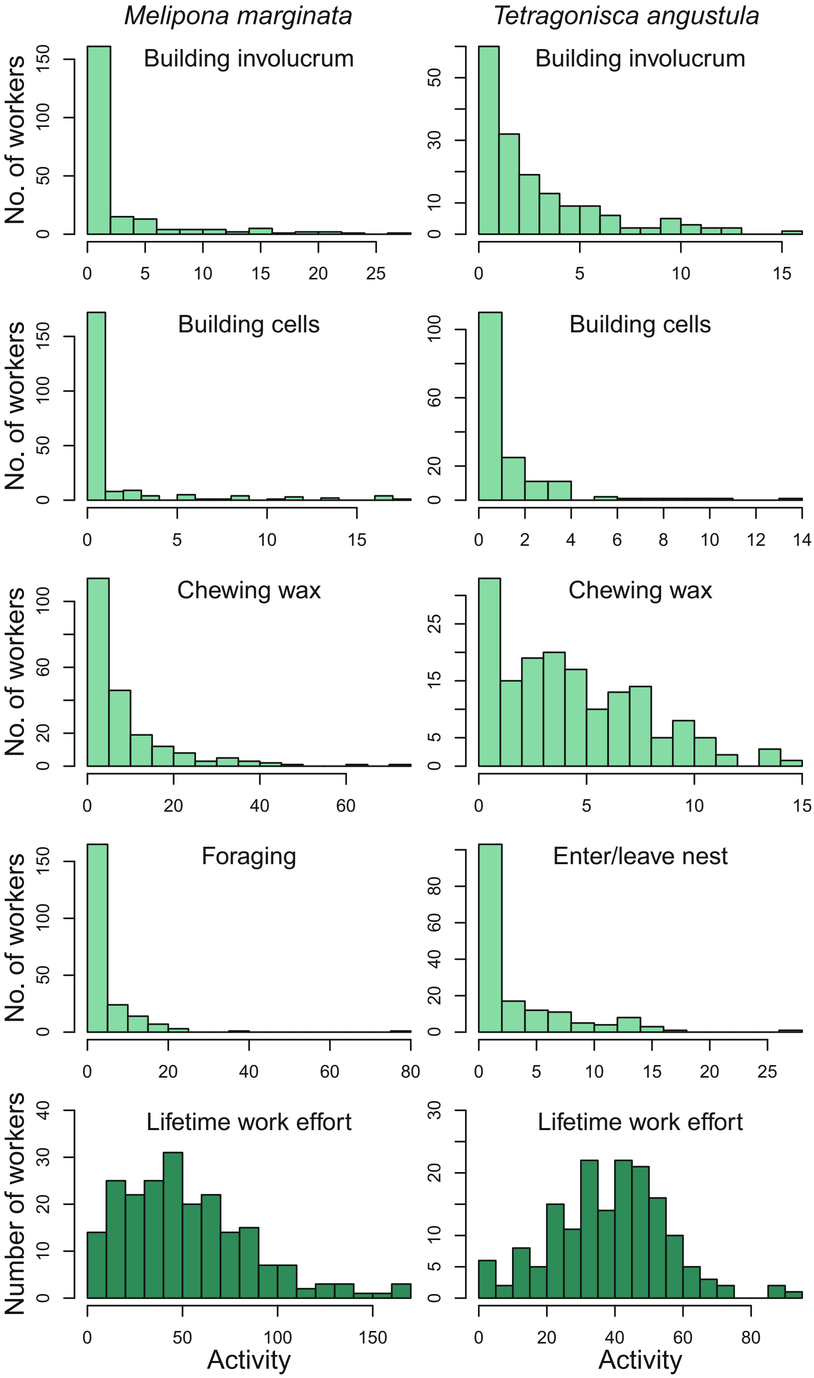
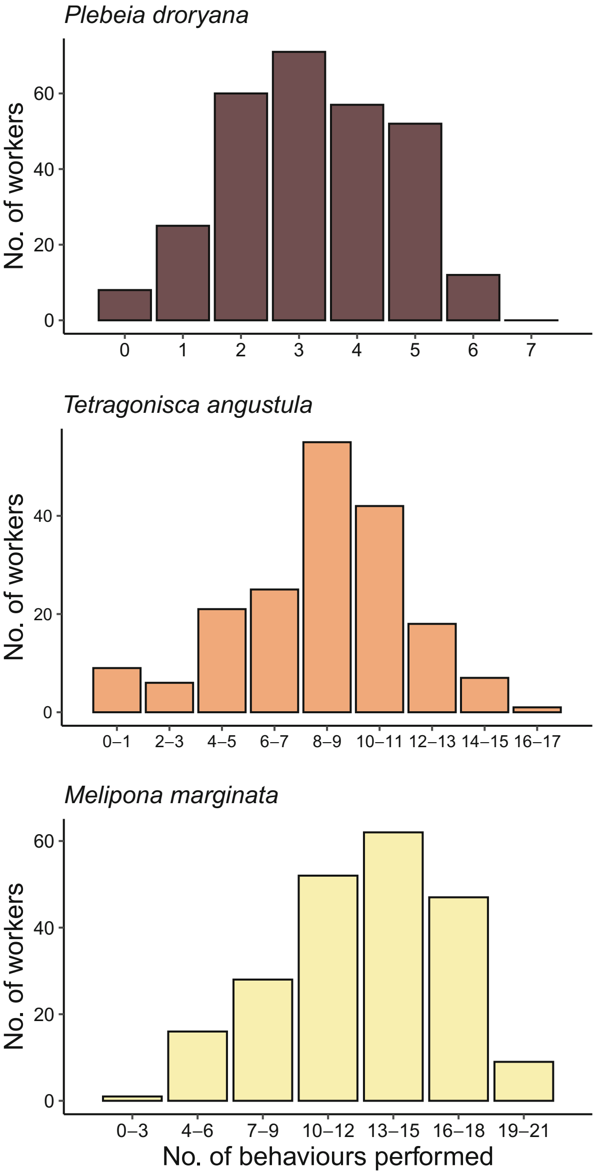


Fig. 6.10 Activity (measured as the number of times a worker was observed performing this behaviour) distribution in *Melipona marginata* and *Tetragonisca angustula* for four different tasks and lifetime work performance. Lifetime work performance is the total number of behavioural acts of all studied behaviours. Data recovered from Mateus et al. (2019) and Hammel et al. (2016)

Fig. 6.11 The total number of (observed) tasks performed by workers of three Neotropical species during their adult life (data from van Benthem et al. 1995; Hammel et al. 2016; Mateus et al. 2019)



return to it afterwards (see also Chap. 4) (van Veen et al. 1997; Veiga et al. 2018). Until they leave their home, however, drones could potentially contribute to colony life without sacrificing mating opportunities. Drones of the Neotropical species *Plebeia remota* (van Benthem et al. 1995), *P. droryana* (Cortopassi-Laurino 1979), *Schwarziana quadripunctata* (Imperatriz-Fonseca 1973), *Melipona beecheii*, *M. favosa*, *M. flavolineata* and *M. marginata* (Roubik 1989; van Veen et al. 1997; Veiga et al. 2018) have been observed to ripen nectar. Some drones were even seen to act as nectar receivers for returning foragers (Kerr 1997). However, since drones have not yet been observed depositing the concentrated honey into honey pots (Kerr 1997), it is not clear whether nectar ripening by males is altruistic or selfish. Nectar dehydration could be a strategy to decrease the weight and increase the energy content of their fuel, which could increase the manoeuvrability of males that are trying to mate with a queen (Cortopassi-Laurino 2007). However, males have been observed to contribute in other seemingly altruistic ways. For example, there are several accounts of males secreting wax (*Melipona compressipes*, *M. quadrifasciata*, *M. marginata*, *M. scutellaris*, *M. rufiventris*, *Nannotrigona testaceicornis*) and helping in building works (Sakagami 1982; Kerr 1997). Kerr (1951) observed *M. marginata* and *Plebeia droryana* colonies with more than a hundred males producing substantial amounts of wax. Kerr (1997) also described the behaviour of *M. compressipes* males as being similar to that of workers, with males removing the wax scales from their tergites and adding them to the wax deposits in the nest. He describes seeing males of *M. rufiventris* and *M. compressipes* using the wax to build small pillars, resource pots and involucrum sheets, but not brood cells. It is unclear how common wax production (which is unknown in honey bee drones) is in stingless bee males and a histological study of males of several species only found evidence for wax glands in the American *Melipona marginata* and the African *Meliponula bocandei*[9] (Cruz-Landim 1963, 1967).

Alarm pheromones produced by the mandibular glands play an important role in stingless bee colony defence (see Chap. 7) (Cruz-López et al. 2007; Schorkopf et al. 2009; Nunes et al. 2014). Schorkopf (2016) recently studied the mandibular glands of *Scaptotrigona depilis* males and found that they contain compounds that are also found in workers, although in smaller quantities. Among them was the alarm pheromone compound 2-heptanol. Simulating attacks on males of *S. depilis* and *S. bipunctata* at the nest entrance caused them to open their mandibles and guard workers to show defensive behaviours, suggesting that attacked males might be able to induce a defensive response in guards (Schorkopf 2016). Males exposed to the male mandibular compounds responded by fleeing.

Other potentially altruistic behaviours performed by males are the feeding of the queen (e.g. *Plebeia droryana*) (Cortopassi-Laurino 1979; Kerr 1997) or other males (*P. droryana*, *Melipona compressipes*, *M. quadrifasciata*, *M. beecheii* and *Scaptotrigona postica*) (Cortopassi-Laurino 1979; Kerr 1997; van Veen et al. 1997) and the apparent incubation of brood (Kerr 1997). Most of these observations are anecdotal and systematic studies on the contribution of males to the total

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[9] It has been noticed that males in *Melipona* can be similar in appearance to queen-worker intercastes (Michael Hrncir, personal communication). Thus, closer examination is required to determine whether these working "males" could be female intercastes.

workload of a colony do not yet exist, but these reports suggest that stingless bee males perform more work than honey bee males (van Veen et al. 1997). Kerr (1997) and Kerr et al. (2004) point out that stingless bee males and worker females are relatively similar in both behaviour and morphology due to their system of sex determination and brood rearing (Chaps. 1 and 5). Thus, stingless bee males might be better equipped for work than their honey bee counterparts. The work contribution of males could also explain why they appear to receive less aggression from workers than honey bee drones (Sakagami 1982), even though the expulsion and even the killing of males by workers has been observed in several species (Kerr 1951). In *Melipona beecheii* and *M. rufiventris*, males were observed to be dragged to the nest entrance by their wings at certain times of the year (von Ihering 1903; Schwarz 1948). In *Tetragonisca angustula*, aggression towards drones increases with the time drones stay inside their nest (Santos 2018).

Koedam et al. (2002) studied the production of wax by young virgin queens in *Melipona bicolor*. The wax produced by queens was of similar composition as the wax produced by workers, but queens produced wax during shorter periods than workers. Wax secretion has also been observed in virgin queens of *Scaptotrigona bipunctata* (Kerr and de Lello 1962) and in *Schwarziana quadripunctata*, virgin queens may even perform building work with wax (Imperatriz-Fonseca 1973).

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Enemies, Dangers and Colony Defence

7

Stingless bee nests contain valuable resources, such as honey, pollen, cerumen, batumen, resin, larval food and finally, the cavity and the bees themselves (Chap. 3). It is, therefore, unsurprising that other animals attempt to gain access to these resources. This, in turn, can lead to substantial predation-related mortality of stingless bee colonies. In Uganda, for instance, 12% of colonies from five species died from predation per year (Kajobe and Roubik 2006) and in a Costa Rican habitat, ~40% of all mortality was caused by predation (Slaa 2006). In an Indonesian population of *Tetragonula minangkabau*, 47% of all colonies died from either predation or parasitism (Inoue et al. 1993). Eltz et al. (2002), on the other hand, found that predation was rarely a cause for colony death in a Malaysian habitat.

In response to these external threats, stingless bees have evolved many fascinating defence traits, but they have also lost the most obvious defence trait, the sting (see also Chap. 1). The reduction of a functional sting during stingless bee evolution seems puzzling given that bee stingers can be a formidable weapon. However, the effectiveness of a sting might have been reduced due to the small body size of stingless bee ancestors (Melo 2020) and it is unclear if the sting would indeed be an effective weapon against the enemies that stingless bees face today. For instance, it is possible that stingless bees face more invertebrate enemies than honey bees and that stinging is less effective against enemies with an exoskeleton.[1] Honey bees are frequently outfought during aggressive interactions with similarly sized *Melipona scutellaris* workers (Schwarz 1948). In honey bees and some wasps, stinging an enemy often leads to considerable injuries and, as a result, death of the stinging insect. Thus, stinging can be costly and the benefits of stinging will depend on the enemy. Additionally, the development of a sting and the production of venom are likely to be energetically costly (Michener 1974). Thus, the loss of a functional

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[1] There are also examples showing that a bee sting can be effective against invertebrate enemies. For example, honey bees targeted by the European beewolf (*Philanthus triangulum*) occasionally manage to kill their attacker with their sting (Tobias Engl, pers. communication).

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Fig. 7.1 Inner side of the mandibles of three stingless bees and the honey bee. More aggressive species have a more painful bite and stronger toothed mandibles (from Shackleton et al. 2015)

stinging apparatus suggests that the costs of having a sting outweighed its benefits (Kerr and de Lello 1962).

The loss of a functional sting is compensated by other defensive traits. Defensive strategies vary greatly among stingless bee species and depend on the enemy. Species with smaller workers will often not attack large vertebrate enemies, but respond aggressively towards arthropods (Johnson and Wiemer 1982; Roubik 1989). Species that do attack large vertebrates often do so in large numbers and bees attempt to enter nostrils, ears, hair or they attack the eyes (Schwarz 1948; Kerr and de Lello 1962; Wille 1983; Roubik 2006; Shackleton et al. 2015). Roubik (1983) found that about half of 40 tested Neotropical species behaved aggressively towards humans. Aggression is particularly strong in species that build exposed nests (Michener 1974; Roubik 1983, 2006) and the most aggressive stingless bees (particularly some *Trigona* species) often continue to bite their victim for lengthy periods even if the latter has moved several hundred metres away from the nest (Schwarz 1948; Shackleton et al. 2015; pers. obs.). The reason for the increased aggression in species with exposed nests could be that they are more easily targeted by vertebrate enemies (Roubik 1983). More aggressive species also tend to have more painful bites and sharper teeth on the inside of the mandibles (Fig. 7.1) (Schwarz 1948; Shackleton et al. 2015). Shackleton et al. (2015) suggest that the unrelenting biting behaviour might be no less deadly for the biting bee than stinging is in honey bees.

There is not only variation in aggression among species but also within species (Roubik 1989). *Melipona fuliginosa*, for example, has been described as gentle by some and aggressive by others (Roubik 1989) and I have encountered *Tetragonisca angustula* colonies, a species that is usually very docile towards humans, which showed great willingness to attack me.

7.1 Who Are the Enemies?

7.1.1 Invertebrate Enemies

Ants

Among the most important enemies of stingless bees are ants (Schwarz 1948; Roubik 1989). *Azteca*, *Camponotus*, *Eciton* and *Pheidole* have been observed to invade and kill stingless bee nests in the Neotropics (Fiebrig 1908; Schwarz 1948; Roubik 1989). As a result, nest entrances often show signs of fights with ants (Fig. 7.2a) and some defensive traits seem to be aimed specifically at ant intruders (see Sect. 7.3). Managed colonies in meliponaries are particularly vulnerable because beekeeping often creates small gaps and holes through which ants can enter colonies (Schwarz 1948). *Atta* leafcutter colonies occasionally kill stingless bees nesting at the base of trees by covering them with excavated earth (pers. obs.). Ants, such as the Asian *Oecophylla smaragdina*, often hunt individual stingless bees at flowers (Fig. 7.2b) and Neotropical *Ectatomma tuberculatum* workers ambush guards (e.g. hovering and standing guards in *Tetragonisca angustula,* see Chap. 6) at the entrance of stingless bee nests (Ostwald et al. 2018).

Phorid Flies

Phorid fly larvae (e.g. *Pseudohypocera*) are a major pest of stingless bee colonies, particularly colonies that are weak or that are frequently manipulated by beekeepers (Nogueira-Neto 1997; Sommeijer 1999; Hernández and Gutiérrez 2001). Adult flies attempt to enter the nest (Fig. 7.2a) through the entrance, most likely attracted by

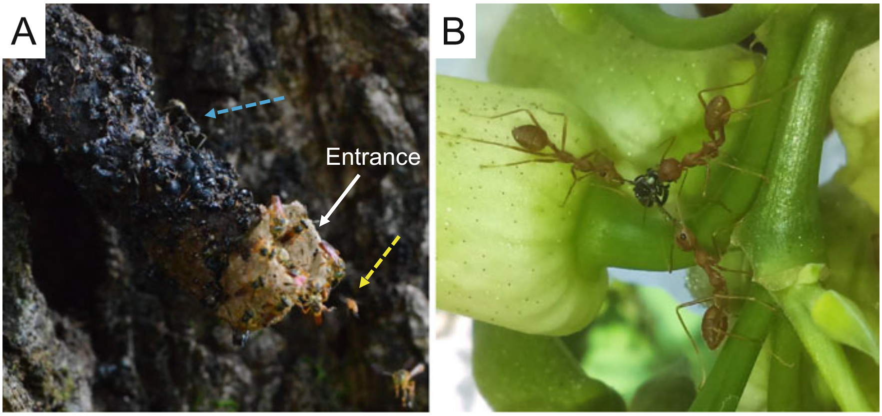

Fig. 7.2 Ants and phorid flies are major dangers for stingless bees. (**a**) Black dead *Camponotus* ants cover the entrance of this *Tetragonisca angustula* nest entrance in Brazil. One ant can be seen walking towards the nest entrance (blue arrow). Simultaneously, a phorid fly (yellow arrow) attempts to enter the colony. (**b**) *Oecophylla smaragdina* weaver ants often hunt for stingless bees at flowers; here, three ants pull on a *Tetragonula* forager captured near blossoms in Sri Lanka (Photos: C. Grüter)

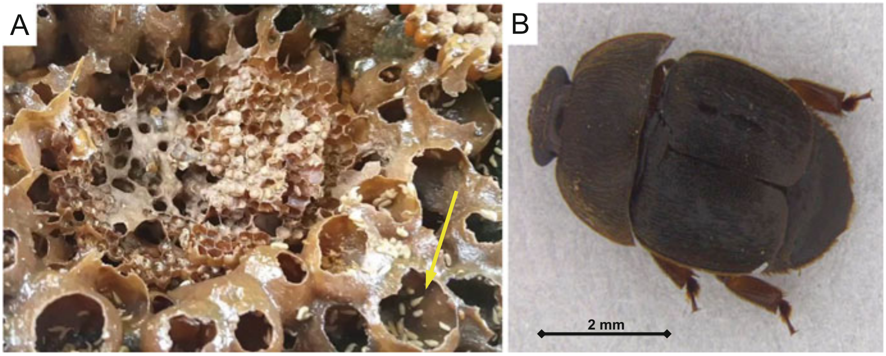

Fig. 7.3 (**a**) A *Melipona scutellaris* colony devastated by phorid fly larvae (yellow arrow) (Photo: Diego Vedovato). (**b**) The small hive beetle *Aethina tumida* (from Al Toufailia et al. 2017)

colony odours (Roubik 1989). Inside the nest, they produce large numbers of larvae that consume honey, pollen and immature bees (Fig. 7.3a) (Nogueira-Neto 1997). Some phorids also target adult foragers or males at aggregation sites (Simões et al. 1980; Brown 1997; Sommeijer et al. 2004; Santos et al. 2014). When Brown (1997) collected males from *Cephalotrigona capitata*, he found that nearly 50% were parasitised by *Apocephalus apivorus* phorid flies. This suggests that parasitism by phorids can represent a significant danger at male aggregations in this species (Brown 1997). It is not currently known whether similar levels of parasitism are common in other species. The Neotropical phorid genus *Melaloncha* (~170 species) targets worker bees near the nest entrance. Females inject an egg into the body of the worker through a membranous area between exoskeleton plates. The growing larvae consume and kill their host before pupation (Simões et al. 1980; Brown 2016). Up to 37% of examined *Scaptotrigona* workers were parasitised in a study by Simões et al. (1980).

Other Arthropods

Termites appear less often in reports about stingless bee enemies, but they have been observed to raid and kill small *Trigonisca atomaria* or weak *Melipona fasciata* colonies (Roubik 1989). Wasps occasionally enter stingless bee nests to steal resources (e.g. *Polybia*) or they hunt for foragers at flowers or for males in aggregations (Camargo and Posey 1990; Rasmussen 2008; Koedam et al. 2009). Up to 50 *Scaptotrigona postica* drones were predated per day by individual *Trachypus boharti* wasps in front of a single nest (Koedam et al. 2009), suggesting that these wasps can represent a significant risk for males in aggregations. In Australia and Asia, *Bembix* sand wasps hunt for *Tetragonula* workers to feed their larvae (Schwarz 1948; Rasmussen 2008). The braconid wasp *Syntretus trigonaphagus* is a parasitoid of the Australian stingless bee *Tetragonula carbonaria* (Gloag et al. 2009). Wasps lay their eggs on the abdomen of workers at the nest entrance or on flowers and wasp larvae subsequently develop in the abdomen of their

Fig. 7.4 A jumping spider (Salticidae) is hunting for guards and foragers at the entrance of a *Plebeia droryana* colony in Brazil. *P. droryana* uses small droplets of resin for defence against smaller enemies (Photo: C. Grüter)

host. Camargo and Posey (1990) report that bumble bees (*Bombus* sp.) have been observed to steal pollen from stingless bees in the Neotropics.

Beetles and their larvae can represent a significant risk for weakened colonies (see Sect. 3.5 for more details). One species that has received increasing attention is the small hive beetle *Aethina tumida* (SHB) (Fig. 7.3b), which is native to sub-Saharan Africa and an emerging threat to honey bee colonies in areas where the SHB has been introduced (Neumann and Elzen 2004). SHB feeds on stored food, larvae and adult bees and can devastate honey bee colonies in a short time period. These beetles seem to naturally occur in some African stingless bees and, as an introduced pest, are now found in colonies of Australian stingless bees (*Austroplebeia australis* and *Tetragonula carbonaria*) and on Cuba in *Melipona beecheii* colonies (see also Chap. 3) (Greco et al. 2010; Halcroft et al. 2011, 2013; Peña et al. 2014; Bobadoye et al. 2018). SHB was recently recorded for the first time in Brazil in honey bee nests (Al Toufailia et al. 2017). Nearby stingless bee colonies did not seem to be affected (Al Toufailia et al. 2017), but the spread of SHB and the vulnerability of Neotropical stingless bees should be closely monitored.

Spiders, and especially jumping spiders (Salticidae), commonly hunt for workers near nest entrances (Fig. 7.4) (Sakagami et al. 1983; Penney and Gabriel 2009; Shackleton et al. 2019). Spiders that come too close to the entrance are often attacked by guards, which causes spiders to retreat temporarily (Schwarz 1948, pers. obs.). In *Partamona helleri*, a species that builds a so-called “toad-mouth” entrance (see Chap. 3; Fig. 3.1), foragers enter the nest at a faster speed if jumping spiders are placed at the entrance, presumably because this reduces the risk of being captured by the waiting spider (Shackleton et al. 2019). Crab spiders (Thomisidae) and orb-weaver spiders (Araneidae) are also known predators of stingless bees (Schwarz 1948; Rasmussen 2008). Other predators that hunt stingless bees at their nest entrance or on flowers include mantises (Mantidae), assassin flies (Asilidae), assassin bugs (Reduviidae) and predatory mites (*Amblyseius*) (Schwarz 1948; Roubik 1989; Rasmussen 2008). Assassin bugs also prey on males in aggregations (Cortopassi-Laurino 2007). Mantis fly larvae of the species *Plega hagenella* can develop inside stingless bee brood cells where they kill the developing bee before pupating in the cell. *Melipona* colonies can be severely harmed by large numbers of *Plega hagenella* larvae (Maia-Silva et al. 2013). Leaf-cutter bees (*Megachile*) have been observed to steal wax or resin from the entrance tubes of *Tetragonula iridipennis* in India (Schwarz 1948). Wax moth larvae (the lesser wax moth *Achroia grisella*) consume wax and pollen from both New World and Old World stingless bees (Hockings 1883; Roubik 1989; Nogueira-Neto 1997; Cepeda-Aponte et al. 2002). Wax moth larvae, like many other pests, seem to thrive in weakened colonies (Cepeda-Aponte et al. 2002).

7.1.2 Vertebrate Enemies

Whether it is through the removal of honey, the destruction of habitat, pesticides, the introduction of non-native competitors or the removal of nests in urban areas, humans represent the biggest threat to stingless bees (see also Chap. 1) (Fowler 1979; Inoue et al. 1993; Antonini and Martins 2003; Cairns et al. 2005; Kajobe and Roubik 2006; Slaa 2006; Freitas et al. 2009; Venturieri 2009; Velez-Ruiz et al. 2013; Pioker-Hara et al. 2014; Barbosa et al. 2015). The removal of honey can, depending on the hunting tradition, lead to the destruction of many nests in a population. Inoue et al. (1993), for instance, found that honey harvesting was the most significant mortality factor in *Tetragonula minangkabau* colonies in a disturbed habitat in Indonesia: nearly 40% of all colony deaths could be attributed to human disturbance. The most dramatic threats to stingless bee communities come from large-scale alterations of the environment, mainly the conversion of natural habitats into urban or intensely farmed land. Habitat loss has led to the reduction of nest densities and the disappearance of species in severely affected areas (see also Chaps. 1 and 3) (Cairns et al. 2005; Slaa 2006; Brosi 2009; Freitas et al. 2009; Pioker-Hara et al. 2014).

Other primates that can threaten stingless bee colonies are chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla beringei*), baboons (*Papio anubis*) and several monkey species (Schwarz 1948; Kajobe and Roubik 2006; Estienne et al. 2017a). In a Ugandan forest reserve, humans and chimpanzees were responsible for 82% of all instances of stingless bee nest predation (Kajobe and Roubik 2006). Particularly, ground nesting species were affected. Estienne et al. (2017a), studying the ground nesting *Plebeiella lendliana* in Gabon, found that chimpanzee attempts to steal honey are more frequent in the dry season when other food sources are scarce. To circumvent the problem of a hard soil, the apes prefer to extract honey from nests that live in softer ground. To be more successful, chimpanzees can use complex tool sets to extract the honey from both ground and tree nesting stingless bees (Boesch et al. 2009; Estienne et al. 2017b).

The largest consumers of stingless bee products are probably elephants (*Loxodonta cyclotis*), which occasionally dig up colonies in the ground (Estienne et al. 2017a), as do honey badgers (*Mellivora capensis*), which open colonies during the night (Schwarz 1948; Estienne et al. 2017a). The Southeast Asian sun bear

(*Helarctos malayanus*) mainly consumes honey not only from *Apis dorsata* but also occasionally from stingless bees, such as *Tetragonilla collina* (Schwarz 1948; Eltz et al. 2002). In the Neotropics, the spectacled bear (*Tremarctos ornatus*) is known to destroy bee nests (Roubik 1989, 2006). Anteaters (*Tamandua* spp.), hog-nosed skunks (*Conepatus* spp.) and armadillo's can open nests to consume its content, the latter mainly those in the ground. The tayra (*Eira barbara*) is a dog-sized Neotropical mustelid who likes to steal honey from nests in the ground and in tree cavities (Schwarz 1948; Roubik 1989, 2006). Its name in Guaraní means "lord of the honey" (Schwarz 1948). Another animal with a well-known liking for stingless bee honey is the eyra cat or jaguarundi (*Herpailurus yagouaroundi*). The name eyra is derived from "ira" or "eira", which means "bee" in Tupi-Guarani languages (Schwarz 1948). Further, notable mammal enemies are the kinkajou or "honey bear" (*Potos flavus*, Procyonidae), the grison or South American wolverine (*Galictis* spp., Mustelidae) and coyotes (*Canis latrans*) (Schwarz 1948). There are anecdotal accounts of monkeys and coyotes extracting honey by dipping their tails into the honey pots (Schwarz 1948). Geckos and other lizards are known to hunt for adult bees at nest entrances (Schwarz 1948; Grüter et al. 2011) and toads (Bufonidae) can consume many adult workers if they can position themselves close to a nest entrance (Roubik 1989; Halcroft et al. 2013).

Woodpeckers (e.g. *Dryocopus lineatus,* Picidae) also occasionally eat stingless bees. Other bird enemies include bee-eaters (Meropidae), woodcreepers (Dendrocolaptidae), drongos (Dicruridae), jacamars (Galbulidae), herons (Ardeidae), kingbirds (Tyrannidae), flycatchers (e.g. *Myiarchus yucatanansis*, Tyrannidae) and swifts (Apodidae) (Schwarz 1948; Rasmussen 2008). In Australia, honeyeaters (Meliphagidae) occasionally add bees to their diet. A particular threat is the honeyguides (Indicatoridae). The African honeyguide species *Indicator indicator* has developed a mutualism with human honey-hunters to the detriment of honey bee and stingless bee nests (e.g. *Hypotrigona araujoi*) (Spottiswoode et al. 2016). Honeyguides will actively guide hunters to bee nests and, after the honey-hunters have opened the nests and removed the honey, the birds eat the leftover wax and larvae (Schwarz 1948; Spottiswoode et al. 2016).

7.1.3 Diseases

Stingless bees seem to be less affected by diseases than the Western honey bee *Apis mellifera* (Schwarz 1948; Nogueira-Neto 1997; Roubik 2006; Medina et al. 2009; Al Toufailia et al. 2016). This could indicate that stingless bees have more effective strategies of disease management and/or that their biology reduces exposure to diseases. For example, the immediate capping of brood cells after oviposition (Chap. 5) means that, unlike in the progressively provisioning honey bee, there is no direct contact between workers and brood, which could reduce the exposure of stingless bee brood to pathogens. Also the fact that brood cells are not reused (Chap. 3) could lower disease risk. Thirdly, the storage of food in distinct food pots, often distant from the brood area (Chap. 3), makes a contamination of brood cells via the collected resources less likely (Medina et al. 2009).

An important behavioural strategy to lessen the impact of diseases is hygienic behaviour. Honey bees open capped cells containing dead and infected larvae and pupae and remove them, which is likely to reduce the risk of transmission within the colony (Rothenbuhler 1964; Spivak et al. 2003; Bigio et al. 2013). Much less is known about hygienic behaviour in stingless bees, but experimental studies on several Neotropical species suggest that stingless bees show efficient hygienic behaviour (Medina et al. 2009; Nunes-Silva et al. 2009; Al Toufailia et al. 2016). When stingless bee brood was experimentally freeze-killed or pin-killed, between 50 and 99% of the dead brood was removed within 48 hours (Medina et al. 2009; Nunes-Silva et al. 2009; Al Toufailia et al. 2016; Jesus et al. 2017). However, there seems to be considerable inter- and intraspecific variation in hygienic behaviour, possibly due to differences in olfactory sensitivity of workers, and the removal of dead pupae seems to be less efficient than the removal of dead larvae (Jesus et al. 2017).

Our understanding of stingless bee health is still limited and there are likely to be many diseases that have not yet been discovered. For instance, Al Toufailia et al. (2016) observed that a considerable proportion of the brood of some *Scaptotrigona depilis* colonies had shrivelled wings (a typical symptom of Deformed Wing Virus, DWV, in honey bees) and, thus, likely suffered from a disease. DWV has indeed been shown to be common in other stingless bees in Argentina, Brazil and Mexico (Guzman-Novoa et al. 2015; Alvarez et al. 2018; Souza et al. 2019; Tapia-González et al. 2019; Guimarães-Cestaro et al. 2020).[2] A recent *Melipona scutellaris* colony decline in a Brazilian population has been linked to Acute Bee Paralysis Virus (ABPV) (Ueira-Vieira et al. 2015), a common virus in honey bees. The virus was detected in all 10 colonies from an affected area, whereas colonies from healthy populations did not carry the virus. In honey bees, symptoms of ABPV include a dark and shiny appearance due to hair loss, trembling body movements and an inability to fly (Miranda et al. 2010).

In southern Brazil, *Melipona quadrifasciata* colony losses occurring at the end of summer have been recorded since many years (Díaz et al. 2017; Caesar et al. 2019). As a result of these losses, *M. quadrifasciata* is now virtually absent in the wild in affected areas (Díaz et al. 2017). Symptoms shown by bees differed among locations and included disorientation, trembling, paralysis and walking with an extended proboscis, which are similar to symptoms shown by honey bees with ABPV and Israeli Acute Paralysis Virus (IAPV) (Díaz et al. 2017; Caesar et al. 2019). Caesar et al. (2019) identified several new viruses in affected colonies, but ABPV and IAPV did not seem to be responsible for the symptoms or the colony losses. Caesar et al.

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[2] Interestingly, *M. subnitida* colonies were mainly infected by the C-variant of DWV, whereas honey bee colonies from similar areas were affected by the A-variant. This raises the question whether DWV in stingless bees is the result of a spillover from honey bees or whether DWV might be a more general hymenoptera virus (Souza et al. 2019).

(2019) discuss the possibility that none of the identified viruses might be responsible for the colony losses, but that viruses replicate opportunistically in colonies weakened by unidentified pathogens or parasites. In sum, there is increasing evidence from several Neotropical areas that viruses that are known to affect honey bees are also found in stingless bee colonies. The effects that these viruses have on managed and wild stingless bee colonies are, however, poorly understood and there is clearly an urgent need for a better understanding of the impacts of these viruses on stingless bee health.

The first bacterial brood disease was recently discovered in the Australian *Tetragonula carbonaria* and the disease may also affect *Austroplebeia australis* (Shanks et al. 2017): colonies infected by *Lysinibacillus sphaericus* show weakened growth, pungent, rotten smell, darkened larvae and lethargic workers (Shanks et al. 2017). European Foulbrood is a well-known brood disease in honey bees, caused by the bacterium *Melissococcus plutonius*, and was recently discovered in *Melipona* colonies in Brazil (Teixeira et al. 2020). The affected colonies suffered from brood loss and some eventually died.

An emerging threat is the transfer of pathogens and diseases from managed honey bee or bumble bee colonies to other bees, e.g. via shared resources or robbing (e.g. Genersch et al. 2006; Meeus et al. 2011; Fürst et al. 2014; Guzman-Novoa et al. 2015; Purkiss and Lach 2019; Teixeira et al. 2020). The microsporidium *Nosema ceranae*, a pathogen that commonly affects honey bees, was detected in various species of stingless bees in Argentina (Porrini et al. 2017), Australia (Purkiss and Lach 2019) and Brazil (Guimarães-Cestaro et al. 2020; but see Nunes-Silva et al. 2016). Workers of the Australian *Tetragonula hockingsi* acquire *N. ceranae* spores from infected honey bees visiting the same flowers. Ingested *N. ceranae* spores germinate in the bee midgut, enter host cells and produce new spores within 2–4 days. *T. hockingsi* bees that ingested *N. ceranae* spores died at a much higher rate than non-infected bees (Purkiss and Lach 2019). European Foulbrood could be transferred from honey bees to stingless bees through the feeding of honey bee honey and pollen, a common practice in stingless beekeeping (Teixeira et al. 2020). However, the possibility that some of these diseases and pathogens represent natural pathogens of stingless bees cannot be excluded (Alvarez et al. 2018).

7.2 Robbing Bees and Robber Bees

One of the main threats to stingless bee colonies is other stingless bee colonies (Nogueira-Neto 1970; Laroca and Orth 1984; Bego et al. 1991; Sakagami et al. 1993; Grüter et al. 2016) and Schwarz (1948) argued that there are more instances of warfare between stingless bee colonies than between stingless bees and any other group of insects. However, this could also be explained by an observer bias (ant invasions could be more frequent at night) and by the fact that stingless bees are often studied in artificial boxes in high-density meliponaries. This is likely to promote warfare among bee colonies.[3] Nonetheless, attacks by other stingless bees do frequently occur in wild nests and this should not be surprising given that stingless bees can make the best use of all the resources raided from a stingless bee nest. Accordingly, cleptobiosis or cleptoparasitism (Iyengar 2008; Breed et al. 2012) is known in many species: more than 30 species have been described to attack other nests (including honey bee nests) (Grüter et al. 2016), either to steal resources or to usurp the nest, i.e. to swarm into an already occupied cavity (e.g. Gloag et al. 2008; Cunningham et al. 2014).[4] About 50 species have been reported to be victims of attacks by other bees (Grüter et al. 2016) and it is likely that robbing is a part of life for many stingless bee colonies. Usurpation as a swarming strategy could be particularly beneficial when competition for food sources or nest sites is intense (Foitzik and Heinze 1998; Quezada-Euán and González-Acereto 2002; Gloag et al. 2008; Rangel et al. 2010). Usurpation seems to be a common strategy in *Tetragonula hockingsi* and *Tetragonisca angustula* and maybe the only mode of swarming in *Lestrimelitta* robber bees (see also Chap. 4) (Schwarz 1948; Gloag et al. 2008; Cunningham et al. 2014; Grüter et al. 2016). A potential risk for invaders is the acquisition of diseases and pathogens from the attacked colonies, as was found in honey bees (Lindström et al. 2008; Breed et al. 2012).

Stealing the provisions of other stingless bee colonies is the obligate lifestyle of robber bees. They are represented by the Neotropical genus *Lestrimelitta* (~23 species, see Chap. 2) (Gonzalez and Griswold 2012) and the African genus *Cleptotrigona* (1 species, *C. cubiceps*) (Eardley 2004). These species are obligate cleptoparasites, which means that they are not visiting flowers to collect nectar or pollen (Müller 1874; Friese 1931; Michener 1946; Sakagami and Laroca 1963; Roubik 1989). Unfortunately, virtually nothing is known about the biology of *Cleptotrigona cubiceps* (see Portugal-Araújo 1958 for a rare description of its behaviour). Robber bee workers have reduced or absent corbiculae (Fig. 1.1) (Friese 1931; Parizotto 2010), but they can still use the hind tibiae to transport nesting material or pollen from raided colonies back to the robber bee nest (von Zuben and Nunes 2014). *Lestrimelitta* has powerful mandibles that allow them to kill workers of attacked colonies with ease (Nogueira-Neto 1970).[5]

The elusive *Trichotrigona* (two species) are probably also obligate robbers. Even though robbing behaviour has not yet been observed, the lack of a pollen comb (rastellum) on the hind legs and the absence of food stores in nests suggest that this genus is cleptoparasitic, possibly exploiting *Frieseomelitta* colonies (see Sect. 3.5.2)

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[3] The risks experienced by manipulated colonies in meliponaries are probably not entirely representative of the risks to wild colonies.

[4] Sometimes, robbers do not enter the colony, but steal cerumen or resin from the entrance tube (Schwarz 1948, pers. obs.). The same applies to Africanised honey bees, which I have often observed stealing resin on or next to entrances of stingless bee nests.

[5] The honey of *Lestrimelitta* robber bees has been described as being toxic, which could be the result of fermentation or the addition of secretions (see Chap. 1) (Friese 1931; Sakagami and Laroca 1963).

(Camargo and Moure 1983; Camargo and Pedro 2007). Overall, approximately 4–5% of all stingless bees are obligate cleptoparasites.

Another species that frequently attacks stingless bee nests is *Melipona fuliginosa,* the largest known stingless bee[6] (Nogueira-Neto 1970; Camargo and Pedro 2008). *M. fuliginosa* colonies can completely destroy strong colonies of other *Melipona* species within hours (A. Vollet-Neto, pers. communication). However, *M. fuliginosa* is not obligate robbers, but may raid other nests under particular environmental circumstances (Nogueira-Neto 1970). Other species that often show up as aggressors in reports of stingless bee attacks are *Oxytrigona* sp. and *Tetragonisca angustula* (Nogueira-Neto 1970; Roubik 1989; Grüter et al. 2016). The latter is somewhat unexpected given that *T. angustula* is a "mere midget" (Schwarz 1948) compared to the species it attacks.

The mortality experienced by attacking and defending colonies varies greatly among species and raids. In many instances involving *Lestrimelitta* robber bees, raids do not lead to fights and mortality of adult workers is close to zero (Nogueira-Neto 1970; Sakagami et al. 1993; Grüter et al. 2016). However, occasionally, robber bee raids inflict heavy losses (e.g. if they involve *Scaptotrigona* or *Tetragonisca angustula* colonies) (Johnson 1987; Nogueira-Neto 1970; Sakagami et al. 1993; Grüter et al. 2016). In raids of the African *Cleptotrigona cubiceps*, mortality depends on the host species. According to Portugal-Araújo (1958), attacks on *Hypotrigona araujoi* lead to much higher mortality than attacks on *H. braunsi*. Large-scale battles can lead to the death of hundreds or even thousands of workers (Schwarz 1948; Nogueira-Neto 1970; Hubbell and Johnson 1977; Johnson 1987; Sakagami et al. 1993; Cunningham et al. 2014; Grüter et al. 2016). For example, the Australian *Tetragonula carbonaria* and *Tetragonula hockingsi* engage in large-scale intraspecific and interspecific battles for nest sites during which thousands of workers from both attacking and defending colonies can die (Cunningham et al. 2014). Schwarz (1948) describes a fight between two *Melipona scutellaris* colonies that resulted in the death of about 3000 bees. Nearly 8000 bees died in an attack of *Lestrimelitta* robber bees on a colony of *Trigona fulviventris* (Johnson 1987). The robber bee colony apparently won this battle but also lost 1162 of its own bees. These costs do not seem to justify an attack on such an aggressive *Trigona* colony and Johnson (1987) speculated that the attack escalated by mistake because the *T. fulviventris* alarm pheromone (nerol) is similar to citral, the putative recruitment pheromone used by *Lestrimelitta* (see below).

Severe levels of mortality on both sides can make sense if the winner wins the ultimate price, a new nest with all its provisions and, indeed, colony death caused by another stingless bee colony is well-known (Müller 1874; Michener 1946; Schwarz 1948; Portugal-Araújo 1958; Sakagami and Laroca 1963; Nogueira-Neto 1970;

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[6] *Melipona fuliginosa* workers weigh ~125 mg (Roubik 1989, Table 2.3). Another species of the *fuliginosa* species group, *M. titania*, is about the same size (Camargo and Pedro 2008). In comparison, the average weight of the giant honey bee *Apis dorsata* foragers ranges from 107 to 118 mg (Tan 2007).

Johnson 1987; Bego et al. 1991; Sakagami et al. 1993; Quezada-Euán and González-Acereto 2002; Pompeu and Silveira 2005; Cunningham et al. 2014; Mascena et al. 2017). Even raids without adult mortality can potentially kill the raided nest because colonies often loose most of their food stores and the removal of larval food from cells kills all eggs and the young larvae developing in the cells (Grüter et al. 2016). As a result, colonies might die weeks or months after an attack. Occasionally, robbers become the robbed and both *Tetragonisca angustula* and *Scaptotrigona pectoralis* have been observed to attack and kill *Lestrimelitta* nests (Schwarz 1948; Sakagami et al. 1993; Nogueira-Neto 1997).

7.2.1 The Organisation of Attacks

We know little about the recruitment and communication processes involved in the coordination of attacks. Studies on *Lestrimelitta* show that robber bee raids involve from a few dozens to more than thousand robber bees, but they usually start with one or a few scouts identifying a suitable target (Wittmann 1985; Sakagami et al. 1993). Shortly afterwards, a larger group of robber bees arrives at the nest of the victims (Sakagami et al. 1993; Grüter et al. 2016; Mascena et al. 2017). Robber bees start to guard the entrance of the attacked colony and often build their own entrance tube (Sakagami and Laroca 1963; Nogueira-Neto 1970; Mascena et al. 2017). Pheromones are likely to play an important role in the recruitment of more robber bees. One pheromone that has been suspected to function as a recruitment signal is citral (Blum et al. 1970; Wittmann et al. 1990; Sakagami et al. 1993; van Zweden et al. 2011; von Zuben et al. 2016), a lemon-like odour produced in the mandibular glands of robber bees. Furthermore, volatiles emitted from the labial glands (mainly the esters hexadecyl acetate and 9-hexadecenyl acetate) also seem to play an important role during attacks of *Lestrimelitta limao* (von Zuben et al. 2016). Labial gland compounds are used by several stingless bee species to recruit food sources (Chap. 10) (Jarau et al. 2004, 2006, 2010; Schorkopf et al. 2007, 2009; Stangler et al. 2009) and *Lestrimelitta* may have co-opted these pheromones for recruitment during raids. In the “firebee” *Oxytrigona* spp., a frequent robber of nest building material and food stores (Roubik et al. 1987; Rinderer et al. 1988; Grüter et al. 2016), cephalic compounds seem to be similarly important during raids (Rinderer et al. 1988). It is not clear, however, if these compounds act as recruitment pheromones or as a repellent of host defenders. The observation that introduced honey bees seem to respond in a disoriented fashion when attacked by *Oxytrigona* supports the latter function (Rinderer et al. 1988).

During a *Lestrimelitta* raid, returning host foragers often do not enter their nest, but remain hovering in front of the entrance or they land on nearby vegetation (Michener 1946; Kerr 1951; Nogueira-Neto 1970; Sakagami et al. 1993). This phenomenon has led to the hypothesis that robber bees produce pheromones that disrupt defensive behaviours (“superseding odour” hypothesis) (Kerr 1951; Moure et al. 1958; Blum et al. 1970). This assumes that the response of the host is in the interest of the attacker, rather than the host. Even though it has been suggested that citral, released from the mandibular glands, is responsible for the host response (Blum et al. 1970), *Frieseomelitta varia* foragers returning to their nest responded to the labial gland compounds of *Lestrimelitta* and stopped entering their colony (von Zuben et al. 2016). The respective functions of labial (esters) and mandibular compounds (citral) during robber bee raids remain poorly understood. Nogueira-Neto (1970) argued that the behavioural response of the host towards robber bee pheromones is in the interest of the victim colony and helps attacked colonies to avoid larger losses ("retreat message" hypothesis). According to this view, the robber bee pheromones function as alarm kairomones (i.e. semiochemicals that affect the behaviour of heterospecifics in ways that benefit the receiver of the message) (Wittmann 1985; Roubik 1989; Kärcher and Ratnieks 2009).

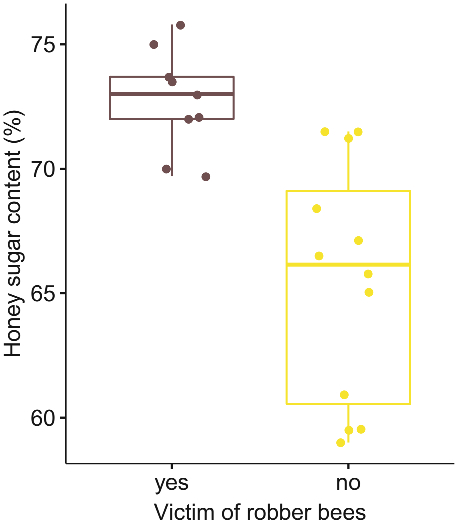
Robber bee raids vary considerably in their duration (Sakagami and Laroca 1963; Nogueira-Neto 1970; Sakagami et al. 1993; Grüter et al. 2016). They can last from a few hours to several days or weeks, until most resources of the victim have been removed. Some colonies are attacked repeatedly (Sakagami and Laroca 1963; Roubik 1989), possibly by the same robber bee colony, suggesting a role of learning in host selection. Nogueira-Neto (1970) hypothesised that learning from past attacks also affects the defensive behaviour of guards. He described a *Plebeia droryana* colony attacked by *Lestrimelitta limao* that seemed to have learned not to attack the invading robbers, possibly through some habituation process (see also Weaver et al. 1975). Nogueira-Neto (1970) also suggested that robber bees might prefer to return to colonies that were successfully raided in the past (see also Sakagami et al. 1993). It would make sense for robbers and victims to acquire information during raids, which helps them to respond more effectively in the future, but learning during raids has not yet been studied experimentally.

In the case of *Tetragonula hockingsi* colonies attempting to usurp *T. carbonaria* nests, fights vary from small skirmishes lasting a few hours to large-scale attacks that last for several weeks and can result in thousands of casualties (Cunningham et al. 2014). The reasons for this variation are not clear, but it is possible that colonies assess each other's strength and aggressor colonies abort attacks if their opponent colony is too strong.

7.2.2 Factors Affecting Vulnerability to Being Raided

Some species appear to be attacked more often by robber bees than others (Nogueira-Neto 1970; Sakagami et al. 1993; Roubik 1989; Quezada-Euán and González-Acereto 2002). For example, the African *Cleptotrigona cubiceps* more frequently attacks *Hypotrigona braunsi* than the sympatric *H. araujoi* (Portugal-Araújo 1958) and *Lestrimelitta* frequently attacks *Plebeia*, *Nannotrigona* and *Scaptotrigona* (Nogueira-Neto 1970; Laroca and Orth 1984; Roubik 1989; Bego et al. 1991; Sakagami et al. 1993; Grüter et al. 2016). This indicates host preferences by robber bees, but there also seem to be regional differences in how vulnerable particular species are (Grüter et al. 2016). Emery's rule states that social parasites are often closely related to their hosts (Emery 1909). Given the wide range of species that are

Fig. 7.5 Sugar content of honey of victim and non-victim stingless bee species inhabiting the same areas (GLS with Brownian motion: t-value = 2.94 and $p = 0.0084$) (modified from Grüter et al. 2016)



attacked by *Lestrimelitta* (including both closely, e.g. *Nannotrigona*, and distantly related species, e.g. *Melipona*), this pattern does not seem to apply in the case of *Lestrimelitta* robber bees. On the other hand, there are suggestions that *Trichotrigona* specialises in stealing the provisions of closely related *Frieseomelitta* colonies (see above).

How attackers choose their victims is still poorly understood, but there is evidence that *Lestrimelitta* robber bees prefer to attack species that produce honey with a higher sugar content (Fig. 7.5) (Quezada-Euán and González-Acereto 2002; Grüter et al. 2016). This is in line with findings showing that the energetic value of resources affects their exploitation in other stingless bees (see Chaps. 8 and 10) (Roubik 1989; Biesmeijer and Ermers 1999; Schmidt et al. 2006) and honey bees (e.g. von Frisch 1967; Seeley 1995). Whether the quantity of food stored in colonies also affects robber bee preferences remains to be investigated. In the context of usurpation, cavity and entrance size are likely to play an important role. For instance, it is unlikely that cavity nesting *Lestrimelitta* colonies attempt to usurp much smaller species because the latter use small cavities or species that nest in the ground or in self-constructed nests.

Invaders could target smaller colonies because they are defended by fewer bees, which is likely to reduce the mortality costs for the attacking colony. Colony size of both attacker and attacked is particularly important if the outcome of attacks follows the "square law" of combats (Whitehouse and Jaffe 1996; McGlynn 2000). Here, workers are killed at a rate that is proportional to the number of opponents and the colony with the greatest number of available fighters will prevail. Nest usurpation in *Tetragonula hockingsi* and *T. carbonaria* seems to fit this scenario (Cunningham et al. 2014). On the other hand, if the size of a particular colony is small because of a disease or parasite, attackers that prefer small colonies risk acquiring these pathogens (Michener 1974; Lindström et al. 2008; Breed et al. 2012). Furthermore, smaller colonies probably have fewer resources, which can favour a preference for larger colonies (Pohl and Foitzik 2011).

Colony size does seem to affect the outcome of raids. For example, *Lestrimelitta limao* has been observed to destroy small *Melipona rufiventris* colonies, whereas strong *M. rufiventris* colonies are more successful at resisting robber bees (Pompeu and Silveira 2005). Likewise, small *Apis mellifera* colonies are less able to prevent robber bee invasion (Nogueira-Neto 1997). However, at species level, colony size does not seem to predict robber bee preferences (Grüter et al. 2016). Another important factor could be bee size, which has indeed been shown to affect the outcome of fights between stingless bees (Dworschak and Bluethgen 2010; Grüter et al. 2012). However, at species level, bee size does not seem to predict whether robber bees frequently attack the colonies of a particular species (Kerr 1951; Grüter et al. 2016) and both *Lestrimelitta* and *Cleptotrigona* robber bees are relatively small compared to the species they frequently attack. For example, *Lestrimelitta limao* weighs ~13 mg and commonly attacks bees that are much larger, such as *Melipona* (~50–100 mg) (Grüter et al. 2016). *Melipona fuliginosa*, on the other hand, might benefit from its large body size and strength when attacking other nests (see above).

Preventing an attack often depends on the ability of entrance guards to recognise an intruder. Intruders might attempt to prevent detection using chemical mimicry, camouflage or “chemical insignificance” (Lenoir et al. 2001; Uboni et al. 2012; Grüter et al. 2018). Quezada-Euán et al. (2013) found that the chemical surface profile of *Lestrimelitta niitkib* is more similar to the chemical profile of their preferred host species than to the profile of non-preferred species. However, it is not yet clear whether this is a case of chemical mimicry or whether robber bees acquire some of the chemical compounds as a result of robbing resources from other colonies. Guards of most colonies that are attacked by robber bees strongly change their behaviour in the presence of robber bees, either by showing aggression or avoidance, which suggests that they are able to detect *Lestrimelitta* robbers (e.g. Nogueira-Neto 1970; Sakagami et al. 1993; van Zweden et al. 2011; Nunes et al. 2014).

7.2.3 Variation in the Defensive Response

While some species mount a very aggressive defence when being attacked by robber bees, others show hardly any aggression (Nogueira-Neto 1970; Sakagami et al. 1993; Grüter et al. 2016). Such a “retaliation vs. toleration” dichotomy has been observed in other animal taxa that face cleptoparasitism (Iyengar 2008). Avoiding fights has the advantage that most adult workers and some of the food stores are spared and costs are relatively predictable. Resources can be partially salvaged by the workers of an attacked colony by ingesting liquid foods during an attack (e.g. *Aparatrigona*, *Nannotrigona* or *Plebeia*) to store it in the communal crop. Thus, workers can use their body for temporary storage and hide in the corners or under the involucrum until the raid is over (Fig. 7.6) (Nogueira-Neto 1970; Grüter et al. 2016).

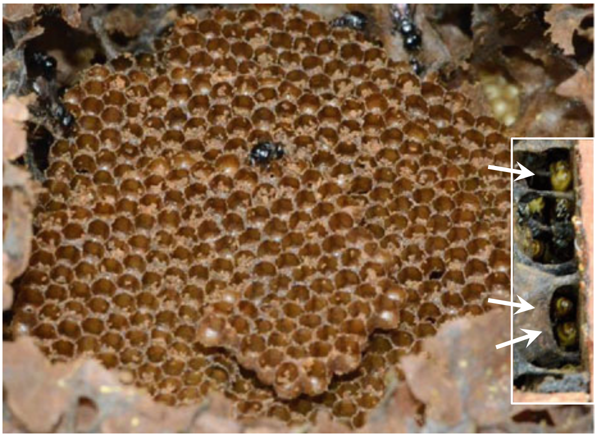


Fig. 7.6 *Aparatrigona* sp. brood nest during an attack of *Lestrimelitta maracaia*, near Belém in Brazil. The robber bees have removed the larval food from brood cells. Workers of the attacked colony are hiding in the corners of the nest box (white arrows). Many workers have extended abdomen, presumably because they store food in their crop (Photo: C. Grüter)

If the attacked colony retaliates, costs could be more unpredictable (Grüter et al. 2016). If aggressive guards can prevent an attack at an early stage, e.g. by killing robber bee scouts, then costs are low. However, if a full-scale invasion cannot be prevented, mortality costs can be very high as colonies lose their food stores, eggs, larvae and many adult workers.

7.3 Defence Strategies

Some of the most intriguing behavioural and architectural traits found in stingless bees are evidently a response to the risk of being attacked by other animals and colonies invest a significant amount of their resources into colony defence, either in the form of guarding, the collection of large amounts of resin or defensive nest structures.

7.3.1 Avoiding Attacks

A potentially very successful strategy is to avoid being attacked in the first place. This could be achieved either by nesting in habitats with fewer enemies, by camouflage, by mimicking other animals or by nesting in close proximity to colonies that offer some protection (Kerr and de Lello 1962; Rech et al. 2013; Grüter et al. 2018). It is not currently known whether stingless bee colonies prefer to nest in safer environments, but there is evidence that the swarming process is occasionally aborted (Chap. 4) and that disturbing nests that are in the process of being established can be a reason for the sudden end of the swarming process (pers. obs.). Thus, the reconnaissance stage of swarming (Chap. 4) could allow scouts to assess whether enemies are present near the new nest site.

Ground nesting species could attempt to nest in cavities that are more difficult to reach. For example, chimpanzees are less likely to find nests if the horizontal distance between the nest chamber and the entrance hole at the surface is larger because this creates uncertainty regarding the location of the nest (Estienne et al. 2017a). Camouflage could include having a very small and inconspicuous entrance or having entrance guards that colour-match the surroundings of the entrance (see also Chap. 6) (Grüter et al. 2017a).

Living in close proximity to termite or ant nests (Chap. 3) could have the advantage that attackers not only have to overcome bee guards but potentially also aggressive ants or termites. Some bee keepers take advantage of differences in aggression and place poorly defended stingless bee colonies close to highly defensive stingless bees (Nogueira-Neto 1997; Rech et al. 2013). Finally, Schwarz (1948) and Kerr (1951) describe several cases of Batesian mimicry, i.e. cases where stingless bees look or behave similar to bees with a sting or wasps. However, it is often not clear if the resemblance is coincidental or indeed a case of Batesian mimicry. One putative case of Batesian mimicry is the dark wing colouration with a white tip found, for example, in some *Frieseomelitta* (e.g. *F. silvestrii*, *F. flavicornis* or *F. varia*), *Tetragonilla* and *Tetrigona* species, which resembles the dark wings with white tips of some social wasps (e.g. *Parachartergus fraternus*) (Kerr 1951; Kerr et al. 1967).

7.3.2 Entrance Guards

If invaders detect a potential victim colony, nest entrance guards will usually attempt to prevent them from entering the nest (Fig. 3.7) by attacking and by recruiting more defenders through the release of alarm pheromones (Cruz-López et al. 2007; Gloag et al. 2008; Schorkopf et al. 2009; Nunes et al. 2014; Jernigan et al. 2018). Stingless bee guards can be very dedicated to their task and perform guarding for many days (compared to about 1 day on average in *Apis mellifera*; Moore et al. 1987). In *Tetragonisca angustula*, a species with guards of larger size (soldiers, see below), guards were seen at the nest entrance for an average of ~5 days (Hammel et al. 2016), but some bees guard for nearly 3 weeks (see also Chap. 6) (Grüter et al. 2011; Baudier et al. 2019). The number of active guards depends on the time of day and is lowest in the early and late hours with daylight (Fig. 6.6) (Grüter et al. 2011; van Oystaeyen et al. 2013). During the night, guards are usually inside the nest (Schwarz 1948), but a few can occasionally be seen inside the entrance tube (Schwarz 1948; pers. obs.) and Michener (1946) described a *Partamona testacea* colony that attacked humans disturbing the nest at night no less ferociously than during the day. Some guards take up the exact same location on or next to the entrance, from morning to afternoon and for several days (Zeil and Wittmann 1989; Kelber and Zeil 1997; Grüter et al. 2011).

Enemy recognition is straightforward in most cases as intruders look and/or smell different and guards can use both visual and chemical cues for recognition (see below). The first signs of an aggressive response by guards towards a potential threat are often the spreading of the wings to a V-shape or wing vibration (Johnson and Wiemer 1982). The next level is the biting of the opponent (Kerr and de Lello 1962;

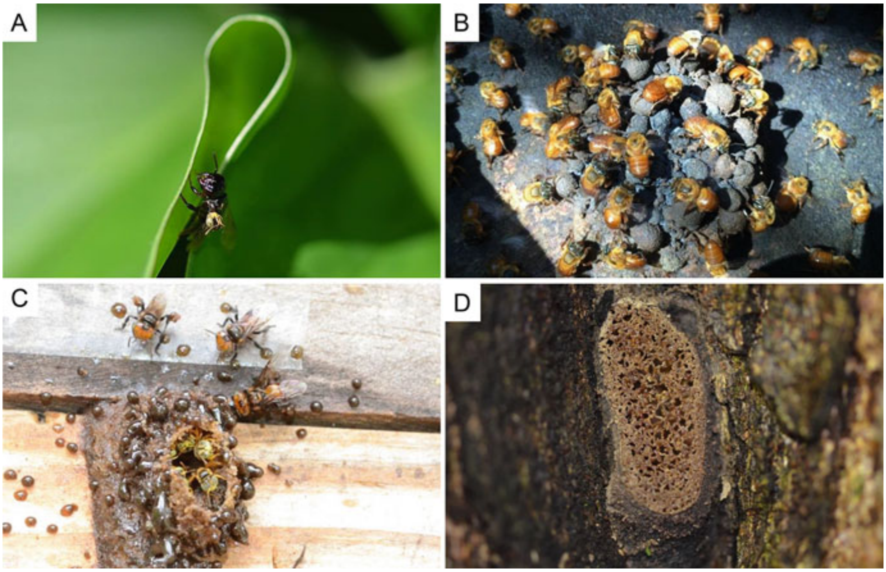


Fig. 7.7 Defence traits in stingless bees. (**a**) A *Tetragonisca angustula* guard biting the base of the wing of a *Lestrimelitta limao* robber bee. The mandibles of the guard remain locked even after decapitation, making the robber bee unable to fly (Photo: C. Grüter). (**b**) A pile of batumen balls in a colony of *Melipona flavolineata* (Photo: Túlio Nunes). (**c**) *Tetragonisca angustula* attacked by *Oxytrigona tataira*. Guards deposit droplets of resin near the nest entrance to deter the enemy (Photo: C. Grüter). (**d**) Many colonies (here *Nannotrigona testaceicornis*) close their entrance at night using soft cerumen. The cerumen cover has small holes that allow the exchange of air (Photo: C. Grüter)

Michener 1974; Shorter and Rueppell 2012; Shackleton et al. 2015). Biting into the base of the wings of opponents is a common and successful strategy to immobilise a winged attacker (Fig. 7.7a) (Schwarz 1948; Sakagami and Laroca 1963; Grüter et al. 2012). Some species, *Oxytrigona* in particular, use caustic chemical secretions against vertebrate enemies (Michener 1974; Rinderer et al. 1988; Roubik 1989). These "fire bees" ("cagafogo" in Brazil[7]) spit formic acid mixed with diketones and other compounds (possibly proteinaceous venoms) on the attacker, which causes a burning sensation and blistering of the skin (Michener 1974; Roubik et al. 1987; Rinderer et al. 1988). In humans, encounters with *Oxytrigona* often lead to permanent skin scars (Michener 1974). Probably connected to this is the observation that "fire bees" have unusually large mandibular glands that occupy a large part of the head capsule (Michener 1974; Roubik et al. 1987). *Oxytrigona* is also unusual in

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[7] "Cagafogo" literally means "shits fire" and is, thus, slightly misleading because the caustic substances are released from cephalic glands, *i.e.* they "spit fire". The name *O. tataira* is derived from the indigenous "tata-ira", "eira-tata" or "ei-tata" ("fire bee" in the Tupi and Guarani languages) (Schwarz 1948).

having a red head (Fig. 7.7), which represents a rare case of warning colouration (aposematism) in stingless bees.

In Australian *Tetragonula*, colonies exhibit defensive swarming in response to attacks by other stingless bee colonies (Gloag et al. 2008). Hundreds of workers swarm in close proximity to the nest entrance. This collective response can be triggered by the presence of a small number of non-nestmate stingless bees near the entrance (Gloag et al. 2008; Cunningham et al. 2014). Defensive swarms could also have the function of a visual display of strength to deter the opponent colony from mounting a full-scale attack (Grüter et al. 2016). A similar mass exodus of workers can be seen in some *Melipona* or *Tetragonisca angustula* colonies in response to the chemical cues of *Lestrimelitta* robber bees (Wittmann et al. 1990; Segers et al. 2016; Jernigan et al. 2018; Campollo-Ovalle and Sánchez 2018).

7.3.3 Alarm Pheromones

It is currently unknown how widespread the use of alarm pheromones is in stingless bees (Roubik 1989; Campollo-Ovalle and Sánchez 2018), but there is little doubt that alarm pheromones are used by many species when under attack. However, only a few of the alarm substances have been identified. A common feature is that they are produced predominantly in the mandibular glands and that they are highly volatile (Blum et al. 1970; Luby et al. 1973; Weaver et al. 1975; Johnson and Wiemer 1982; Smith and Roubik 1983; Cruz-López et al. 2005, 2007; Schorkopf et al. 2009; Alavez-Rosas et al. 2019). Lindauer and Kerr (1958) proposed that substances from the mandibular glands are used as both alarm pheromones and trail pheromones in foraging (“one gland-two function” hypothesis), but more recent research does not support a role of mandibular gland compounds as trail pheromones (Chap. 10) (e.g. Jarau et al. 2004; Schorkopf et al. 2009; Solórzano-Gordillo et al. 2018). Instead, mandibular gland compounds predominantly function as alarm pheromones, whereas trail pheromones often originate from the labial glands (Chap. 10).

Alarm pheromones can have a strong smell, which—depending on the species—is perceived as pleasant or unpleasant by humans (e.g. “rancid-butter” or “blue cheese”-like in some *Oxytrigona*, *Scaptotrigona* and *Trigona* species, but pleasantly lemon or rose-like in others[8]) (Schwarz 1948; pers. obs.). In *Trigona fulviventris*, nerol seems to be the key compound of the alarm pheromone (Johnson and Wiemer 1982). Citral, with its pleasant lemon-like smell, seems to function as an alarm pheromone in *Geotrigona subterranea* in a dose-dependent fashion (Blum et al. 1970; Schorkopf et al. 2009). In two *Scaptotrigona* species and in *Tetragonisca angustula*, benzaldehyde is an important component of the alarm pheromone (Luby et al. 1973; Wittmann et al. 1990; Jernigan et al. 2018). In *Melipona triplaridis*,

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[8] Stingless bee odours can also make colonies more vulnerable as natives in Australia and South America use these odours to locate stingless bee nests (Schwarz 1948, pp. 80).

skatol, which has a strong faecal odour, might function as both an alarm pheromone and a deterrent for vertebrates (Smith and Roubik 1983; Wille 1983). Skatol is also released by army ant raiding parties and might therefore have a strong deterrent effect on smaller enemies that are potential victims of army ants (Roubik 1989). Geraniol acetate and farnesyl acetate induce aggression in *Melipona beecheii* workers exposed to concentrations that are similar to those found in mandibular extracts of this species (Cruz-López et al. 2005). Some of the identified compounds also function as alarm substances in other social insects or are known repellents, e.g. benzaldehyde in some ants and honey bees (Luby et al. 1973; Schorkopf et al. 2009). The ketone 2-heptanone (smells like blue cheese) is very likely to be a key component of the alarm pheromone of *Oxytrigona mediorufa* (Cruz-López et al. 2007). *Melipona solani*, *Trigona spinipes* and *Scaptotrigona depilis* (and possibly many other species) also use 2-heptanol as a key alarm compound (Smith and Roubik 1983; Schorkopf et al. 2009; Leonhardt 2017; Alavez-Rosas et al. 2019). The fact that 2-heptanol is used in phylogenetically distant species suggests that it might be more widely used as an alarm pheromone compound in stingless bees.

Alarm pheromones are not only released into the air but may often be actively transferred to the body of the enemy, e.g. by biting or rubbing, thereby providing a chemical beacon for attacking nestmates (Roubik 1989). At least in some species, males also release alarm pheromones when under attack, e.g. at male aggregations or at colony entrances, to which both workers and other males respond, either by attacking (workers) or by fleeing (males) (Engels and Engels 1984; Sommeijer and De Bruijn 1995; Schorkopf 2016).

7.3.4 Entrance Blocking and the Use of Resin

Other behavioural responses are the blocking of the entrance or the tunnels leading to the brood area and food pots. *Melipona seminigra* workers make small balls of mud (Kerr 1984), whereas *Melipona flavolineata*, *M. fasciata* and *M. panamica* make balls of batumen or hardened resin (Fig. 7.7b) to block the entrance, e.g. during attacks of *Lestrimelitta* or when attacked by the facultative robber *M. fuliginosa* (Roubik 1983, 2006; Nunes et al. 2014). *Lestrimelitta* itself has been observed to block its entrance with pieces of wax and resin during ant attacks (Kerr and Lello 1962), whereas *Trigona cilipes* mainly uses resin for entrance blocking (Kerr et al. 1967). Similarly, guards of the African *Apotrigona nebulata* use resin balls to narrow their entrance tunnel when attacked by ants (Darchen 1969). *Hypotrigona braunsi* workers reportedly block the entrance of their nest during attacks of *Cleptotrigona cubiceps* robber bees by pouring honey into the entrance tube (Portugal-Araújo 1958). The honey, which is subsequently collected by the attackers, seemed to stop the attack (Portugal-Araújo 1958). In species with small entrance holes, e.g. *Frieseomelitta*, guards can block the entrance with their heads (Schwarz 1948; Sakagami et al. 1993).

Another commonly observed phenomenon is that nest guards carry sticky substances in their corbiculae or in their mandibles (resins and wax in varying ratios), which they attach onto attackers in order to immobilise them (Sakagami 1982; Roubik 2006; Lehmberg et al. 2008; Gastauer et al. 2011; Nunes et al. 2014). Sticky substances are used effectively in this way against ants, bees, wasps and humans (Schwarz 1948; Camargo and Pedro 2003; Alves et al. 2018). Guards also deposit sticky droplets near the entrance hole (Fig. 7.7c) (Roubik 2006; Alves et al. 2018). Some Australian and Asian *Tetragonula* species and the Asian *Lophotrigona canifrons* have taken this a step further and build a soft and sticky ring around the outer rim of the entrance tube (Hockings 1883; Kerr and de Lello 1962; Inoue et al. 1993), which Schwarz (1948) likened to a moat of a medieval castle. This seems to be effective against ants, such as the common Asian weaver ant *Oecophylla smaragdina* (Schwarz 1948; Inoue et al. 1993; Duangphakdee et al. 2009). Additionally, non-volatile chemicals that are present in resin can have a repellent effect and might deter some ants from crossing the resin barrier (Wang et al. 2018). The importance of resin against ants is highlighted by the observation that ant attacks led to a sharp increase in resin foraging in *Tetragonilla collina* and *Tetragonula melanocephala* (Leonhardt and Blüthgen 2009).

Different body parts of foragers and guards are often covered in sticky resinous substances (Fig. 6.6a) (Gastauer et al. 2011; Grüter et al. 2012) and Asian species are known to carry resin-derived compounds on their cuticle (Leonhardt et al. 2009, 2011a, 2015). The function of these compounds is not yet well known, but they could make stingless bees less palatable for predators such as ants, spiders or wasps (Lehmberg et al. 2008; Drescher et al. 2014; Leonhardt et al. 2015). For instance, choice experiments involving several Asian stingless bee species showed that ants prefer to prey on bees that were washed with a solvent that removes plant-derived compounds compared to un-washed bees (Lehmberg et al. 2008).

Resin is known to have antimicrobial properties and both ants and bees may use resin for protection against pathogens (see also Chap. 3) (Messer 1985; Christe et al. 2003; Chapuisat et al. 2007; Simone-Finstrom and Spivak 2010; Drescher et al. 2014; Brütsch et al. 2017). Resin, cerumen and mud are also used by some species to cover unwanted inquilines inside the nest (Chap. 3). The Australian *Austroplebeia australis* and *Tetragonula carbonaria*, for example, "mummify" adult small hive beetles (SHBs) (*Aethina tumida*) (Greco et al. 2010; Halcroft et al. 2011). *Tetragonisca angustula* mummifies even large beetles (Schwarz 1948; Nogueira-Neto 1997) and ants can often be seen "glued" to the entrance tube in this species (Fig. 7.2a).

At night, some species close their entrance with a soft and porous layer of cerumen (Fig. 7.7d) (Michener 1946; Schwarz 1948; Kerr and de Lello 1962; Sakagami et al. 1973; Wille and Michener 1973; Roubik 1983; Roubik 2006; Grüter et al. 2011; Rasmussen and Gonzalez 2017). Among the 40 Neotropical species examined by Roubik (1983), 17 (43.5%) closed their entrance at night. In *Tetragonisca angustula*, only about half of the colonies closed their entrance, and more colonies left their entrance open after a rainy day (Grüter et al. 2011). Night temperature seems to be another factor that determines if and how colonies close their entrances at night (Schwarz 1948). I have also observed that on rainy days, entrances remain closed for longer in the morning and in *Austroplebeia australis*, entrances can remain closed for several days in cold weather (Wille and Michener 1973). These observations highlight that climatic conditions (possibly via their effect on foraging activity) and the need for ventilation determine whether and for how long colonies close their entrances. Given that the cap is often very soft, it is possible that its aim is mainly to keep out small parasites (e.g. phorid flies), ants or insects that enter by mistake. In *Lestrimelitta* or *Scaura*, workers can close the entrance within seconds by pulling the soft rim of the entrance inwards until only a narrow gap is left (Roubik 1989; pers. obs.).

7.3.5 Nestmate and Non-nestmate Recognition

An effective colony protection relies on the guards' ability to discriminate between their own nestmates and bees from other nests that might attempt to enter the colony by mistake (drifting), to steal resources (robbing), to usurp the nest or in an attempt to parasitise reproduction (Roubik 1989; van Oystaeyen et al. 2013; Cunningham et al. 2014; Grüter et al. 2016, 2018). Workers of most studied stingless bee species are able to distinguish between nestmates and non-nestmates and they show aggression towards non-nestmates (Kirchner and Friebe 1999; Breed and Page 1991; Suka and Inoue 1993; Inoue et al. 1999; Jungnickel et al. 2004; Buchwald and Breed 2005; Couvillon and Ratnieks 2008; Nunes et al. 2008; Kärcher and Ratnieks 2009; Leonhardt et al. 2010; Nascimento and Nascimento 2012; Wittwer and Elgar 2018; Bobadoye 2019). This discrimination occasionally occurs also at food sources, e.g. when foragers either avoid or attack bees from other colonies (Johnson and Hubbell 1974; Nagamitsu and Inoue 1997; Hrncir and Maia-Silva 2013).[9] Odours perceived by guards via antennal receptors help them to decide whether a bee should be allowed to enter the nest or repelled and attacked. Visual cues can be used to recognise heterospecifics (Bowden et al. 1994).

Even though we still have little information about the identity of the chemical compounds that guards use to differentiate between nestmates and non-nestmates, existing evidence suggests that cuticular hydrocarbons (CHCs), fatty acids and esters (and possibly floral oils) play important roles in recognition in stingless bees (Jungnickel et al. 2004; Buchwald and Breed 2005; Nunes et al. 2008; Leonhardt et al. 2010; Nascimento and Nascimento 2012; Martin et al. 2017). This suggests that the classes of recognition cues used by stingless bees are similar to those used by honey bees (Breed 1998; Buchwald and Breed 2005; van Zweden and D'Ettorre 2010).

CHCs cover the body of most insects. They create a semi-fluid waxy layer that is important for waterproofing, protection against microbial infection, recognition and signalling (e.g. van Zweden and D'Ettorre 2010; van Oystaeyen et al. 2014; Menzel et al. 2017). The main hydrocarbon classes found in cuticular profiles of bees are

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[9] Howard (1985) describes that aggression among bees from different colonies is particularly strong at resin collection sites. Interestingly, some colonies seem to defend larger resin collection sites.

alkanes, alkenes and alkadienes consisting of chains of 19–33 carbon atoms (Breed 1998; Abdalla et al. 2003; Jungnickel et al. 2004; Nunes et al. 2008, 2009a, b; Leonhardt et al. 2009, 2011a; van Zweden and D'Ettorre 2010; Nascimento and Nascimento 2012; Martin et al. 2017; Balbuena et al. 2018). Terpenes are a biosynthetically different class of hydrocarbons that are also particularly prominent on the cuticle of some stingless bees (Leonhardt et al. 2009, 2011a, b, c, 2013; Balbuena et al. 2018; Kämper et al. 2019). CHCs are either synthesised de novo and, therefore, are likely to have a genetic component or are acquired from the environment, e.g. via food, resin, nestmates or nest structures (Pianaro et al. 2007; Guerrieri et al. 2009; Leonhardt et al. 2009, 2011a, 2013; van Zweden and D'Ettorre 2010; Jones et al. 2012; Gutiérrez et al. 2016; Kämper et al. 2019). In honey bees (*Apis mellifera*), alkenes seem to be more important as CHC recognition cues than alkanes (Dani et al. 2005), whereas in the stingless bee *Trigona fulviventris*, both alkenes and n-alkanes affected aggression (Buchwald and Breed 2005).

In honey bees, wax combs play an important role as a source of the colony odour (Breed et al. 1988; Breed 1998; Downs and Ratnieks 1999; D'Ettorre et al. 2006; Couvillon et al. 2007) and the swapping of wax combs among colonies leads to increased acceptance of non-nestmates from the colonies involved in the swap (D'Ettorre et al. 2006). The main constituents of bee wax are hydrocarbons (~15% of wax content in honeybees and ~60–90% in stingless bees), esters (~35% in honeybees and ~6–25% in stingless bees) and fatty acids (~12% in honeybees and ~2–6% in stingless bees) (see also Chap. 3) (Blomquist et al. 1985; Milborrow et al. 1987; Breed 1998; Koedam et al. 2002; Pianaro et al. 2007). This composition makes wax and, therefore, cerumen prime candidates as a source of recognition cues in stingless bees (see also Buchwald and Breed 2005). But whether cerumen (see Chap. 3) mediates the colony odour in stingless bees remains unclear. Exchanging the cerumen entrance tubes on which *Tetragonisca angustula* guards stand did not increase the acceptance of non-nestmates from tube-donating colonies 24 hours after the swap (Jones et al. 2012). Furthermore, the chemical composition of entrance tube material (presumably mostly cerumen) and cuticular profiles differ greatly in several Asian species (Leonhardt et al. 2011a). On the other hand, *Frieseomelitta varia* workers that had contact with cerumen from another colony received significantly more aggression from their nestmates (Nunes et al. 2011), suggesting that cerumen odour cues contribute to the cuticular profile of bees.[10]

To distinguish between a nestmate and a non-nestmate, guards compare the odour (the cuticular odour profile or "label") of the incoming bee with a stored template, which is the memorised colony odour (van Zweden and D'Ettorre 2010). One challenge for guards is that nestmates vary in their chemical profiles and non-nestmates may carry some of the same odour cues on their bodies (Jungnickel

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[10] The authors also explored if the odour profile used in recognition is affected by the collected food, but feeding different colonies with identical food did not lead to a reduction in aggression towards non-nestmates, suggesting that variable diets do not explain the rejection of non-nestmates (see also Downs et al. 2001).

et al. 2004; Nunes et al. 2008, 2009a, b; Leonhardt et al. 2009, 2011b; Ferreira-Caliman et al. 2010; Martin et al. 2017; Balbuena et al. 2018). Especially in the case of conspecific intruders, differences in cuticular odour cues are often quantitative rather than qualitative (e.g. Leonhardt et al. 2009; Nunes et al. 2009a, b; Nascimento and Nascimento 2012) and avoiding mistakes is more challenging. If colony odours are very similar, entrance guards will accept workers from another colony more frequently (Nunes et al. 2008; Nascimento and Nascimento 2012).[11] In *Tetragonilla collina*, for example, workers of neighbouring colonies show a substantial overlap in their chemical profiles and aggression among non-nestmates is virtually absent (Leonhardt et al. 2011b).

A guard bee deciding whether to allow an incoming bee to enter the nest can make two kinds of mistakes. She could accept a non-nestmate (acceptance errors) or reject a nestmate (rejection errors) (van Zweden and D'Ettorre 2010). The two types of errors are likely to be connected: selection for a low rate of acceptance errors is likely to increase the rate of rejection errors because guards become more restrictive overall (van Zweden and D'Ettorre 2010). The individual behavioural thresholds that lead to acceptance or rejection can change relatively rapidly, e.g. depending on the risk of being invaded, among other factors (Reeve 1989; Inoue et al. 1999; Downs and Ratnieks 2000; Couvillon et al. 2008a).

Interestingly, the presence of familiar cues on non-nestmates has less of an effect on guard aggression than the additional presence of unfamiliar cues on nestmates (Couvillon and Ratnieks 2008; Ratnieks et al. 2011; Jones et al. 2012). In the first case, aggression towards non-nestmates remains high, whereas in the second case, aggression towards nestmates increases. This suggests that bees recognise (or pay attention to) chemical differences rather than similarities (van Zweden and D'Ettorre 2010). Mechanistically, it could be explained by a desensitisation of receptor neurons in antennal sensilla (Ozaki et al. 2005) or by habituation in higher brain centres (van Zweden and D'Ettorre 2010) towards the continuously experienced nestmate odour profile. Since non-nestmates (and nestmates treated with non-nestmate odour cues) have an odour profile that is different from the colony odour profile that the guards are exposed to on a daily basis, these unfamiliar profiles will induce responses in antennal sensilla or higher brain centres of guards. This interpretation of guard behaviour has been termed the "undesirable-present" (U-present) model of recognition (van Zweden and D'Ettorre 2010). However, changing the CHC profile of bees does not always increase aggression. Leonhardt et al. (2010) reported that the addition of certain terpenes (in particular sesquiterpenes) to the odour profile of Bornean stingless bee species lowered aggression towards the manipulated bees. The authors suggest that these terpenes have an "appeasement" effect and may explain why some stingless bees

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[11] The response of guards is often not binary but graded, depending on the degree of the mismatch between the template and the label of the incoming bee (van Zweden and D'Ettorre 2010). Some non-nestmates are only mildly aggressed or intensely groomed, whereas others are immediately bitten and pulled by all guards within reach (pers. obs. in *Tetragonisca angustula*).

(e.g. *Tetragonilla collina*) often nest in close proximity without showing aggression. The mechanism behind this putative appeasement effect remains to be studied.

Recognition accuracy (or motivation to attack) seems to differ considerably among species (Jones et al. 2012) and among colonies of the same species (Grüter et al. 2017b) and ranges from accepting most non-nestmates (e.g. *Melipona scutellaris* and *Tetragonilla collina*) (Breed and Page 1991; Leonhardt et al. 2010) to nearly 100% rejection (e.g. *Tetragonula minangkabau* and *Tetragonisca angustula*) (Suka and Inoue 1993; Inoue et al. 1999; Kärcher and Ratnieks 2009; Jones et al. 2012). This inter- and intraspecific variation could be explained by (1) ecological factors, e.g. differences in the risk of being robbed that translates into different aggression levels, (2) species-specific differences in how much odour profiles overlap among colonies or (3) variation in the ability to perceive odour differences, e.g. due to variation in the number and sensitivity of antennal sensilla (Downs and Ratnieks 2000; Quezada-Euán et al. 2013; Grüter et al. 2017b; Leonhardt 2017). However, because different studies used different methodologies, species differences are difficult to interpret. Methodologies differ in terms of the number of discriminating bees (individuals vs. groups of guards), task or age group (guards, foragers or random in-nest workers as discriminating bees), the duration of observations (from seconds to hours), the discrimination context (at the nest entrance, inside the nest or in artificial containers, boxes or tubes), time of year and whether observations were done blind. These different methodologies are likely to affect the probability to observe aggression (Couvillon et al. 2013; van Wilgenburg and Elgar 2013). For example, *Tetragonisca angustula* guards accepted only 13.3% of non-nestmates when tested at the nest entrance, i.e. in a natural context, but accepted 60–70% of non-nestmates when tested in a petri dish (Couvillon et al. 2013) and in *Tetragonula carbonaria*, aggressive behaviour depended strongly on the presence of nest odours (Wittwer and Elgar 2018).

As is the case in honey bees, newly emerged bees from other colonies are not usually aggressed (Bassindale 1955; Suka and Inoue 1993; Kirchner and Friebe 1999; Nunes et al. 2011; but see Inoue et al. 1999), most likely because their cuticular odour profile is not yet well developed ("blank slate" hypothesis) (Breed et al. 2004) and/or because the compounds that are present on very young bees are not used in nestmate recognition (Nunes et al. 2011). Cuticular odours not only increase in quantity as bees age, but the relative quantities of cues also change and often are correlated with particular life stages and/or behavioural roles (Abdalla et al. 2003; Nunes et al. 2009a, b, 2011; Ferreira-Caliman et al. 2010; Balbuena et al. 2018). Cuticular compounds also differ between workers, queens and drones (Abdalla et al. 2003; Nunes et al. 2009a, b; Ferreira-Caliman et al. 2013), which makes them potential carriers of information, e.g. about queen presence (see also Chap. 4).

7.3.6 Morphological Adaptations: Large Guards and Soldiers

Several species have guards of increased body size (see also Chap. 6) (Grüter et al. 2012, 2017a; Segers et al. 2015; Wittwer and Elgar 2018). In some species (*Tetragonisca angustula*, *T. fiebrigi* and possibly *Frieseomelitta longipes*), guard size shows little overlap with forager size and guards form a distinct soldier caste (or sub-caste) (Grüter et al. 2012, 2017a; Baudier et al. 2019). The most pronounced size differences between guards and foragers have been found in *Tetragonisca angustula* (Figs. 6.6 and 6.7) (Grüter et al. 2012; Segers et al. 2015, 2016; Hammel et al. 2016). Guards have relatively small heads (i.e. negative allometry), but relatively large hind legs. *Tetragonisca angustula* is a prime target of *Lestrimelitta* robber bees (Nogueira-Neto 1970; Bego et al. 1991; Sakagami et al. 1993; Grüter et al. 2016) and it has been hypothesised that soldiers have evolved in response to robber bee attacks (Grüter et al. 2012, 2017a; Segers et al. 2016). For instance, *T. angustula* guards respond aggressively to objects that visually (black) or chemically (e.g. citral or crushed heads) resemble *Lestrimelitta* workers (Wittmann 1985; Bowden et al. 1994; van Zweden et al. 2011; Segers et al. 2016; Campollo-Ovalle and Sánchez 2018; Baudier et al. 2020). If *T. angustula* colonies are exposed to the volatiles emitted by robber bees, dozens or even hundreds of bees often exit the nest to fight their enemies (Wittmann 1985; Wittmann et al. 1990; Segers et al. 2016; Campollo-Ovalle and Sánchez 2018). Interestingly, guard antennae seem to be more sensitive to citral (but not to a floral odour) than forager antennae (Balbuena and Farina 2020). Furthermore, *T. angustula* colonies in areas with more robber bees have more soldiers and an experimental exposure of *T. angustula* colonies to the crushed heads of *Lestrimelitta limao* led to a long-term increase in the number of soldiers at the colony entrance (Segers et al. 2016). Robber bees might not only be a driving force behind soldier evolution in *T. angustula*, but Neotropical stingless bees in general: species that are known targets of *Lestrimelitta* robber bees are more likely to have guards of increased size than species that are not known to be targets of robber bees (Fig. 7.8) (Grüter et al. 2017a).

Tetragonisca angustula soldiers can be divided into two groups (see also Chap. 6). The first group hovers close to the nest entrance while facing the flight corridor leading to the entrance (Fig. 6.4) (Zeil and Wittmann 1989; Kelber and Zeil 1997; van Zweden et al. 2011; Shackleton et al. 2018; Baudier et al. 2019, 2020; *Tetrigona apicalis* might be another species with two types of guards, Burgett et al. 2020). Hovering guards often briefly approach incoming bees, probably for closer visual inspection. Hovering guards coordinate their respective positions so that a similar number of bees hover on the left and the right side of the entrance tube (Shackleton et al. 2018). Having hovering guards aids in the early detection of potential threats compared to species without hovering guards (van Zweden et al. 2011). The second group of soldiers stands on, in or near the entrance tube (Wittmann 1985; Kärcher and Ratnieks 2009; Grüter et al. 2011; van Zweden et al. 2011). Soldier size is an important predictor of defensive performance. During fights with larger *Lestrimelitta limao* workers, soldier size correlates positively with the time it takes robber bees to defeat *T. angustula* soldiers (Grüter et al. 2012).

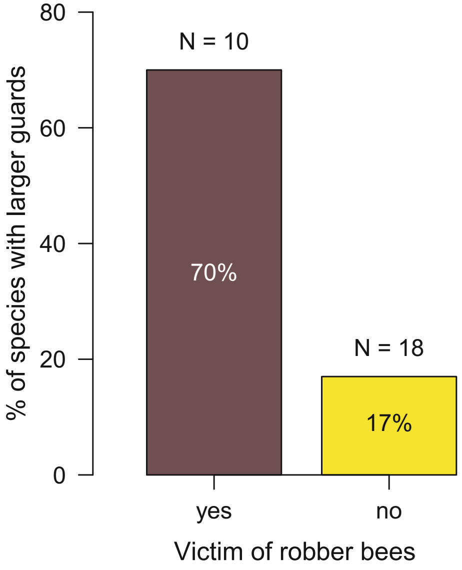

Fig. 7.8 Neotropical stingless bee species that are known to be victims of *Lestrimelitta* robber bees are ~4 times more likely to have guards of increased size than species that are not known to be a robber bee victim (modified from Grüter et al. 2017a)

Larger guards also seem to be better at recognising conspecific non-nestmates, presumably because larger guards have larger antennae that can accommodate more sensory sensilla (Grüter et al. 2017b; see also Wittwer and Elgar 2018; Month-Juris et al. 2020).

7.3.7 Architectural Defences

Entrance architecture has important consequences for colony defence. Narrower entrances are easier to defend, e.g. by blocking, but restrict foraging traffic (Biesmeijer et al. 2007; Couvillon et al. 2008b). Larger entrances not only facilitate foraging but also make it easier for invaders to gain access to the nest. Possibly in response to this risk, species with relatively large entrances often have a larger number and more aggressive entrance guards (Biesmeijer et al. 2007; Couvillon et al. 2008b). *Partamona* species that build "toad-mouth" entrances (see Fig. 3.1) seem to have solved this conflict between defence and foraging efficiency by building two entrance holes in sequence (Couvillon et al. 2008b). Bees first enter the very large entrance hole at great speed, before bouncing off the ceiling of the entrance to fall into the smaller entrance hole (Shackleton et al. 2019).

Some *Partamona* and *Ptilotrigona* species build intricate structures made from mud and resin adjacent to the nest entrance (Fig. 3.6) (Michener 1974; Roubik 1989; Camargo and Pedro 2003). It has been speculated that this vestibule could distract and delay attackers, provide space for large numbers of guards or discourage anteaters (Michener 1974; Roubik 1989; Camargo and Pedro 2003). Even more intriguing are so-called false nests adjacent to the real nest, which can contain empty cells, food pots and involucrum sheets (Roubik 1989; Camargo and Pedro 2003). Camargo and Pedro (2003) observed that during a *Lestrimelitta rufa* attack on a *Partamona vicina* colony, robbers occupied the false nest, while the actual brood chamber remained unharmed. A delay in the attack could provide more time to block the access tunnel to the brood and food chambers, as was observed in *Partamona ferreirai* following a disturbance (Camargo and Pedro 2003). Thus, false or decoy nest chambers might help protect the brood nest during robber bee raids.

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Foraging

8

Stingless bees need different types of resources to rear brood, build nest structures and defend their colony. This has major consequences for tropical ecosystems because the collection of pollen, the main protein source for larvae, and carbohydrates in the form of floral nectars lead to pollination. Worldwide, thousands of plant species are likely to benefit from stingless bee pollination (Chap. 9). Stingless bees differ somewhat from honey bees in that non-floral resources (e.g. resinous materials, fruit juice and carrion; see below) account for a significant proportion of foraging trips (Roubik 1989; Lorenzon and Matrangolo 2005).

8.1 What Do Stingless Bees Collect?

8.1.1 Protein

Pollen

Pollen is the main protein source for bees. Some flowers offer pollen as their only reward, whereas others offer both pollen and nectar (Vogel 1983; Roubik 1989). However, pollen not only provides proteins (~10–60% of the dry weight) and amino acids but also contains water (often around 20%, but up to 50%), vitamins (e.g. A, B, C, D and E), carbohydrates (up to 40% of dry weight), lipids (1–20%), terpenes, steroids or carotenoids (Solberg and Remedios 1980; Vogel 1983; Roubik 1989; Roulston and Cane 2000; Vossler et al. 2010; Vossler 2015). The carotenoids, terpenes and lipids are often found in the outermost layer of pollen, the pollenkitt (Roulston and Cane 2000). The amount of carbohydrates found in bee-collected pollen is partly the result of bees adding regurgitated nectar or honey to the collected pollen loads (see below) (Solberg and Remedios 1980; Roulston and Cane 2000). Pollen has a characteristic odour, which can be learned and is, subsequently, used to locate flowers of the same type (studied mainly in honey bees and bumble bees: von Frisch 1967; Vogel 1983; Dobson 1987; Dobson et al. 1996; Pernal and Currie 2002; Arenas and Farina 2012, 2014; Muth et al. 2016a, b).

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The nutrient composition of pollen is highly conserved within species, but varies greatly among plant species (Roulston et al. 2000; Roulston and Cane 2000). Collecting pollen with higher protein content is potentially beneficial, e.g. because higher protein content has a positive effect on body size (Quezada-Euán et al. 2011). Whether stingless bees discriminate among different types of pollen based on their nutritional value is not yet known. In general, bees do not seem to pay a lot of attention to protein content *per se* (Roulston et al. 2000; Pernal and Currie 2002; Vossler 2015; Beekman et al. 2016; Zarchin et al. 2017) and honey bees are known to occasionally collect non-nutritional substances like brick, carbon and saw dust, instead of pollen (von Frisch 1967; Shaw 1990). However, honey bees show a preference for pollen containing macronutrients that are complementary to what the colony has collected in the past (Hendriksma and Shafir 2016; Zarchin et al. 2017) and there is evidence that a higher diversity of pollen sources enhances honey bee health (Alaux et al. 2010; Pasquale et al. 2013; Dolezal et al. 2019). Whether stingless bee colonies also attempt to achieve a diverse pollen diet to balance nutritional needs is currently unknown.

The front basitarsi and the proboscis are the principal structures involved in the removal of pollen from the anthers of flowers (Michener et al. 1978). Large-bodied *Melipona* can extract pollen by rapidly and repeatedly contracting their flight muscles while grabbing the anthers. This leads to the release of pollen from the anthers and is accompanied by a characteristic buzzing noise, which is why this behaviour is also called buzz collection or buzz pollination (Wille 1963; Sommeijer et al. 1983; Roubik 1989; Nunes-Silva et al. 2013; Vallejo-Marín 2019). There are also instances of stingless bees (mainly *Trigona*) damaging the anthers or other floral tissues to reach the pollen (or nectar), so-called pollen (or nectar) robbing, often without pollinating the plant (Willmer and Corbet 1981; Roubik 1982b, 1989; Renner 1983; Roubik et al. 1985; Tezuka and Maeta 1995; Murphy and Breed 2008a; Rego et al. 2018). The holes created by robbing stingless bees can then be used by other bee species or hummingbirds (Roubik 1989, see also his Table 2.5). One morphological feature that helps *Trigona* species to rob pollen and nectar is that they have strong and toothed mandibles (Fig. 7.1) (Shackleton et al. 2015). These mandibles, which can be used very effectively against enemies, are ideal tools to perforate the anthers. Pollen and nectar robbing can impact the reproductive success of plants, e.g. by deterring effective pollinators, such as hummingbirds, thereby reducing fruit set (McDade and Kinsman 1980; Roubik 1982b; Murphy and Breed 2008a). Rego et al. (2018) argue that the negative impact of pollen robbing by *Trigona fulviventris* bees on *Eriocnema fulva* in the Atlantic rain forest, in combination with habitat loss, could put this threatened plant species at risk of extinction.

Bees mainly use their front and middle legs to transfer the pollen grains from the different body parts to the corbiculae (see Michener et al. 1978 for more detailed descriptions of the involved grooming behaviours) where they are mixed with regurgitated liquids. The transfer of pollen to the pollen baskets often occurs while bees are hovering, as this frees up the middle and hind legs for the pollen-transfer movements (Schwarz 1948; Wille 1962; Roubik 1982a; Baumgartner and Roubik 1989; Camargo and Roubik 1991; Noll et al. 1996; Breed et al. 1999). For this

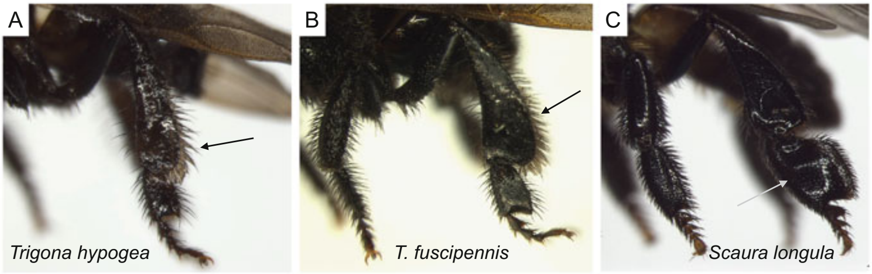

Fig. 8.1 Hind legs of the obligate necrophagous Neotropical bee *Trigona hypogea* (**a**) and the pollen collecting *T. fuscipennis* (**b**). The corbiculae of *T. hypogea* are reduced compared to *T. fuscipennis* (black arrow). (**c**) Bladder-like hind basitarsus (arrow) of *Scaura longula*, which may help foragers gather pollen that has dropped to the ground or on vegetation (Photos by Kátia Aleixo)

purpose, bees have grooming brushes or comb-like structures on the forelegs, the hind- and mid-basitarsi and tibiae (Roubik 1989). Inside the nest, pollen packages are then dropped into the pollen pots with a single movement of the mid legs by the pollen carrying bee (Roubik 1989).

Animal Protein

Several species are known to collect meat from dead animals (e.g. lizards, frogs, birds and mammals of various sizes) (Schwarz 1948; Wille 1962; Roubik 1982a; Baumgartner and Roubik 1989; Camargo and Roubik 1991; Noll et al. 1996; Breed et al. 1999; Mateus and Noll 2004). For example, when Baumgartner and Roubik (1989) set out baits of dead fish and beef liver in different habitats in Peru, they found foragers from ~30 stingless bee species visiting these baits. Some of the bees might use meat as a nest building material or as a source of salts, but at least three closely related *Trigona* species (*Trigona crassipes*, *T. hypogea* and *T. necrophaga*) have permanently switched from using pollen protein to protein collected from dead animals or abandoned insect brood (Roubik 1982a; Camargo and Roubik 1991; Noll 1997; Mateus and Noll 2004). One benefit of using animal protein might be that it is easier to digest than protein in pollen grains (Cruz-Landim and Serrao 1994).

Animals that have been dead for a while or that are infested with fly larvae are ignored by carnivorous bees (Roubik 1982a, 1989). The workers of these three *Trigona* species have reduced corbiculae (Fig. 8.1) (corbiculae, in these species, are still used to collect resin, but not pollen) and they lack the typical hairs on the mouthparts that help foragers extract pollen from anthers (Roubik 1982a, 1989; Camargo and Roubik 1991). The glandular secretions of carnivorous bees make up most of the brood food and have a protein content of ~20%. These protein rich secretions are also stored in storage pots instead of pollen (Roubik 1982a). Foragers of *Trigona hypogea* also do not seem to visit flowers for nectar, but instead collect fruit juice or sugar-rich liquids from extrafloral nectaries (Noll et al. 1996). This suggests that obligate carnivorous stingless bees are non-pollinating bees (Camargo and Roubik 1991).

The efficient recruitment of these three *Trigona* species (Chap. 10) (Noll 1997; Jarau et al. 2003) means that colonies can monopolise carrion and, thanks to their sharp, toothed mandibles, reduce small corpses to skeletons within hours. Roubik (1982a) describes that bees appear to use digestive enzymes secreted from cephalic glands to pre-digest the meat at the carrion. Additionally, these secretions have been observed to deter ants and flies (Noll 1997). Bees then carry the liquefied material in their crop and regurgitate it inside the nest. Beneficial microbes (several *Bacillus* species) found in glandular secretions and in larval food are thought to play an important role in metabolising the proteins and in producing antibiotics (Gilliam et al. 1985; Camargo and Roubik 1991; Noll et al. 1996).

Fungal Spores

Foragers of some species consume spores of fungi (Roubik 1989; Oliveira and Morato 2000). In the Amazon region, several *Trigona* species have been observed to lick the spore containing gleba of stinkhorn fungi (Phallaceae) or they carry spores back to the nest in their corbiculae (Burr et al. 1996; Oliveira and Morato 2000). Oliveira and Morato (2000) suggested that fungus spores represent an important protein source for *Trigona crassipes*. The Asian *Tetragonilla collina* has been observed to collect the spores of the mould *Rhizopus* (Mucoraceae) (Eltz et al. 2002). Overall, little is known about whether fungal spores are an important source of protein in stingless bees, but the finding that several *T. collina* colonies collected substantial quantities of mould spores on consecutive days suggests that fungal spores may temporarily be a significant part of their diet (Eltz et al. 2002). Whether stingless bee brood development is affected by a diet of fungal spores is not known (Shaw 1990; but see Menezes et al. 2015). Fungal spores are thought to have a slightly lower protein content compared to many types of pollens, but they could represent an attractive alternative to pollen if it is available in considerable quantities during certain periods (Shaw 1990; Eltz et al. 2002).

8.1.2 Carbohydrate Sources

Nectar

Nectar is secreted by many plants to attract and reward pollinators. It is the main carbohydrate source for many bees, including most stingless bees. The three dominant sugars in nectars are the disaccharide sucrose and the two monosaccharides fructose and glucose (Vogel 1983; Chalcoff et al. 2017), with sucrose being the most dominant sugar of the three in the majority of nectars (Chalcoff et al. 2017).[1]

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[1] Note that this is different from stingless bee honeys, where sucrose occurs only in minor quantities (Chap. 1), suggesting a biochemical conversion of sucrose into other types of sugars during the process of honey production.

Additionally, there can be traces of several other mono-, di- and oligosaccharides including mannose (which can be harmful to honey bees and stingless bees at higher concentrations, Zucoloto and Penedo 1977), pentose, maltose, melibiose or raffinose. These sugars often appear in plant species-specific ratios. It is not well known if stingless bees have preferences for particular sugar ratios, but Biesmeijer et al. (1999a) found that *Melipona beecheii* and *M. fasciata* preferred sucrose over glucose and fructose, which also seems to be the case in honey bees (Değirmenci et al. 2018). However, choice tests in honey bees also suggest that sugar composition is less important than sugar concentrations (Vogel 1983). Nectars can also contain minor quantities of yeast, carbon acids, lipids, proteins, amino acids, vitamins (e.g. Vitamin C), alkali metals (e.g. potassium) and alkaloids like caffeine and nicotine (Vogel 1983; Kretschmar and Baumann 1999; Singaravelan et al. 2005). Some of these compounds are likely to be the result of microbial activity, by-products of plant metabolism or deterrents/attractants of particular groups of visitors (e.g. Vogel 1983; Kim and Smith 2000; Afik et al. 2014; Couvillon et al. 2015). For example, caffeine (found in *Coffea* and *Citrus* nectars) can have a stimulating or deterring effect on honey bee foragers, depending on the concentration (Kretschmar and Baumann 1999; Singaravelan et al. 2005; Wright et al. 2013; Couvillon et al. 2015; Thomson et al. 2015). Plants might add caffeine in low concentrations to enhance visitation by some pollinators (Couvillon et al. 2015) or deter others by adding caffeine in high concentrations (Singaravelan et al. 2005). Little is known about the effects of these compounds on stingless bee foraging behaviour (Leonhardt 2017), but caffeine did not affect the foraging activity of *Plebeia droryana* in Brazil (Peng et al. 2019). The lack of a response to caffeine could be the result of local adaptation in *P. droryana* due to its potentially harmful effects (Couvillon et al. 2015) or it could indicate differences between stingless bee and honey bee neurophysiology (Peng et al. 2019).

Amino acids present in nectar did not affect the foraging behaviour of some stingless bee species (Roubik et al. 1995; Gardener et al. 2003), but *Melipona fuliginosa* and *M. panamica* foragers avoided sucrose solutions containing alanine, arginine, glutamic acid, glycine, proline and serine (Roubik et al. 1995). Sugar solutions containing potassium near or above the levels found naturally in avocado (*Persea americana*) nectar also reduced foraging in *Frieseomelitta nigra* and *Nannotrigona perilampoides* (Afik et al. 2014).

Bees perceive sugar as a reward and contact with the antennae or tarsi induces the reflexive extension of their tongue (the proboscis extension response or PER) (Takeda 1961; Menzel 1999; Scheiner et al. 2004). The sugar concentration that induces bees to extend their proboscis to drink a sugar solution (sugar response threshold) provides information about the gustatory sensitivity and reward evaluation of a bee. When comparing the two Neotropical stingless bees *Melipona quadrifasciata* and *Scaptotrigona depilis* with Africanised honey bees in Brazil, Mc Cabe et al. (2007) found that bees of all three species started to respond to solutions containing, on average, between 3% and 10% sugar. A similar sucrose sensitivity was found in the two African species *Meliponula bocandei* and *Axestotrigona ferruginea* (Henske et al. 2015). In *Melipona eburnea*, on the other hand, most bees failed to show the PER to solutions containing 10% sucrose

(Amaya-Márquez et al. 2019). The sucrose response threshold (SRT) is not fixed during the lifetime of a bee, but varies with age, prior experience, nectar availability and season (honey bees: Lindauer 1948; Scheiner et al. 2003; Pankiw et al. 2004; stingless bees: Mc Cabe et al. 2007).

Little is known about the neurobiological basis of reward perception in stingless bees. In honey bees (and other insects), biogenic amines like octopamine and dopamine are important mediators of reward perception and learning (Mercer and Menzel 1982; Scheiner et al. 2002; Perry and Barron 2013). There is evidence that octopamine plays similar roles in how stingless bees perceive rewards. *Melipona scutellaris* foragers show a higher sensitivity towards sucrose after octopamine feeding (Mc Cabe et al. 2017) and *Plebeia droryana* foragers increase their foraging rate and recruitment intensity after octopamine ingestion (Peng et al. 2020).

Most plants offer nectars with sugar concentrations above the detection threshold of bees (Gut et al. 1977; Southwick et al. 1981; Vogel 1983; Roubik and Buchmann 1984; Roubik 1989; Chalcoff et al. 2006, 2017). For example, the sugar concentrations of liquids collected by stingless bees in Panama ranged from 9% to 72% (Roubik and Buchmann 1984; Roubik et al. 1986; see also Roubik et al. 1995), from 0% (water) to 75% in Brazil (Fig. 8.2) (I'Anson Price 2018) and from 9% to 67% in Ugandan species (Kajobe 2007a). This sugar concentration can affect the quantity of solution that is collected by foragers on a foraging trip.[2] In some species, foragers fill their crop more when collecting sweeter food (*Melipona triplaridis*), whereas others collect smaller quantities (*M. fuliginosa* and *Scaptotrigona depilis*) when resources contain more sugar. Finally, there are also species where sugar content does not seem to affect crop load behaviour (*Melipona micheneri* and *M. fasciata*) (Roubik and Buchmann 1984; Schmidt et al. 2006). This interesting phenomenon remains poorly understood in stingless bees, but could be linked to the recruitment behaviour of a species: foragers that can recruit nestmates could decide to return half-empty after finding a high-quality food source in order to recruit additional nestmates to this food source quicker ("information transfer hypothesis", Roces and Núñez 1993; Dornhaus et al. 2006; Bollazzi and Roces 2011).

Foragers are expected to consider both benefits and costs in their foraging decisions (e.g. von Frisch 1967; Heinrich 1975; Schmid-Hempel et al. 1985; Roubik 1989; Seeley 1995).[3] As one would expect, stingless bees show a preference for food

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[2] Even though foragers can carry about 50% of their body weight in the form of liquids in their crop (Roubik and Buchmann 1984; Schmidt et al. 2006), they usually return with only a partially filled crop (honey bees: Schmid-Hempel et al. 1985), most likely due to the increased energy and time costs that are associated with filling the crop while foraging.

[3] Which currency stingless bee foragers prioritize has received little attention. Honey bees assess patch quality based on the net energy efficiency, (gain-costs)/costs, rather than the net rate of energy intake (gain-costs)/time (Schmid-Hempel et al. 1985; Seeley 1986), possibly because of the relatively high metabolic costs of food transport in bees (Wolf and Schmid-Hempel 1989). Other factors that are likely to impact the preferred currency are the risk of predation and competition or the value of information to nestmates (Núñez 1982; Nieh 2010). The currency used by bees is likely to affect how long bees continue to forage in a patch.

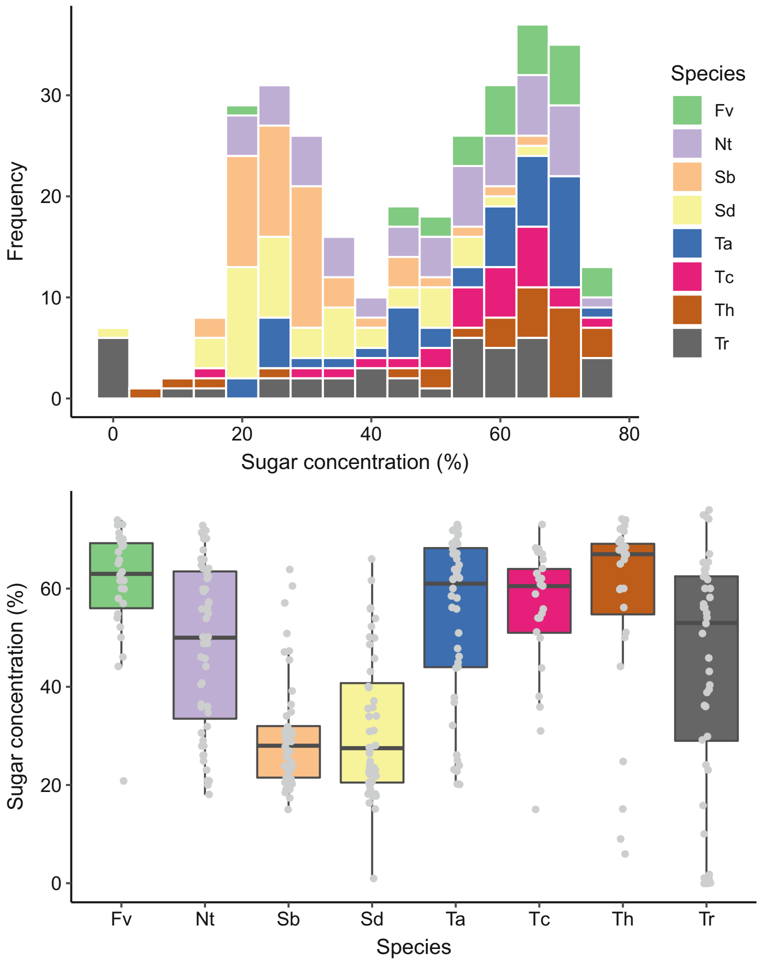

Fig. 8.2 Sugar concentrations of liquid food obtained from the crop of foragers from eight stingless bee species (I'Anson Price 2018). Fv = *Frieseomelitta varia*, Nt = *Nannotrigona testaceicornis*, Sb = *Scaptotrigona bipunctata*, Sd = *Scaptotrigona depilis*, Ta = *Tetragonisca angustula*, Tc = *Tetragona clavipes*, Th = *Trigona hypogea* and Tr = *Trigona recursa*

sources that contain more sugar (Roubik and Buchmann 1984; Biesmeijer et al. 1999a, b; Biesmeijer and Ermers 1999; León et al. 2015; Peng et al. 2019; Silva et al. 2019): all else being equal, sweeter food sources increase recruitment (Chap. 10), foraging tempo (e.g. by reducing hive-stay time) and the probability to return to and aggressively defend a food source (Johnson and Hubbell 1974; Biesmeijer and Ermers 1999; León et al. 2015; Schorkopf et al. 2016; Krausa et al. 2017; Peng et al. 2019). However, solutions with very high sugar concentrations are more difficult to exploit due to their higher viscosity (von Frisch 1967; Vogel 1983; Roubik and Buchmann 1984; Schmidt et al. 2006; Kim et al. 2011). This physical constraint leads to an optimal sugar concentration that is below the maximum concentrations found in natural nectars. Most bees, including stingless bees and honey bees, use “viscous dipping” (of the proboscis into nectar) as a drinking technique. Due to this technique, the optimal sugar concentration for stingless bees is thought to be around 55–60% and does not depend greatly on body size (Roubik and Buchmann 1984; Biesmeijer et al. 1999a; Kim et al. 2011). Body size might, however, have a positive effect on the drinking speed (Roubik 1989, Table 2.3). For example, the large *Melipona fuliginosa* (~125 mg body weight) ingests a 27–30% sucrose solution at a rate of 2.6 μl/s, whereas the much smaller *Trigona pallens* (~10 mg body weight) achieves 0.3 μl/s (Roubik 1989). Bees can use tricks to exploit higher concentrations and even dried sugar. Honey bees, for example, are known to add saliva to the dried nectar and then suck up the sweet solution (Simpson and Riedel 1964).

Despite an optimal sugar concentration of 55–60% (Kim et al. 2011), there is variation among stingless bee species in the sugar content of the nectars they collect and some species consistently collect liquids with a much lower sugar concentration (Fig. 8.2) (Roubik et al. 1995; Biesmeijer et al. 1999a, b; I’Anson Price 2018). *Melipona beecheii* collects nectar that is more concentrated than the nectar collected by the sympatrically occurring *M. fasciata*, even if they collect nectar from the same plant species (Biesmeijer et al. 1999a, b). Similarly, Roubik and Buchmann (1984) found that *Melipona compressipes* and *M. marginata* collected nectars of higher sugar content than *M. fasciata* and *M. fuliginosa*. *Scaptotrigona bipunctata* and *S. depilis* collected liquids of considerably lower sugar concentrations (20–30%) compared to six sympatric species in Brazil (Fig. 8.2) (I’Anson Price 2018). *Scaptotrigona depilis* is also unusual in that recruitment behaviour does not depend strongly on the sucrose concentration *per se* (Chap. 10) (Schmidt et al. 2006). This highlights that there are other factors that determine the attractiveness of a food source, including food source distance, nectar flow rate, competition or morphological constraints (e.g. variation in body size, colouration or tongue length) (see Sect. 8.6).

Plant Sap and Fruit Juice

Carbohydrates from plant sap or fruit juice represent important resources for some stingless bees, especially during periods when floral resources are scarce (Fig. 8.3) (Wille 1962; Roubik 1989; Figueiredo 1996; Koch et al. 2011; Vijayakumar and Jeyaraaj 2016; Santos et al. 2019). Next to sugars, plant sap can contain amino acids, proteins, vitamins and minerals (Roubik 1989). Plant sap is collected either in the form of honeydew secreted by aphids, scale insects (Coccidae and Pseudococcidae) and membracids or directly from openings in the plant (Roubik 1989; Camargo and Pedro 2002; Santos et al. 2019). In the case of the treehopper *Aethalion reticulatum*, the species most frequently found in associations with Neotropical stingless bees (Santos et al. 2019), *Trigona* bees stimulate the release of honeydew droplets by walking over the nymphs and adults and antennating their abdomen (Oda et al. 2009; Barônio et al. 2012; Santos et al. 2019). Stingless bees may have to compete with ants for access to honeydew and offer protection to their providers in return (Oda

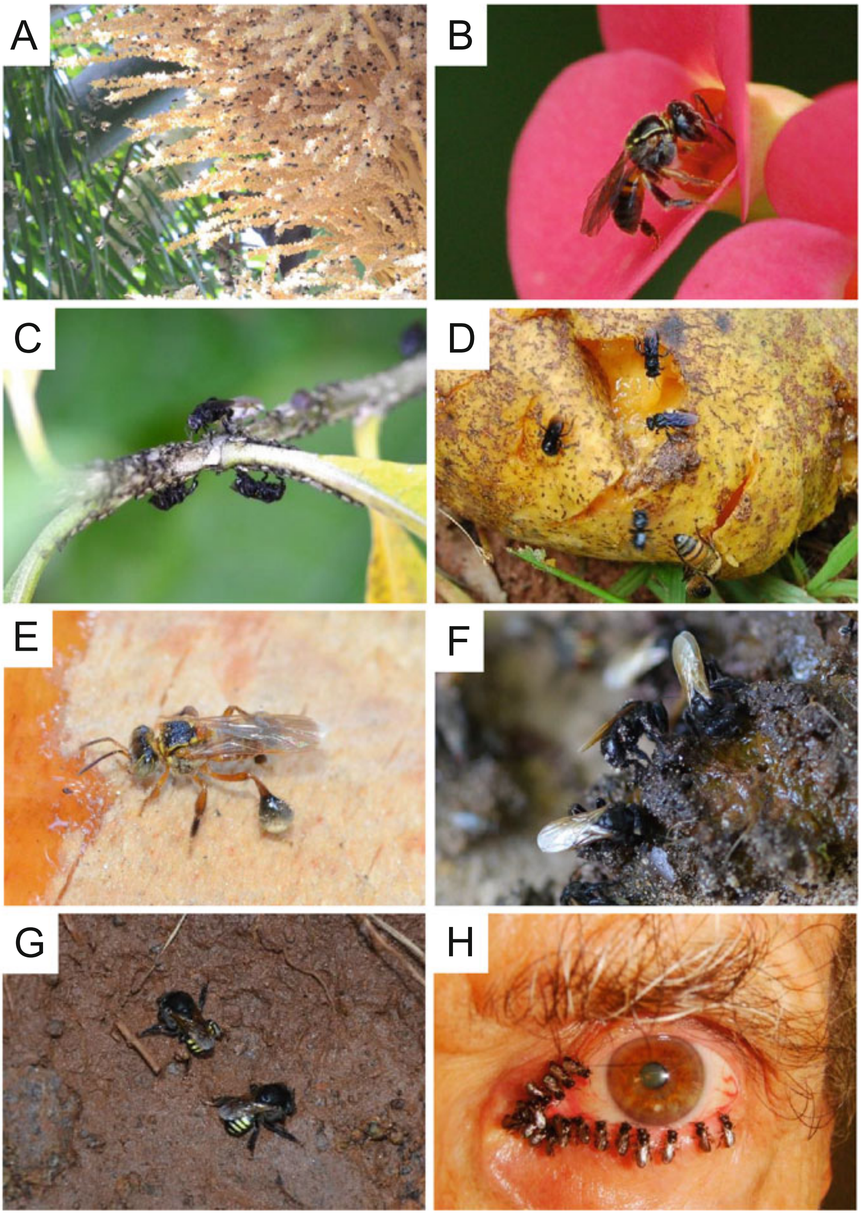

Fig. 8.3 Stingless bees collecting different types of resources. (**a**) *Trigona hyalinata* collecting pollen from a palm tree. (**b**) *Plebeia droryana* collecting nectar from *Euphorbia milii*. (**c**) *Trigona* sp. collecting honeydew. (**d**) *Trigona spinipes* collecting juice from a fallen Mango (next to an Africanised honey bee). (**e**) *Frieseomelitta varia* collecting resin from an injured tree. (**f**) *Trigona hyalinata* collecting animal faeces. (**g**) *Melipona quadrifasciata* collecting mud. (**h**) *Lisotrigona furva* collecting human tears (Photos a–g by c. Grüter taken in São Paulo state, Brazil; photo h taken from Bänziger 2018)

et al. 2009; Koch et al. 2011; Barônio et al. 2012). For example, the Malagasy stingless bees *Liotrigona mahafalya* and *L. madecassa* compete with the ants *Monomorium destructor* and *Paratrechina longicornis* for access to honeydew secreting mealybugs (see also Sect. 8.6) (Koch et al. 2011).

Another resource collected by stingless bees is fruit juice and fruit pulp, either from fruits that are still on trees or from ripe fruits that have fallen to the ground (Fig. 8.3) (Roubik 1989; Peruquetti et al. 2010). Fruit juice contains lower amounts of sugar (usually 8–25%) than many nectars (White and Stiles 1985; Peruquetti et al. 2010; Shackleton et al. 2016), but can often be collected much quicker, making it a potentially inexpensive (in terms of energy and time expenditure) resource to collect (Shackleton et al. 2016).

8.1.3 Resin and Other Sticky Plant Materials

Plant resins not only mainly consist of sticky, insoluble terpenes (mono-, di-, tri- and sesquiterpenes) but may also contain other compounds such as gallic acid (Velikova et al. 2000; Patricio et al. 2002; Leonhardt et al. 2011). Resins are secreted in liquid form by some flowers, fruits and trees, often in response to injuries and as a deterrent against herbivorous insects (Roubik 1989). Stingless bees, especially *Trigona*, occasionally stimulate the secretion of resin (and other sticky substances) for days or weeks by biting into buds, young leaves, flowers or bark of plants (Schwarz 1948; Wille and Michener 1973; Howard 1985; Reyes-González and Zamudio 2020).

A colony usually collects resins from multiple plant species, but some plants seem to be preferred (e.g. Dipterocarpaceae in Asia and Caesalpinioideae and Papilionoideae in the Americas), possibly because of the antimicrobial activity of their resins (Leonhardt et al. 2009; Leonhardt and Blüthgen 2009; see Bassindale 1955 and Gastauer et al. 2011 for detailed descriptions of the behaviours involved in resin collection). Species differ somewhat in their preferences for plant resins (Roubik 1989; Patricio et al. 2002). For example, several *Melipona* species show a preference for resins produced by *Vismia* fruits (Roubik 1989). Collecting resin from multiple plant species could be beneficial if mixtures of resins offer better protection against potentially dangerous organisms than single types of resins (Drescher et al. 2014; see also Kämper et al. 2019).

Some flowers actively secrete resin from floral structures to attract pollinators (from the families Clusiaceae and Euphorbiaceae) (Armbruster 1984; Murphy and Breed 2008b). In these cases, resin has replaced nectar and pollen as the main rewards.[4] Floral resins may have the advantage that they stay liquid for longer (up to several weeks) and can, therefore, be collected and stored for longer time periods than resins from tree injuries, which often harden within hours following secretion (Armbruster 1984). Stingless bees (e.g. *Melipona*, *Scaptotrigona* and

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[4] Resin robbing by some *Trigona* species, particularly *T. fulviventris*, has also been observed (Murphy and Breed 2008b).

Trigona) have also been observed to collect latex, a milky liquid that coagulates when exposed to air following injury (Absy and Kerr 1977; Roubik 1989; Pereira and Tannús-Neto 2009). Latex is probably used as a nest building material and is known to have antimicrobial properties (Pereira and Tannús-Neto 2009).

8.1.4 Other Resources

Stingless bees collect many additional materials, such as mud, wood pulp, leaves, bark, little stones, seeds, epicarp and faeces (Fig. 8.3) (Schwarz 1948; Wille 1983; Baumgartner and Roubik 1989; Roubik 1989, 2006; Bastos Garcia et al. 1992). These are used mainly as building materials (Chap. 3).[5] In urban areas, bees occasionally collect paint and other man-made materials (Wille and Michener 1973; pers. obs.). The collection of these substances remains largely unstudied. Mud, for example, is an important resource for many *Melipona* species, but little is known about the natural history and regulation of mud collection. An exception is a study by Costa-Pereira (2014) who found that *Melipona* foragers (Fig. 8.3) prefer mud that is not too humid, possibly because excess water adds weight. Larger foragers were able to collect larger loads (Costa-Pereira 2014), but it is currently unknown whether mud collecting bees are larger than nestmates that collect other types of resources.

The deliberate and accidental transport of seeds can lead to bee-mediated seed dispersal, as is the case with the seeds of the Australian eucalypt *Corymbia torelliana*, which are transported by Australian stingless bees attempting to collect the resin from the seed capsules (Wallace and Trueman 1995; Wallace and Lee 2010). A single colony can collect thousands of seeds over the course of a year. Workers then often remove the seeds from their nest and discard them in the vicinity, leading to *C. torelliana* trees growing near stingless bee nests (Wallace and Trueman 1995). Collected seeds can also be used as a building material (Chap. 3) and, when incorporated in the nest entrance, can germinate (Bastos Garcia et al. 1992).

Urine, sweat or muddy water are probably collected because they contain essential minerals and salts (Wille 1962; Roubik 1989, 2018; Bänziger et al. 2009; Karunaratne et al. 2017) that function as important nutrients in physiological processes. Since these substances exist only in small amounts in nectar and pollen, bees often actively supplement their diet by collecting these more unusual materials. For instance, when offered a choice between deionised water, mineralised water and sucrose solution, *Trigona silvestriana* foragers collected the mineralised water almost as much as the sucrose solution, but showed little interest in deionised water (Dorian and Bonoan 2016). Calcium chloride ($CaCl_2$), sodium chloride (NaCl) and potassium chloride (KCl) were particularly attractive. In the case of the collection of bird, mammal and reptile tears (Fig. 8.3), a phenomenon observed in

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[5] See Michener et al. (1978) for detailed descriptions of the behavioural motor sequences involved in the collection of materials such as mud or cerumen.

several Asian species, it has been hypothesised that bees might be interested in proteins as well as salts (Bänziger et al. 2009; Karunaratne et al. 2017; Bänziger 2018). Some Neotropical species (*Tetragona, Melipona* and several *Trigona*) have been observed to collect plant oils (Roubik 1989; Simpson et al. 1990; Lorenzon and Matrangolo 2005; Ferreira et al. 2019). The biological function of oil collection remains unclear, but Simpson et al. (1990) suspected that oil is used as building material, rather than for its lipid content (see also Roubik 1989).

8.2 Spatio-temporal Distribution of Resources

Tropical flowers tend to bloom for shorter time periods than flowers in temperate environments (Primack 1985; Roubik 1989; Bawa 1990). For example, the average flower longevity in tropical dry forests and tropical rain forests was estimated to be 1.1 and 1.3 days, respectively, and most species have flowers that last no longer than 3 days (there are, however, also tropical flowers that bloom for several days or even weeks) (Primack 1985). In temperate forests, flowers lasted on average 2.5–6.9 days, depending on the time of year (Primack 1985). This means that stingless bee foragers might have to search for new floral resources more frequently than temperate honey bees or bumble bees, which is likely to affect the importance of recruitment communication (e.g. Schürch and Grüter 2014) and the dynamics of learning and memory processes (e.g. Menzel 1985).

Another important feature of flower phenology is the synchrony of flowering within and among species. Plants with short flowering periods often produce large numbers of flowers simultaneously (mass-flowering), whereas plants with longer lasting flowers produce only a few flowers per day over longer periods (steady-state flowering) (Rathcke and Lacey 1985). The latter is more common in understory plants, whereas mass-flowering is found in many trees. Mass-flowering trees are thought to represent the most important food source for many stingless bee species (see also Chap. 9) (Kleinert-Giovannini and Imperatriz-Fonseca 1987; Wilms et al. 1996; Ramalho 2004; Hrncir and Maia-Silva 2013b; Roubik and Moreno Patiño 2018), but flowering shrubs, epiphytes, lianas, herbs and small trees also play important roles as food sources (Roubik and Moreno Patiño 2018). Asynchrony in flowering among species could arise from plant-plant competition for pollinator visits and selection against interspecific pollen transfer. Asynchrony within species could affect gene flow by promoting interplant movement (Roubik 1989). Both synchronised and more random flowering are found in plant communities (Rathcke and Lacey 1985).

During a particular day, flowers show periodicity in the amount (and quality) of pollen, nectar and floral fragrances they produce (Butler 1945; Vogel 1983; Kajobe 2007a). Bees adjust to these fluctuations and increase visitation in periods when sugar concentration or nectar flow is highest (Kleber 1935; Butler 1945; Vogel 1983). Similarly, stingless bees often collect pollen in the early morning when this resource has its highest availability (see below) (Roubik 1989). It should be noted, however, that the time of day when plants produce the highest amount of nectar does not necessarily coincide with the time of highest sugar concentration and depends on whether flowers are in shaded or sunny locations (e.g. Biesmeijer et al. 1999b).

8.3 Foraging Specialisation

Individual foragers often show a preference for one particular kind of resource (Sommeijer et al. 1983; Biesmeijer and Tóth 1998; Hofstede and Sommeijer 2006; Leonhardt et al. 2007; Gomes et al. 2015; Gostinski et al. 2017; Mateus et al. 2019). For example, Sommeijer et al. (1983) followed *Melipona favosa* foragers and found that most collected either exclusively nectar or exclusively pollen and only ~25% collected both types. In *Melipona beecheii,* about 50% of all foragers specialised on either pollen or nectar during their foraging career and the other half collected more than one type of resource (Biesmeijer and Tóth 1998). In the latter case, foragers would often collect pollen in the morning and nectar in the afternoon (see also below). In *Plebeia tobagoensis* studied in a greenhouse, 71% of all foragers specialised on one type of resource (pollen, nectar, water or resin) during a 3-day observation period (Hofstede and Sommeijer 2006). Interestingly, individuals that were flexible in their food choice were overall less efficient than specialists: flexible foragers collected smaller pollen loads and/or they needed more time to collect sucrose solution, which supports the hypothesis that individual specialisation increases task performance (but see Biesmeijer and Tóth 1998). Pollen foragers—and to a lesser extent resin and mud foragers—leave their nest carrying concentrated nectar in their crop (Leonhardt et al. 2007; Harano et al. 2020).[6] This nectar probably has two functions. First, it serves as fuel for the flight and, second, as binding material that helps bees attach pollen grains to the corbiculae (Leonhardt et al. 2007). In *Melipona marginata*, the most pronounced behavioural specialisation was found in mud collecting bees, possibly because this specialisation reduces the risks of a contamination of food stored in food pots by microorganisms present in collected mud (Mateus et al. 2019). In honey bees, foragers collecting different resources differ in their sensitivity to sugar rewards (Scheiner et al. 2004; Simone-Finstrom et al. 2010). Balbuena and Farina (2020) recently provided the first evidence for similar differences in stingless bees: *Tetragonisca angustula* pollen foragers had a lower sucrose response threshold (SRT) than non-pollen foragers, consistent with SRT differences between pollen and nectar foragers in honey bees.

The decision to collect nectar or pollen is likely to also depend on colony conditions, such as the amount of stored food. The removal of honey from *Melipona fasciculata* nests led to an increase in nectar collection that lasted several days (Gostinski et al. 2017) and experimentally adding pollen to nests of *M. subnitida* caused a reduction of pollen collection within 24 h (Maia-Silva et al. 2016). This

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[6] Nectar foragers left the hive without any detectable nectar in their crop in *Melipona subnitida*, suggesting that they collected nectar from food sources that were very close to the nest (Harano et al. 2020).

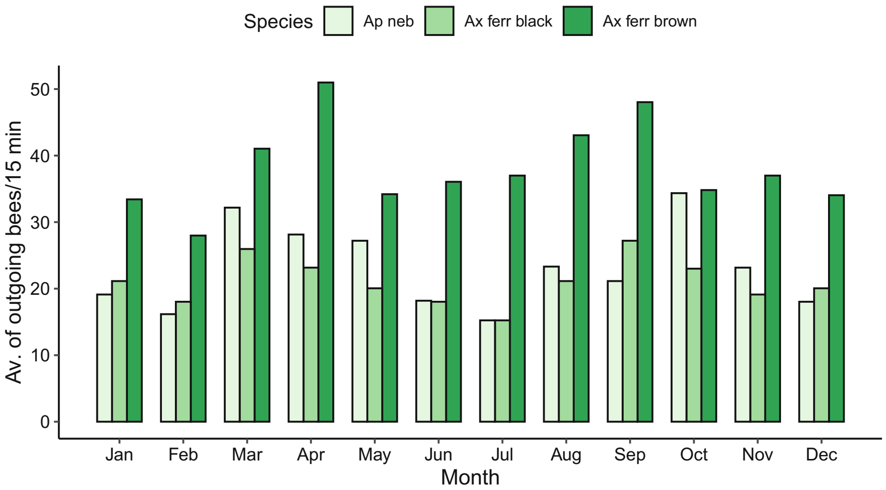

Fig. 8.4 Mean number of bees leaving the nest during 15 min of observation during a 12-month study period. Species are *Axestotrigona ferruginea* (reddish brown morph), *A. ferruginea* (black morph) and *Apotrigona nebulata* (modified from Kajobe and Echazarreta 2005)

suggests that foragers acquire information about the amount of stored food and adjust resource collection accordingly. However, an experimental reduction of pollen stores had no immediate effect on pollen collection in *M. subnitida*. Maia-Silva et al. (2016) argue that a temporary shortage of pollen might have less severe consequences in species with mass provisioning than in species with progressive provisioning (honey bees). In the latter case, a sudden lack of pollen could lead to the death of all larvae, whereas in stingless bees, a lack of pollen could lead to a temporal interruption of brood rearing, but without any harm to the existing brood.

8.4 Foraging Activity

8.4.1 Activity During the Season

In some species, foraging is possible throughout the year (Fig. 8.4) (e.g. *Apotrigona nebulata* in Uganda, Kajobe and Echazarreta 2005; *Melipona beecheii* in Mexico, Di Trani and Villanueva-Gutiérrez 2018; *Partamona cupira* in Panama, Wolda and Roubik 1986, *Tetragonula carbonaria* in Australia, Heard and Hendrikz 1993), whereas in others, foraging is reduced or ceases completely during some months, either due to low temperatures, resource scarcity, extreme draught or strong rain (see also Chap. 5) (Roubik 1982c; van Benthem et al. 1995; Ferreira Junior et al. 2010; Nascimento and Nascimento 2012; Halcroft et al. 2013; Maia-Silva et al. 2015; Hrncir et al. 2019). Nearly all species show at least some level of seasonality in their foraging activity. This seasonality directly affects the amount of stored resources as colonies build up more reserves when food is more abundant (see also Chap. 5)

(Roubik 1982c; Nascimento and Nascimento 2012; Maia-Silva et al. 2015; Aleixo et al. 2017; Hrncir et al. 2019). For example, *Melipona subnitida* colonies living in the Caatinga, a tropical dry forest in north-eastern Brazil, experience long periods of drought during which they invest little in foraging or brood production and exist in a state of lockdown until foraging conditions suddenly improve (Maia-Silva et al. 2015; Hrncir et al. 2019).[7] The same is found in *Austroplebeia australis*, where foraging stops almost completely during periods of resource scarcity, which are not uncommon in the semi-arid habitats in inland Australia where these bees live (Halcroft et al. 2013). Not only the quantity of food sources depends on the time of year but most likely also the quality: the nectar collected by *Hypotrigona gribodoi* and *Axestotrigona ferruginea* in Uganda contains more sugar during the dry season than during the rainy season (Kajobe 2007a; see also Campbell et al. 2019).

8.4.2 Activity During the Day

Foraging activity is not evenly distributed over the day and depends on light conditions, wind, humidity, temperature and bee body size (Fowler 1979; Imperatriz-Fonseca et al. 1985; Baumgartner and Roubik 1989; Roubik 1989; Heard and Hendrikz 1993; Hilário et al. 2000, 2001; Pick and Blochtein 2002; Teixeira and Campos 2005; Ferreira Junior et al. 2010; Sung et al. 2011; Nascimento and Nascimento 2012; Keppner and Jarau 2016; Macías-Macías et al. 2017; Layek and Karmakar 2018). Somewhat different from honey bees, some stingless bees also forage when it is raining (Baumgartner and Roubik 1989; Kajobe and Echazarreta 2005; Keppner and Jarau 2016). These external factors can affect flight activity directly, e.g. by affecting energy and water loss during flight, or indirectly through their effects on the production of pollen and nectar by flowers.

Species differ considerably in the time of highest activity, even if they inhabit the same environment (Fig. 8.5) (Roubik 1983, 1989; Wolda and Roubik 1986; Keppner and Jarau 2016; I'Anson Price 2018). Some colonies may show distinct activity peaks, whereas others have a relatively constant foraging activity during daytime. A late afternoon peak around 4–5 p.m. (e.g. in *Tetragona clavipes*, *Trigona fuscipennis*, *T. recursa*, *Friesella schrottkyi* or *Plebeia droryana*) seems to be a more widespread phenomenon in stingless bees (Roubik 1989, pp. 131). It could indicate that foraging conditions improve around this time in many Neotropical environments or it could indicate an increase in orientation flight activity in novice foragers in the late afternoon (Roubik 1989).[8] An unusual pattern is found in *Partamona helleri* and *P. orizabaensis*, where activity is often highest in the early

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[7] In the Caatinga, draughts can last for several years, forcing colonies to maintain minimum levels of brood production for extended time periods, which makes this a particularly challenging habitat for stingless bees (Hrncir et al. 2019).

[8] In *Apis mellifera*, young bees often leave their hive in the afternoon to defecate. Stingless bees, however, defecate inside their nest.

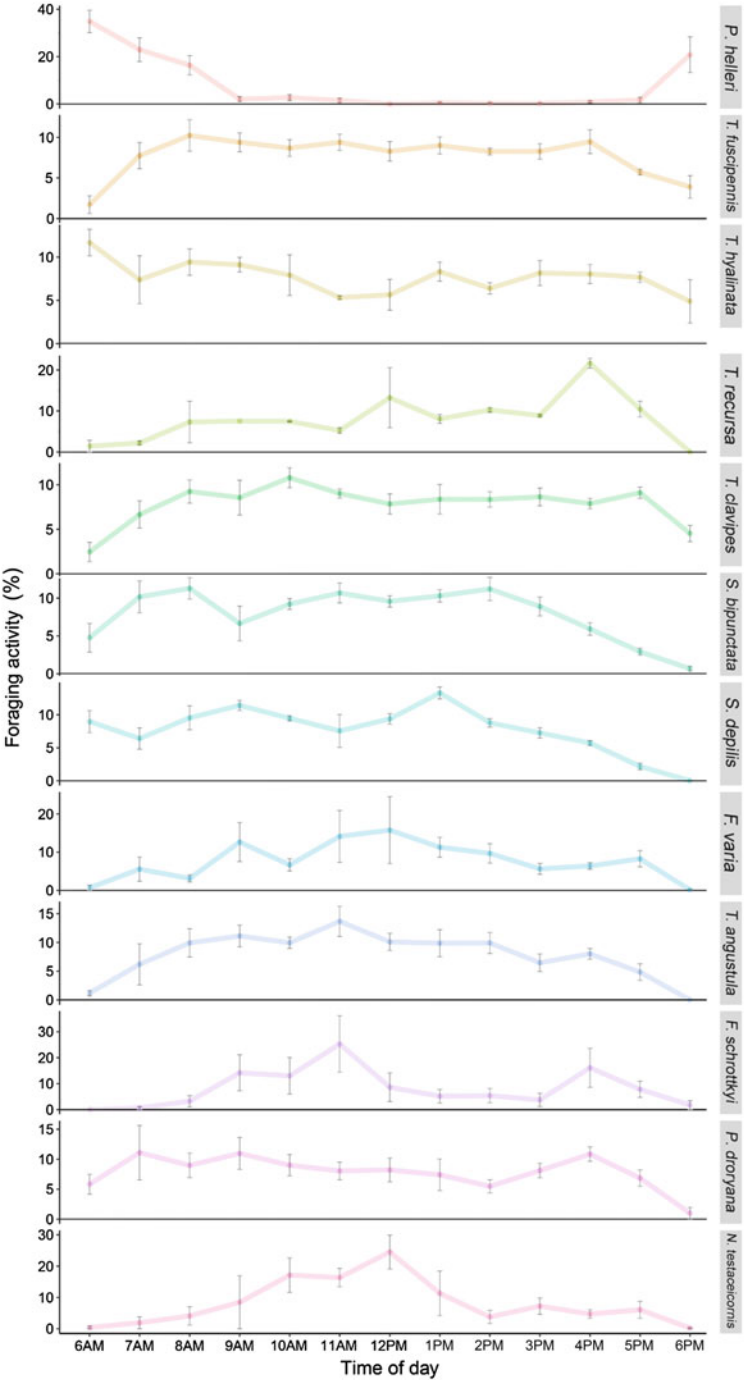

Fig. 8.5 Foraging activity of 12 sympatric species in São Paulo state, Brazil, measured on the same days. Data were collected on two days in late summer (end of rainy season). Data show the mean percentage of total daily foraging activity ± SE (from I'Anson Price 2018)

morning and in the late afternoon, with reduced foraging during most of the day (Fig. 8.5) (Keppner and Jarau 2016; I'Anson Price 2018). It is not currently clear why these *Partamona* species show this unusual pattern of activity.

Species with mass recruitment (see Chap. 10) can collect a significant proportion of their food during relatively short bursts of activity (Roubik et al. 1986; Roubik 1989). Even though such activity bursts may often only last an hour, colonies showing such activity patterns collect a substantial proportion of their food sources during these periods (Roubik et al. 1986; Roubik 1989).

A common observation in Neotropical stingless bees is a relatively clear temporal separation in the collection of pollen and nectar: pollen is mainly collected in the early morning, whereas nectar, resin or mud collection is most intense around noon and in the afternoon (Sommeijer et al. 1983; Roubik and Buchmann 1984; Hilário et al. 2000; Pierrot and Schlindwein 2003; Nascimento and Nascimento 2012; but see Hilário et al. 2001). The postponed onset of nectar collection could be explained by the observation that the sugar content of nectars is often lower in the morning and increases over the day (Fig. 8.6) (Roubik and Buchmann 1984; Roubik et al. 1995; Kajobe 2007a). On the other hand, stingless bees in Uganda did not show such a temporal sequence of pollen and nectar collection. Instead, pollen was collected throughout the day (Kajobe and Echazarreta 2005).

The Role of Bee Morphology

Smaller bees are likely to cool down more during flight because of relatively higher heat convection (Pereboom and Biesmeijer 2003), while larger bees have a higher risk of overheating when foraging in sunlight (Pereboom and Biesmeijer 2003; Hrncir and Maia-Silva 2013a, b). Thus, temperature and body size are key factors determining foraging activity (e.g. Fowler 1979; Roubik 1989; Heard and Hendrikz 1993; Corbet et al. 1995; Hilário et al. 2000, 2001; Teixeira and Campos 2005; Sung et al. 2011; Hrncir et al. 2019; Souza-Junior et al. 2020). Large species, such as *Melipona*, become active at temperatures as low as 11 °C, whereas the much smaller *Friesella schrottkyi* and *Plebeia lucii* require 21–22 °C for foraging (Hilário et al. 2000; Teixeira and Campos 2005; but see Sung et al. 2011). Other relatively small species, like *Tetragonisca angustula*, *Tetragonula carbonaria* or *Plebeia pugnax*, start foraging at 14 °C to 20 °C (Heard and Hendrikz 1993; Hilário et al. 2001; Malerbo-Souza and Halak 2016).

Body size also affects the size and sensitivity of sensory organs in bees, e.g. eye or antenna size (Jander and Jander 2002; Spaethe et al. 2007; Streinzer et al. 2016; Grüter et al. 2017b). Smaller eyes have a lower light sensitivity (Streinzer et al. 2016), which makes foraging under dim-light conditions more difficult for small-bodied species (Streinzer et al. 2016). This can contribute to species differences in the time of foraging activity and the choice of flower patches (Willmer and Corbet 1981; Biesmeijer et al. 1999a, b; Teixeira and Campos 2005). Large stingless bee species like *Melipona* start foraging early, sometimes before sunrise (Pereboom and Biesmeijer 2003; Teixeira and Campos 2005; Streinzer et al. 2016). Smaller bees,

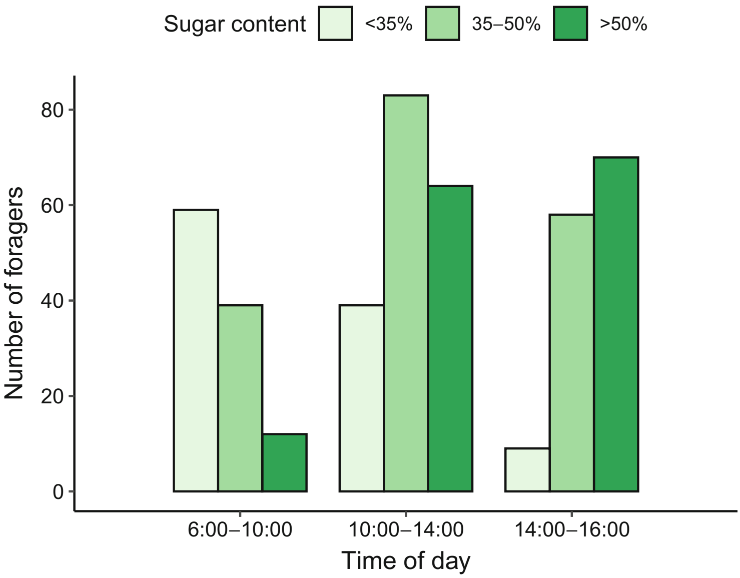

Fig. 8.6 Relationship between time of day and the sugar content of liquids collected by four species of *Melipona* in Panama (data from Roubik and Buchmann 1984, their Table 9)

like the Neotropical *Friesella schrottkyi*,[9] show little activity before 8 or 9 a.m. (Pereboom and Biesmeijer 2003; I'Anson Price 2018).

Body colouration is another morphological feature that affects foraging activity and flower patch exploitation (Hrncir and Maia-Silva 2013b). The yellowish *Melipona beecheii* prefers sunny patches, whereas the darker *M. fasciata* selects more shady patches (Biesmeijer et al. 1999a, b). This can result in differences in the sugar content of the collected nectar (see Sect. 8.1). Morphological properties might also affect the biogeographic distribution of bees. Lighter coloured bees have an advantage in open and very hot environments, whereas dark colouration might be beneficial in wet or mountainous habitats (Willmer and Corbet 1981; Pereboom and Biesmeijer 2003). Species occurring at higher altitudes (>1500 m) are often dark and of medium to large size (Pereboom and Biesmeijer 2003).

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[9] Because of this late onset of foraging (often colonies show little activity before 11 a.m.), *F. schrottkyi* is also called "mirim-preguiça" or "lazy bee" in Brazil.

8.5 Foraging Range and Trip Duration

The foraging range of a colony has important community ecological consequences because it affects competition among colonies and the population genetics of the visited plant species. Additionally, the foraging range of a colony is likely to affect foraging success in a disturbed and fragmented habitat.

Stingless bees are expected to prefer food sources that are closer to their nest (León et al. 2015; Souza-Junior et al. 2020), as this reduces flight costs and lowers exposure to environmental hazards, such as predation or high temperatures. Estimates of the maximum flight range of stingless bee foragers are mostly based on studies using artificial feeders or homing studies (Michener 1974; Roubik and Aluja 1983; Van Nieuwstadt and Iraheta 1996; Silva et al. 2014; Campbell et al. 2019; Nunes-Silva et al. 2020). In the latter case, foragers are captured at the nest, marked and then released at various distances from the nest. If bees are able to return to their nest, it is assumed that the release distance was within the maximum flight range of the bee. The proportion of bees returning to their nest often decreases with increasing distance (Roubik and Aluja 1983; van Nieuwstadt and Iraheta 1996; Nunes-Silva et al. 2020). When artificial feeders are used, bees that are accustomed to collect sucrose solution from artificial feeders are trained to visit feeders at increasing distances until they stop returning to the feeder. This distance is then considered the maximum foraging distance. Van Nieuwstadt and Iraheta (1996) compared the two methods with four stingless bee species and found that the two methods provide comparable results (but see Silva et al. 2014), even though the mark, release and recapture method leads to larger estimates of maximum foraging distances (about +300 m).

Maximum foraging distance estimates range from ~120 m in the Asian *Tetragonula iridipennis* to ~4000 m in the American *Melipona subnitida* (Table 8.1). Both *Melipona fasciculata* and *M. subnitida* foragers were occasionally able to find their way home after being released several km from their nest (Silva et al. 2014; Nunes-Silva et al. 2020). However, *M. fasciculata* foragers that were released more than about 2500 m from their nest needed many hours to return, suggesting that they were lost. This highlights that it is important to also record the time that foragers need to return to their nest to interpret the data obtained from homing experiments (Nunes-Silva et al. 2020). The data shown in Fig. 8.7 underline that flight range increases with body size (van Nieuwstadt and Iraheta 1996; Araújo et al. 2004; Greenleaf et al. 2007).

The two methods used to estimate foraging range have their limitations. First, they may lead to inaccurate estimates of the maximum foraging range and, second, they provide limited insight into the most common foraging distances (Breed et al. 1999; Greenleaf et al. 2007; Kuhn-Neto et al. 2009). For example, whether foragers continue to visit a feeder at a particular distance will depend on their motivation, which in turn is likely to depend on alternative foraging options and the season. The success rate of homing foragers, on the other hand, is likely to depend on the prior foraging experience of the bee. A forager that is able to return from 500 m from a northerly direction, e.g. because she has been foraging in that area on previous days,

Table 8.1 Maximum foraging distance (m), head width (mm) and colony size in stingless bees

Species	Max. flight range	Head width	Method	Reference
Cephalotrigona capitata	1700 m	2.5 mm[a]	Mark and recapture	Roubik and Aluja (1983)
Melipona fasciculata	2300 m	4.5 mm[b]	Mark and recapture	Araújo et al. (2004), Nunes-Silva et al. (2019)
Melipona fasciata	2400 m	4 mm[c]	Mark and recapture	Roubik and Aluja (1983)
Melipona fuliginosa	2000 m	4.9 mm[c]	?	Wille (1983)
Melipona mandacaia	2100 m	3.5 mm[c]	Artificial feeder	Kuhn-Neto et al. (2009)
Melipona marginata	800 m	2.9 mm[d]	?	Wille (1983)
Melipona quadrifasciata	2000 m	3.9 mm[d]	?	Araújo et al. (2004)
Melipona subnitida	4000 m	3.7 mm[b]	Both	Silva et al. (2014)
Nannotrigona perilampoides	620 m	1.8 mm[e]	Both	van Nieuwstadt and Iraheta (1996)
Partamona cupira	800 m	2.5 mm[e]	Both	van Nieuwstadt and Iraheta (1996)
Plebeia droryana	540 m	1.6 mm[b]	?	Araújo et al. (2004)
Plebeia mosquito	540 m	2 mm[f]	Artificial feeder	Michener (1974, pp. 153)
Scaptotrigona postica	860 m	2.5 mm[d]	Mark and recapture	Campbell et al. (2019)
Tetragonula iridipennis	120 m	1.6 mm[g]	Artificial feeder	Lindauer (1957)
Tetragonula minangkabau	430 m	1.7 mm[h]		Inoue et al. (1985)
Tetragonisca angustula	850 m	1.8 mm[b]	Both	van Nieuwstadt and Iraheta (1996)
Trigona amalthea	980 m	3.2 mm[a]	Artificial feeder	Michener (1974, pp. 153)
Trigona corvina	760 m	2.4 mm[e]	Both	van Nieuwstadt and Iraheta (1996)
Trigona silvestriana	800 m	2.7 mm[a]	?	Wille (1983)
Trigona spinipes	840 m	2.6 mm[d]	Artificial feeder	Michener (1974, pp. 153)

[a]Schwarz (1948), [b]Grüter et al. (2017a), [c]Estimated from Schwarz (1932), [d]Based on photos provided by Kátia Aleixo, [e]van Nieuwstadt and Iraheta (1996), [f]von Ihering (1903), [g]Rasmussen (2013), [h]Sakagami and Inoue (1985)

The method "both" indicates that training with artificial feeders and mark and recapture methods were used. Estimates of colony size are based on Table 1.3

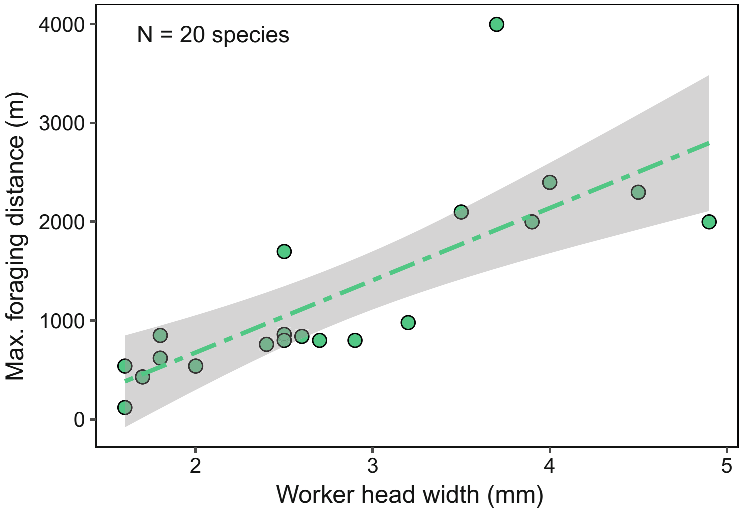

Fig. 8.7 Relationship between bee size (head width) and the estimated maximum foraging distance for 20 species of stingless bees (linear regression, $R^2 = 0.57$, $p < 0.0001$) (see Table 8.1). Grey area represents the 95%-confidence interval

may not be able to return from a 500 m release site in different locations. Thus, actual colony flight ranges might exceed those suggested by homing studies. More importantly, however, estimates of maximum foraging distances provide little information about where the majority of foragers collect food and whether and how the distribution of foraging distances depends on the environment (Roubik 1989; Campbell et al. 2019). Honey bees, for example, can visit artificial feeders at distances of up to 12 km, but most foraging usually occurs at distances below 2.5 km (von Frisch 1967; Steffan-Dewenter and Kuhn 2003; Couvillon et al. 2014). Breed et al. (1999) used baits to estimate the foraging distances of *Trigona amalthea* and *T. corvina*. Their data suggest that most foraging occurs within 200 m from the nest, which is considerably closer to the nest than the suggested maximum foraging range (760–980 m) (Table 8.1). Roubik (1989, pp. 90) proposes the rule of thumb that most foraging takes place within one third to one-half of the maximum foraging range.[10]

Foraging distances strongly depend on the environment and the season (in honey bees: Visscher and Seeley 1982; Waddington et al. 1994; Beekman and Ratnieks 2000; Steffan-Dewenter and Kuhn 2003; Couvillon et al. 2014). For instance, Campbell et al. (2019) found that the foraging distances of *Scaptotrigona postica* foragers in the eastern Brazilian Amazon were shorter during drier months than during the wet season. Interestingly, foraging distances correlated negatively with

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[10] The meat eating *Trigona hypogea* was observed to collect wasp brood 800 m from their nest (Noll 1997), which is close to what would be expected to be the maximum foraging distance for a bee of this size.

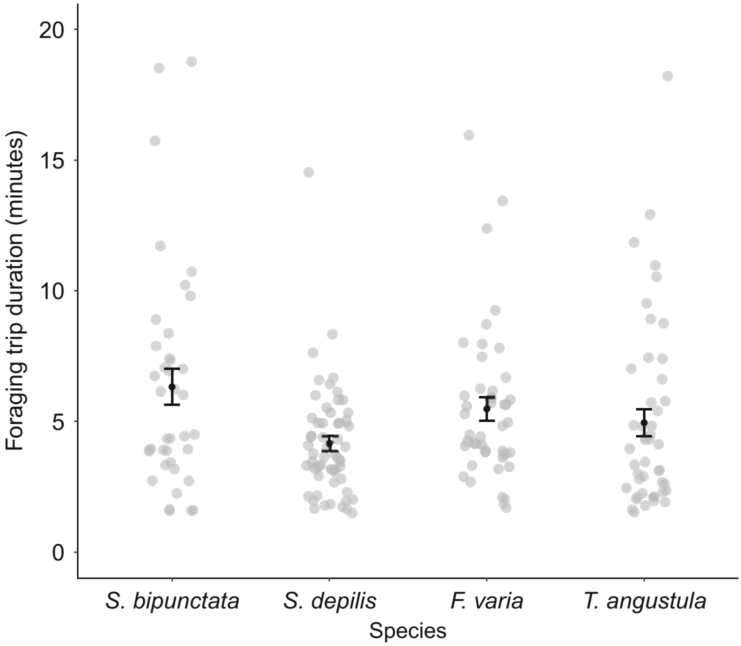

Fig. 8.8 Average foraging trip duration of foragers from four species (mean ± SE), *Scaptotrigona bipunctata, S. depilis, Frieseomelitta varia* and *Tetragonisca angustula* (from I'Anson Price 2018)

the sugar concentration of the collected liquids. This indicates that poor foraging conditions, such as those encountered during the Amazonian wet season, force foragers to travel further to find food (Campbell et al. 2019).

Most foraging trips last from a few minutes to less than an hour (Roubik 1989; Harano et al. 2020). I'Anson Price (2018) studied the duration of nest excursions in four small-bodied species in São Paulo state at the end of the wet season and found that bees left the nest for less than 20 min, with an average foraging trip duration of 5–7 min (Fig. 8.8). Similar durations (4–8 min) were found for pollen collection trips by *Partamona* sp. foragers (Wille and Orozco 1975) and foraging trips of *Melipona costaricensis* lasted on average 4 min (range 1–10 min) (De Bruijn and Sommeijer 1997). This is considerably shorter than the foraging trip durations of temperate honey bees, which usually last from 10 to 100 min (Park 1926; Butler et al. 1943; von Frisch 1967). These data reinforce the idea that, despite the impressive maximum foraging distance estimates shown in Table 1.8, stingless bee foragers usually exploit food close to their nest.

8.6 Competition and Resource Partitioning

8.6.1 Competition Among Stingless Bees

Many tropical habitats are home to a large number of colonies, sometimes belonging to dozens of stingless bee species (see also Chap. 3). An extreme example was recently described by Roubik (2018) who found ~100 stingless bee species coexisting in a small parcel (<10 km^2) of the Yasuní National Park in the Ecuadorian Amazon. This, in combination with their generalist (polylectic) foraging habits, means that the food sources that can be used by different species are likely to overlap, creating significant potential for foraging competition (Sommeijer et al. 1983; Imperatriz-Fonseca et al. 1989; Ramalho et al. 1989; Wilms et al. 1996; Eltz et al. 2001; Biesmeijer and Slaa 2006; Teixeira et al. 2007; Kajobe 2007b; Leonhardt and Blüthgen 2009; Dworschak and Bluethgen 2010; Lichtenberg et al. 2010, 2017; Hrncir and Maia-Silva 2013a, b). Overall, dietary niche overlap (i.e. the joint use of resources by two species, see e.g. Colwell and Futuyma 1971) between two sympatric stingless bee species usually lies between 0.2 and 0.4 (can be >0.5) (Wilms et al. 1996; Roubik and Moreno Patiño 2018), but may vary depending on the foraging conditions and the season (Eltz et al. 2001). The Neotropical genus *Melipona* seems to have a somewhat distinct diet compared to other stingless bees, possibly because their larger body size allows them to exploit different floral resources (see also Chap. 9) (e.g. Ramalho et al. 1989).[11]

Studies in different tropical habitats suggest that the overlap in diet is greater in taxonomically more related stingless bee species (Sommeijer et al. 1983; Ramalho et al. 1989; Wilms et al. 1996; Nagamitsu and Inoue 1997; Biesmeijer and Slaa 2006) and most pronounced in colonies of the same species (Sommeijer et al. 1983; Eltz et al. 2001; but see Roubik and Moreno Patiño 2018). As a result, competition is stronger among more closely related species, which could explain why stingless bee species assemblages often contain a larger diversity of genera than expected from the number of species, i.e. species in assemblages appear to be phylogenetically more distant than expected (Biesmeijer and Slaa 2006).

Competition implies that there are costs for all or some of the involved individuals that lead to a reduction in survivorship or foraging success. For example, competition among stingless bees at food sources reduces the time that foragers spend at these food sources and the size of pollen and nectar loads (Johnson and Hubbell 1974). Competition does not require individuals to interact directly with each other. Instead, foragers may experience reduced rewards because other foragers have already exploited a resource. This “exploitation competition” is likely to be common in stingless bees, but can be hard to quantify without careful experiments (e.g. Balfour et al. 2015). Alternatively, foragers may interact directly with each

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[11] Morphological differences (e.g. in size) are probably also the reason why bumble bees show the smallest overlap with stingless bees in terms of the food sources they exploit (Biesmeijer and Slaa 2006).

other at resources, thus preventing each other from foraging efficiently, so-called "interference competition". Direct interactions range from threatening body postures (opening of mandibles and spreading out of the wings to a V-position) to deadly fights (Johnson and Hubbell 1974; Roubik 1989; Nagamitsu and Inoue 1997; Dworschak and Bluethgen 2010; Lichtenberg et al. 2010). The severity of agonistic behaviours can increase with increasing food source quality (Johnson and Hubbell 1974) and body size asymmetry (Dworschak and Bluethgen 2010). In the latter case, larger bees occasionally outcompete smaller bees (but see Lichtenberg et al. 2010). Due to the conspicuousness of these direct interactions, most studies on competition among stingless bees have dealt with this interference competition. For example, when studying foraging at artificial baits, Baumgartner and Roubik (1989) observed that the relatively small, but rapidly recruiting *Trigona fuscipennis* was found first at baits, but was then replaced by the larger *Cephalotrigona capitata.* A third, non-aggressively foraging species (named as *Scaptotrigona xanthotricha)* was only able to land after the first two more aggressive species became less abundant at the food source (aggressive foragers in the Neotropics often belong to the genus *Trigona*, e.g. *T. amalthea, T. corvina, T. fuscipennis, T. hyalinata, T. sylvestriana* and *T. williana*, see e.g. Johnson and Hubbell 1974; Howard 1985). When studying competition among Asian stingless bees, more aggressive species arrived later at artificial food sources, which could indicate that less-aggressive species are better at discovering food sources (see below) or that they avoid direct interactions by foraging earlier in the day (Nagamitsu and Inoue 1997). It should be noted that aggressive interactions are less common at natural food sources than at artificial baits and resin collection sites (see e.g. Howard 1985; Leonhardt and Blüthgen 2009; Dworschak and Bluethgen 2010).

Niche Partitioning

Given the large potential for competition, how can so many different stingless bee species coexist in the same environment? A large number of behavioural and morphological traits affect whether and how stingless bees compete with each other. For example, less competitive species could shift their foraging time or their diet in order to avoid competition with other species (e.g. Nagamitsu and Inoue 1997; Keppner and Jarau 2016; Lichtenberg et al. 2017). As a result, the realised food niche of a species will be different from its fundamental food niche, i.e. the food niche a species would occupy in the absence of competitors (Biesmeijer and Slaa 2006; Hrncir and Maia-Silva 2013a, b). Adjustments in diet due to competition could explain the observation that the niche breadth of species decreases as the number of sympatric stingless bee increases (Biesmeijer and Slaa 2006). According to Johnson and Hubbell (1974), niche switch could also explain why small-bodied stingless bees seem to specialise on small-flowered, over-dispersed plants.

Morphological traits can have complex effects on resource exploitation. As discussed above, body size and colour affect when foragers are active and, thereby, contribute to resource partitioning. Proboscis length is another morphological trait that affects flower preferences (Corbet et al. 1995; Biesmeijer and Slaa 2006).

Species with a longer proboscis have access to flowers with a deeper corolla and, thus, a potentially larger variety of flower nectars (Hrncir and Maia-Silva 2013a).

A common feature of many aggressive, highly competitive species is that they are mass recruiters with large colony sizes (Lichtenberg et al. 2010). They typically use pheromone trails to recruit nestmates to resources (Chap. 10). Mass recruitment allows colonies to monopolise food sources and exclude competitors (Hubbell and Johnson 1978; Hrncir 2009; Hrncir and Maia-Silva 2013a). However, not all mass-recruiting species are similarly aggressive. *Scaptotrigona*, *Partamona* and some *Trigona* species are not particularly aggressive, but exclude other competitors through their sheer number (Biesmeijer and Slaa 2004; Lichtenberg et al. 2010; Hrncir and Maia-Silva 2013a; Keppner and Jarau 2016). On the other hand, allocating a large number of foragers to a high-quality resource might lower a colony's ability to discover new food sources (Johnson and Hubbell 1974; Hubbell and Johnson 1978). When Hubbell and Johnson (1978) laid out artificial baits consisting of scented sucrose solutions in Costa Rica, the solitarily foraging, small-bodied *Nannotrigona perilampoides*, *Plebeia frontalis* and *Trigonisca* sp.[12] discovered the baits much quicker than the dominant, mass-recruiting (or group-foraging) *Scaptotrigona pectoralis* and *Trigona fuscipennis*. Such a discovery-dominance trade-off is well-known in ants (e.g. Lebrun and Feener 2007; Bertelsmeier et al. 2015) and is thought to allow species that differ in their foraging strategy, but overlap in their diet to coexist in the same habitat (Hubbell and Johnson 1978; Kneitel and Chase 2004; Lebrun and Feener 2007). For instance, dominant mass recruiters and low-density specialists can coexist in the same environment if foraging options range from large, aggregated patches to small, highly dispersed plants (Hubbell and Johnson 1978; Biesmeijer and Slaa 2006). Empirical support for such a scenario comes from observations on *Trigona fulviventris* and *T. fuscipennis* foragers that were foraging together on a population of the shrub *Cassia biflora* (Johnson and Hubbell 1975). The mass-recruiting *T. fuscipennis* mainly exploited large, clumped high-quality patches of *C. biflora*, whereas the more solitarily foraging *T. fulviventris* visited isolated and more widely spaced *C. biflora* plants. This pattern is likely to be the outcome of differences in evolved foraging strategies in combination with differences in competitive abilities (Johnson and Hubbell 1975; Lichtenberg et al. 2017). A suspected result of mass recruitment is that mass recruiters might have a narrower diet than solitarily foraging species (Lichtenberg et al. 2017).

Competition could also be avoided if bees occupy different strata, e.g. in vertically structured tropical forests: lower strata could be occupied by less aggressive bees foraging on smaller, scattered food sources, whereas dominant bees forage at higher levels where mass-flowering trees provide clumped, highly profitable

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[12] Called *T. testaceicornis*, *T. frontalis* and *T. buyssoni* in Hubbell and Johnson (1978). However, neither *N. testaceicornis* nor *Trigonisca buyssoni* exist in Costa Rica according to Camargo and Pedro (2013); my suggestions are based on distribution information recovered from Camargo and Pedro (2013).

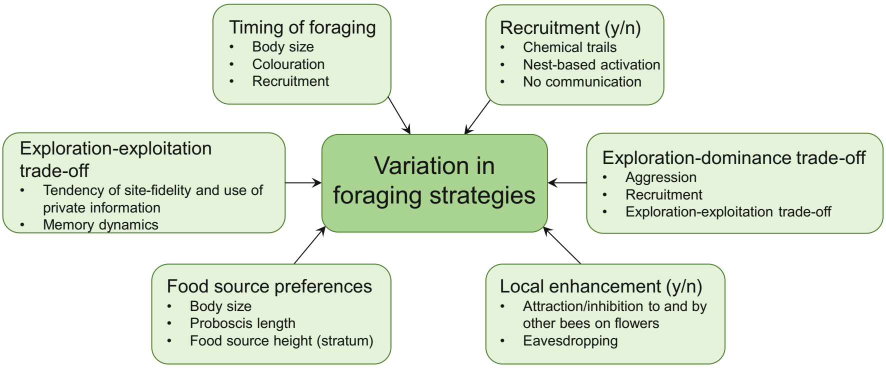

Fig. 8.9 Behavioural and morphological traits contribute to the foraging strategy of a stingless bee species. Variation in these traits facilitates niche partitioning and the coexistence of species in the same habitat

foraging bonanzas. While Nagamitsu and Inoue (1997) found no support for resource partitioning across strata based on dominance, there is evidence that different species consistently forage at different heights: in Malaysia, *Tetragonula fuscobalteata* was often found on canopy flowers, whereas *T. melanocephala* prefers understory flowers (Nagamitsu et al. 1999). Similarly, *Partamona cupira* in Panama was predictably associated with a higher stratum, whereas *Trigona fulviventris* preferred to forage closer to the ground (Roubik 1993; see also Ramalho 2004). *Trigona corvina* showed no specialisation in height in the same environment.

Resource partitioning at patches can also occur because foragers of some less competitive species opportunistically collect pollen that has fallen on petals, leaves or the ground due to the foraging activity of more dominant species (Wille 1963; Roubik 1989; Pick and Blochtein 2002; Hrncir and Maia-Silva 2013b; C.G., pers. obs.). For these often smaller foragers, picking up the leftovers of other stingless bees seems to be a successful foraging strategy and Roubik (2018) suggests that the bladder-like hind basitarsus with long rows of bristles found in some *Scaura* are adaptations that help foragers collect pollen that has fallen on surfaces (Figs. 1.1 and 8.1c). These examples highlight that the coexistence of many species of stingless bees in the same habitat is possible because of their great diversity in behaviour, morphology, physiology and lifestyle (summarised in Fig. 8.9).

8.6.2 Competition with Honey Bees

A potentially significant group of competitors are the honey bees (Apini). In the Neotropics, *Apis mellifera* (mostly the Africanised honey bee) has been introduced and, thus, represents a relatively new competitor. This means that competitive strategies that have evolved over millions of years in a particular community are now tested in a new context, against a new competitor with a broad diet, large body size, large colony size and a sophisticated recruitment communication (von Frisch

1967; Roubik 1989). Unsurprisingly, this has led to worries that Africanised honey bees might put pressure on Neotropical stingless bee populations (Roubik 1978; Wilms et al. 1996).

Africanised honey bees are the result of an escape of African *Apis mellifera scutellata* swarms in 1957, which were collected by Warwick Kerr in southern Africa and kept in an apiary in São Paulo state. The daughters of these African queens soon mated with drones of European sub-species, such as *A. m. ligustica,* which have been introduced to the Neotropics in the seventeenth century. The resulting bees were more productive, more aggressive and better adapted to the tropical climate than their European relatives and, as a result, spread rapidly throughout the tropical parts of South America before reaching Central America in the early 1980s.

There is considerable overlap in the food sources used by stingless bees and honey bees (e.g. Koeniger and Vorwohl 1979; Sommeijer et al. 1983; Roubik 1978, 1983; Wilms et al. 1996; Wilms and Wiechers 1997; Biesmeijer and Slaa 2006; Kajobe 2007b), and the colony foraging success of at least some stingless bees seems to be negatively affected by the foraging activity of introduced Africanised honey bees (Roubik 1978, 1983; Roubik et al. 1986). For example, Roubik (1978) experimentally increased the number of Africanised honey bee colonies in a habitat in French Guiana less than two years after Africanised honey bees first appeared in the area. He found that the presence of honey bees had a negative effect on foraging of stingless bees visiting some plant species but not others. When honey bee colony numbers were experimentally increased in a lowland forest in Panama, stingless bee colony foraging success was reduced in 4 of 12 studied stingless bee species (Roubik et al. 1986). Particularly, the foraging activity at high quality food source patches seemed to be affected by the presence of honey bees (Roubik et al. 1986), most likely due to the ability of *Apis mellifera* to recruit efficiently to high quality food patches (von Frisch 1967).

Observations at natural food sources provide further evidence that honey bees can negatively affect the foraging of Neotropical stingless bees. *Spondias mombin* is a tropical plant that is visited by both honey bees and stingless bees. Honey bees collect pollen earlier in the day, therefore removing this resource for the later arriving *Scaptotrigona tubiba* (Carneiro and Martins 2012; see also Menezes et al. 2007). While competition between honey bees and stingless bees might often be a case of "exploitation competition" (Roubik et al. 1986; Carneiro and Martins 2012), Cairns et al. (2005) have observed that honey bees in Mexico aggressively displaced stingless bees from food sources. Roubik (1980) observed occasional aggression of Africanised honey bees towards *Melipona* in French Guiana at natural food sources and artificial feeders. Such interference competition is likely to increase when food sources are scarce and stingless bees no longer have the option of switching to alternative resources. Thus, negative impacts of honey bee colonies on stingless bees are expected to be stronger or found exclusively in environments where human alterations of the environment have led to a decrease in food availability for bees (Cairns et al. 2005).

The effects of *Apis mellifera* on Neotropical stingless bees are likely to be species specific and some aggressive mass-recruiting stingless bees (e.g. some *Trigona*) are able to displace honey bees from food sources as long as honey bees are not too numerous (Roubik 1980; Cairns et al. 2005). Other species seem to be able to avoid competition by visiting smaller and more dispersed food sources. It has also been suggested that large mass-flowering trees play an important role in mitigating competition between honey bees and stingless bees (and among stingless bees themselves) because they represent food sources that are too large to be monopolised by one colony and, thus, allow species with different foraging strategies to forage without too much interference (Wilms et al. 1996; Hrncir and Maia-Silva 2013a).

Some species, e.g. *Trigona pallens* and *Tetragona clavipes*, are able to forage alongside honey bees by partitioning food sources (Roubik 1980) and a more long-term evaluation suggested that, overall, invasive honey bees have had little impact on native bee abundance in Panama and French Guiana (Roubik 2000). Likewise, a 17-year study on Barro Colorado Island in Panama found no negative impact of the arrival of honey bees on the abundance of stingless and other native bees (Roubik and Wolda 2001). The authors argue that the relatively small size of the island (16 km^2) means that the density of honey bee colonies remained relatively low. In summary, while there is evidence that introduced honey bees can have negative effects on the foraging activity of some stingless bees, it remains to be seen whether honey bees indeed contribute to a decline in Neotropical stingless bees. Assessing the ecological impact of introduced honey bees in the Neotropics is complicated by the fact that honey bees are often associated with humans and are, thus, more common in strongly human-modified environments (Stout and Morales 2009). Understanding the impact of honey bees on native bees requires disentangling the different effects of human alterations of the environment and honey bee activity.

Much less is known about the competitive interactions between honey bees and stingless bees in Africa and Asia where honey bees are native (but see Koeniger and Vorwohl 1979). Honey bees and stingless bees show considerable overlap in the resources they collect in Uganda, which suggests that these two groups might compete (Kajobe 2007b). A study performed in Cameroon, however, found that honey bees and the generalist stingless bee *Meliplebeia ogouensis* foraged on different food sources, suggesting resource partitioning (Tropek et al. 2018). When studying interactions at artificial food sources in Sri Lanka, the small *Tetragonula iridipennis* seemed to be dominant over the dwarf honey bee *Apis florea* and *A. cerana* (Koeniger and Vorwohl 1979) and the authors argue that the much shorter flight range of *T. iridipennis* could explain why this species aggressively defends the food sources that can be found within its smaller territory. Competition among stingless bees and Asian honey bees might vary considerably with season because some Asian honey bee species perform seasonal migrations, leading to highly variable colony densities (Koeniger and Vorwohl 1979).

8.6.3 Competition with Other Animals

Competition with ants is particularly common when collecting honeydew or when foraging at carrion, while competition with flies has been observed at carrion as well (Roubik 1982a, 1989; Baumgartner and Roubik 1989; Figueiredo 1996). Ants and stingless bees occasionally compete at nectar sources: *Ectatomma ruidum* actively prevented *Trigona fulviventris* foraging on *Justicia aurea*, which restricted the stingless bees to foraging either early in the morning or later in the afternoon when the ants were absent (Willmer and Corbet 1981). When bees compete with flies at carrion, bees actively chase away flies (e.g. blow flies) that attempt to lay eggs on the corpses of dead animals (Roubik 1982a; Baumgartner and Roubik 1989). Stingless bees occasionally compete with hummingbirds for nectar. *Trigona ferricauda*, for example, aggressively defends *Pavonia dasypetala* flower patches against *Phaethornis supercilious* hummingbirds in Panama (Roubik 1982b).

8.7 Learning During Foraging

The transition from in-nest work to foraging is often preceded by orientation flights that allow aspiring foragers to learn the visual surroundings of the nest (honey bees: Capaldi et al. 2000). Subsequently, bees can use both path integration and landmark-based learning to travel between the nest and a food source (e.g. von Frisch 1967; Zeil and Wittmann 1993; Chittka et al. 1995; Capaldi et al. 2000). A crucial aspect of navigational learning is the perception of the position of the sun ("sun compass") or, when the sun is not directly visible, the perception of polarised skylight (von Frisch 1967; Rossel and Wehner 1986). This, in combination with the ability to estimate the travelled distance and height based on "optic flow", allows bees to compute their position relative to the position of their nest or a resource (Srinivasan et al. 2000; Hrncir et al. 2003; Eckles et al. 2012). Optic flow refers to the extent by which the image of the surroundings has moved across the insect eye during flight (Srinivasan et al. 2000; Hrncir et al. 2003). Bar a few exceptions (e.g. Zeil and Wittmann 1989, 1993; Hrncir et al. 2003),[13] navigation in stingless bees remains a little explored research area. As a result, we know little about the relative importance of landmarks, features of the skyline, shapes of the canopy or celestial cues in stingless bee navigation.

To attract bees to their flowers, many plants produce fragrances, conspicuous colours and shapes (Vogel 1983). The production of fragrances is often temporarily correlated with the opening of flowers and the offering of pollen and nectar (Vogel 1983), which makes them suitable stimuli for associative reward-based learning. Floral scents usually consist of many different compounds that occur in relatively stable species-specific ratios. This allows bees to discriminate among plant species

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[13] Zeil and Wittmann (1993) show that *Tetragonisca angustula* uses landmarks in the vicinity of the nest to locate the nest, rather than the nest itself.

and learn about the rewards provided by particular types of plants (Vogel 1983). More than 1700 floral volatile organic compounds have been identified, mainly belonging to the fatty acid derivatives, benzenoids, phenylpropanoids, terpenoids and nitrogen- and sulphur-containing compounds (Knudsen et al. 1993; Muhlemann et al. 2014).

Like humans, stingless bees have trichromatic vision[14] and they very efficiently learn the colours and shapes of flowers or the time periods when flowers produce nectar or pollen (Beling 1929; von Frisch 1967; Menzel et al. 1993; Slaa et al. 1998, 2003). *Melipona fasciculata*, for instance, learned to inspect artificial food sources at the correct time of day after just one day of training (Jesus et al. 2014). This time-place learning remains an understudied phenomenon in stingless bees (but see Breed et al. 2002; Murphy and Breed 2008a; Jesus et al. 2014), but is likely to be important for the decision to initiate foraging (Biesmeijer et al. 1998; Breed et al. 2002) and arrive at food sources before competitors (Hrncir and Maia-Silva 2013a, b; Jesus et al. 2014).

8.7.1 Associative Learning

A key process underlying the learning of floral features is associative learning. In associative learning, a bee learns that the perception of a stimulus (or set of stimuli, such as a colour, odour or the shape of the food source) predicts a reward or a punishment (the latter two stimuli are referred to as unconditioned stimuli or US). The strength of the association between an odour or a colour (conditioned stimulus or CS) and the unconditioned stimulus depend on various factors, including the number of foraging trips to a food source, the temporal correlation between the CS and the US, the salience of the CS (e.g. some odours or colours are easier to learn, e.g. due to sensory biases) and the strength of the US (e.g. the sugar content of the nectar) (e.g. Rescorla and Wagner 1972; Menzel et al. 1993; Menzel 1999).

Associative learning has been studied extensively in honey bees using the proboscis extension response (PER) paradigm (and more recently the sting extension response, SER) in the laboratory or using Y-mazes in free flying bees (e.g. Bitterman et al. 1983; Menzel 1999; Giurfa 2007; Vergoz et al. 2007; Giurfa and Sandoz 2012). During typical PER conditioning, a human observer touches the antennae of a harnessed bee with a sucrose solution, which induces the bee to extend her proboscis (unconditioned response, UR). Bees are then fed a small sample of the sucrose solution, while simultaneously being exposed to an odour (Takeda 1961; Bitterman

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[14] Many studied bee species, including stingless bees, have colour vision with ultraviolet (UV), blue (B) and green (G) sensitive photoreceptors (trichromatic vision). The object and colour discrimination ability of the stingless bee species studied so far is somewhat lower than that in honey bees and bumble bees, which could be explained by the smaller size of the tested species (Spaethe et al. 2014; Streinzer et al. 2016; Dyer et al. 2016b).

et al. 1983; Menzel 1999; Giurfa 2007). This is a form of classical (or Pavlovian) conditioning. Learning experiments with free flying bees involve operant conditioning, i.e. a bee learns to associate its own actions with a reward or a punishment (Giurfa 2007).[15] This research has shown that a small number of learning events can establish life-long memories about associations between the CS and the US. For example, Menzel (1968) found that honey bees that received a sucrose reward on either a blue or an orange background on three subsequent visits show a preference for the rewarded colour for the rest of their lives. In olfactory conditioning, a single learning trial might often be sufficient to induce long-term memory in honey bees, which lasts several days (Villar et al. 2020).

The first attempts to study classical olfactory conditioning in stingless bees were not very successful: *Melipona scutellaris* was unable to associate an odour with a reward using the PER paradigm (Abramson et al. 1999, see Roselino and Hrncir 2012 and Amaya-Márquez et al. 2019 for similar results with *M. eburnea* and *M. scutellaris*). The Australian species *Austroplebeia australis*, *Tetragonula carbonaria* and *T. hockingsi*, on the other hand, were able to learn odours in a PER setup (Frasnelli et al. 2011).[16] Mc Cabe et al. (2007) and Mc Cabe and Farina (2010) studied classical conditioning of the proboscis extension in *Melipona quadrifasciata*, *Scaptotrigona depilis* and *Tetragonisca angustula* and found that only *M. quadrifasciata* was able to associate a reward with a particular odour and show the conditioned response (proboscis extension). *T. angustula* foragers were only able to learn to discriminate between a rewarded and an unrewarded odour if they experienced the rewarded odour inside their nest prior to the PER conditioning (Mc Cabe and Farina 2010). The ability of *M. quadrifasciata* to learn to discriminate between a rewarded and an unrewarded odour ("differential conditioning"[13]) was significantly less pronounced than in Africanised honey bees (Mc Cabe et al. 2007). Differential conditioning was used to show that two African stingless bees, *Meliponula bocandei* and *Axestotrigona ferruginea* (reddish brown morph), can learn to associate an odour with a reward in a PER paradigm (Henske et al. 2015). Odour discrimination was, however, higher in *A. ferruginea* than in *M. bocandei* and highest in *Apis mellifera*.

As discussed by Mc Cabe et al. (2007), these learning differences among species could point to differences in foraging behaviour and strategy. For example, it is plausible that some species have a more specialised diet (see also Chap. 9) and do not frequently experience situations where they have to learn new odours. Furthermore, foragers of some species might rely less on floral odours and more on visual stimuli or trail pheromones during foraging. *Scaptotrigona depilis*, for example, deposits

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[15] In differential conditioning, two CS are used, one associated with a reward and the other unrewarded or punished. The two CS (e.g. odours) are presented in a randomized order and the bee has to learn to respond (show the PER) to the rewarded odour, but not to the other (Bitterman et al. 1983).

[16] The authors also show lateralisation of the left and right antennae. Memory retrieval after 1 h was better when odours were presented to the right antennae. The opposite was true when memory retrieval was tested after 5 h (Frasnelli et al. 2011).

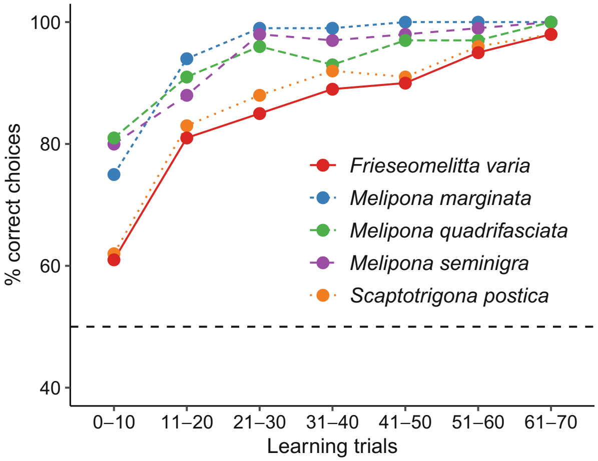

Fig. 8.10 Learning performance of free flying bees trained to collect food at a feeding station that offered two food sources on different backgrounds. Ten bees per species were rewarded (60% sucrose solution) on either a blue or a yellow background. The landing decision (blue vs. yellow) of each bee was recorded for 70 trials (data from Pessotti and Lé'Sénéchal 1981). The dotted line represents a 50% (or random) choice probability

pheromone trails to recruit nestmates to food sources (Chap. 10). Thus, associative learning of floral odours might be less important during foraging in this species.

There could also be methodological reasons explaining the lower learning performance observed in some stingless bee species. The PER has been developed and optimised to study classical conditioning in honey bees, and this learning paradigm might not be equally suited for stingless bees (Mc Cabe et al. 2007; Henske et al. 2015; Nocelli et al. 2018; Amaya-Márquez et al. 2019). In order to better understand associative learning in stingless bees under controlled laboratory conditions, modified versions of the PER should be developed, which are optimised for particular stingless bee species. For example, Amaya-Márquez et al. (2019) used a PER paradigm of freely moving bees kept in mini-cages and recorded better learning than when using the traditional PER method (see also Nocelli et al. 2018).

Learning paradigms using free flying bees seem to be more suited to study learning in stingless bees. Several studies tested the ability of Neotropical stingless bees to learn an association between a colour, a shape or a pattern and a sucrose reward at an artificial feeding station. These studies show that the foragers of all species quickly learned to discriminate between rewarded and unrewarded colours, shapes and patterns (e.g. Fig. 8.10) (Pessotti and Lé'Sénéchal 1981; Menzel et al. 1989; Villa and Weiss 1990; Moreno et al. 2012; Sánchez and Vandame 2012; Balamurali et al. 2018; Aguiar et al. 2020; Koethe et al. 2020). Slower learning was found in *Frieseomelitta varia*, whereas *Melipona* species learned very efficiently (Pessotti and Lé'Sénéchal 1981). Mc Cabe and Farina (2010) studied olfactory learning in free flying *Tetragonisca angustula* foragers and, likewise, found that foragers preferred an odour that they have experienced in association with a reward in previous foraging trips.

Foragers can also learn to associate rewards with the footprints (e.g. cuticular hydrocarbons) left on food sources by foraging conspecifics. If footprints were associated with rewards, *Melipona scutellaris* and *Scaptotrigona mexicana* foragers acquired a preference to land on food sources covered in footprints, whereas foragers avoided visited feeders if food prints were previously associated with empty food sources or food sources offering decreasing rewards (see also Chap. 10) (Sánchez et al. 2008; Roselino et al. 2016). These findings support the interpretation that the apparent inability of some stingless bees to learn associations in the PER setup are due to methodological factors, rather than a poor ability of stingless bees to learn associations between food source features and rewards.

The role of associative learning when no obvious reward is present, e.g. during the collection of mud, resin, faeces or pulp, is not well understood. It is possible that these substances are perceived as rewards (US) (e.g. the water content in these resources) by the bees collecting these materials. An interesting future research avenue would be the study of reward perception and learning processes in bees that collect these alternative resources.

Associative learning involving food odours does not only occur when bees are foraging, but may already occur inside the nest when bees share food *via* trophallaxis or when they sample the food stores (in honey bees: Farina et al. 2005; Grüter et al. 2006; Arenas et al. 2007, 2008; Balbuena et al. 2012). *Melipona quadrifasciata* that encountered scented food inside their colony showed better discrimination ability in a differential PER conditioning involving the odour that was present in the circulating food (Mc Cabe and Farina 2009). Data also indicate that recruits to scented food sources acquire olfactory memories about the food scent while interacting with recruiting bees inside the nest, possibly during trophallaxis (Mc Cabe and Farina 2009; Mc Cabe et al. 2015). *Tetragonisca angustula*, on the other hand, does not show a preference for a scented food source if bees were exposed to the same odour inside their colonies (Mc Cabe and Farina 2010). Interestingly, odours do not necessarily have to be paired with a reward to be learned inside the nest. Reichle et al. (2010) found that odours that were present in the nest air were subsequently preferred by *Scaptotrigona pectoralis* foragers at artificial feeders. The effects of odour exposure on foraging decisions were further explored in a controlled laboratory setting in *Melipona scutellaris* (Roselino and Hrncir 2012): harnessed bees that were repeatedly exposed to unrewarded, scented air were later attracted to a food source of this scent. This suggests that odour familiarisation inside the nest might be an important driver of food source preferences (see also Reichle et al. 2011).

8.7.2 Site Fidelity

Learning about food source features helps foragers to return to a profitable food source (Biesmeijer and Ermers 1999; Jesus et al. 2014; Peng et al. 2019). Honey bees are known to return to the same location for days and weeks (Ribbands 1949; Al Toufailia et al. 2013) and extended site fidelity is also likely to occur in stingless bees (Biesmeijer et al. 1998; Biesmeijer and Ermers 1999; Hrncir and Maia-Silva 2013b). Site fidelity is potentially less important in stingless bees than in temperate honey bees because tropical flowers are often relatively short-lived (see Sect. 8.2). As a result, stingless bee foragers might have to search for new food sources more frequently than honey bee foragers. This is even more pronounced in foragers collecting animal protein from dead animals. On the other hand, foragers collecting mud, honeydew or plant resins might visit the same locations for long time periods (Leonhardt and Blüthgen 2009). Indeed, resin collection sites are often visited for numerous days or even weeks (Schwarz 1948; Leonhardt and Blüthgen 2009).

Experienced foragers that have become temporarily inactive, e.g. due to rain or during the night, often resume foraging after they have received information that their food type is rewarding again, e.g. by tasting a food sample from a returning forager or smelling a familiar odour on the body of other bees (see also Chap. 10) (Biesmeijer et al. 1998; Biesmeijer and Ermers 1999; Hrncir and Maia-Silva 2013b). For example, when studying foraging decisions in *Melipona beecheii* and *M. fasciata*, Biesmeijer et al. (1998) found that the decision to initiate, continue or stop foraging mainly depended on previous experience and on interactions with returning foragers. Novice foragers, on the other hand, relied more on information provided by successful nestmate foragers.

8.7.3 Flower Constancy

Flower constancy means that pollinators show a tendency to visit flowers of only one kind during a foraging trip (Grant 1950; Free 1970; Waser 1986; Chittka et al. 1999; Grüter and Ratnieks 2011) and was already described by Aristotle in 340 BC. It can be the result of flower clustering, learning about flower types and innate preferences for particular floral features (Marden and Waddington 1981; Chittka et al. 1997; Hill et al. 2001; Grüter and Ratnieks 2011; Dyer et al. 2016a; Pangestika et al. 2017). As a group, stingless bees do not seem to have clear-cut innate preferences for particular colours. The preferred colours, e.g. bee-green or bee-blue, seem to vary somewhat among species, background colours or experimental setups (Dyer et al. 2016a; Koethe et al. 2016, 2018; Balamurali et al. 2018). Flower constancy is significant for plants because it facilitates pollination (Waser 1986; Goulson 1999), but whether flower constancy is also in the best interests of bees or the result of learning and information processing constraints is still debated (Waser 1986; Chittka et al. 1999; Goulson 2000; Hill et al. 2001; Grüter et al. 2011; Grüter and Ratnieks 2011; Ishii and Masuda 2014). The "learning constraint" hypothesis argues that pollinators are flower constant because of limitations of the nervous system. For instance, learning how to forage on a new flower type might interfere with the memory about how to forage on previously visited flower types. Related to this is the idea that flower constancy is required so that pollinators can establish a "search image" for a particular flower type, which helps them to forage efficiently at this flower type

(Waser 1986; Chittka et al. 1999; Goulson 2000; Grüter and Ratnieks 2011; Ishii and Masuda 2014). An alternative view is that flower constancy is an adaptive strategy that is the result of foragers balancing the benefits of visiting the current flower type and the costs of acquiring information about alternative options (Chittka et al. 1999; Grüter and Ratnieks 2011). The different hypotheses are not necessarily mutually exclusive and there is empirical support for both the "learning constraint" view and the hypothesis that flower constancy is an adaptive strategy given the limited information that pollinators have about the different foraging options ("costly information hypothesis") (e.g. Chittka et al. 1999; Grüter and Ratnieks 2011; Ishii and Masuda 2014).

Flower constancy seems to be the rule in stingless bees (Ramalho et al. 1994; Slaa et al. 1998, 2003; Pangestika et al. 2017; Layek and Karmakar 2018; Cholis et al. 2020). For instance, Ramalho et al. (1994) observed foragers belonging to nine Neotropical species and found that 97% of all foragers visited just one flower species during a foraging trip. There seem to be differences among species in the degree of flower constancy and stingless bees appear to be somewhat less flower constant than *Apis mellifera* (but similar to *Apis cerana*) when tested in similar experimental setups (Slaa et al. 1998). Various factors have been suggested to explain why a species is more or less flower constant, including competition, colony size, bee size, recruitment method, dietary niche, habitat diversity and stability (Marden and Waddington 1981; Slaa et al. 1998, 2003; Grüter and Ratnieks 2011). One challenge for meaningful comparisons among species has been the use of different experimental setups (Slaa et al. 1998, 2003).

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Importance for Pollination

9

More than 90% of all tropical flowering plants (Angiosperms) are pollinated by animals (Ollerton et al. 2011). Bees represent the most significant group of pollinators in both new and old world tropics (Bawa 1990; Momose et al. 1998; Corlett 2004; Klein et al. 2007; Michener 2007; Giannini et al. 2015; Ollerton 2017) and among them, stingless bees are a particularly prominent group of flower visitors in various environments (Fig. 9.1). For example, Ramalho (2004) recorded flower visiting bees in the Brazilian Atlantic Rainforest and found that stingless bees were responsible for ~70% of all visits even though they represented only 7% of all bee species (see also Wilms et al. 1996). In a Costa Rican habitat, ~50% of the bees that were observed foraging were stingless bees, while accounting for 16% of the recorded bee species (Brosi et al. 2008). Similarly, stingless bees represented ~52% of all bees on flowers in different Mexican habitats (Cairns et al. 2005). Gutiérrez-Chacón et al. (2018) sampled bees at forest edges and in pastures in the Columbian Andes and found that 49% belonged to the Meliponini (11% were *Apis mellifera*), while amounting to 17.5% of all bee species (Fig. 9.1). In these five studies, stingless bees represented half of all recorded individuals, honey bees represented ~18% and other bees ~29%. Stingless bees are probably similarly important in Asia: in a lowland forest in Malaysia, ~25% of all flowering plant species were estimated to be exclusively or partially pollinated by stingless bees (11% were pollinated by Asian *Apis* species) (Momose et al. 1998; Sakai et al. 1999). As a group, stingless bees visited the largest number of plant species of any animal pollinator group in this area (Momose et al. 1998).

There are different reasons for the importance of meliponine bees in tropical environments, including their sheer number, their morphological diversity, their diversity in foraging strategies, their generalist foraging habits (polylecty) and their flower constancy during foraging trips (see Chap. 8). Reported nest densities and colony sizes (Chaps. 1 and 3) suggest that there are often more than a million individual stingless bees inhabiting a square kilometre of tropical habitat. Diversity in morphology and behaviour means that they can collect pollen and nectar from a wide range of flowering plants. However, not all flower visits lead to pollination

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Fig. 9.1 Abundance of stingless bees, honey bees (*Apis mellifera*) and other bees on flowers and on food baits in different locations in tropical America. Wilms et al. (1996) and Ramalho (2004) found that stingless bees represented 44% and 70% of all flower visitors in a Brazilian Atlantic Rainforest, respectively. In the Columbian Andes, 49% of all recorded bees at forest edges and in pastures were Meliponini (Gutiérrez-Chacón et al. 2018), while stingless bees accounted for 46% of bees recorded on flowers in a mountainous Colombian habitat (Cely-Santos and Philpott 2019). In Costa Rica and Mexico, stingless bees represented ~50% of all sampled foraging bees (Cairns et al. 2005; Brosi et al. 2008)

(Heard 1999). This is obvious in the case of nectar and pollen robbers, but also small bees that visit large flowers may consume pollen without contacting the stigma ("pollen consumers"). By removing pollen that could otherwise be collected by effective pollinators, stingless bees could occasionally also have a negative impact on plant fitness (Roubik 1989). Even legitimate flower visits will often not lead to pollination, e.g. because the stigma is no longer receptive (Roubik 1989) and careful study is necessary to determine the effectiveness of bees for pollination.

While most foraging is done by the female workers (Chap. 6), Boongird and Michener (2010) observed that males in some Asian species (e.g. *Tetrigona apicalis,* *Tetragonilla collina*, *Tetragonula fuscobalteata* and *T. pagdeni*) carry pollen or resin on their hind tibiae. In these species, drones have tibiae that are similar in size and shape when compared to those of workers ("isopodal"). Closely related species with "anisopodal" hind tibiae (different from workers) were never seen carrying pollen. Quantitative observations are necessary to confirm whether this phenomenon is more common and explore the potential ecological significance of pollen collection by males (Boongird and Michener 2010).

Numerous studies have analysed the diet of stingless bees, either by surveying them on flowers, by identifying pollen types removed from returning foragers or by recording the pollen types stored in pollen or honey pots (e.g. Table 9.1). Pollen can end up in honey pots because nectar foragers imbibe pollen grains that are dislodged from flowers during the collection of nectar or because pollen grains adhere to the body of nectar foragers and, upon return to the nest, end up in food pots (e.g. Maia-Silva et al. 2018). These studies show that colonies collect pollen and nectar from a wide range of species (Fig. 9.2). *Tetragonisca angustula*, for example, visited 175 different plant species in a study that included honey samples from Bolivia, Panama and Peru (Roubik and Patiño 2013, 2018). *Scaptotrigona hellwegeri* foragers collected pollen from 165 plant species (from 57 families) in two Mexican locations during a 12-month period (Quiroz-García et al. 2011). *Tetragonula iridipennis* colonies in West Bengal in India had 117 different types of pollen from 49 families in their pollen loads and honey pots (Layek and Karmakar 2018; see also Roopa et al. 2017), while *Trigona fulviventris* exploited 95 plant species in lowland Costa Rica (Roubik 1989). These and other studies show that colonies of some stingless bees (e.g. the Asian *Tetragonula iridipennis* or the American *Plebeia droryana* and *Trigona spinipes*) collect food from a wider range of plants than honey bee colonies living in the same area (Koeniger and Vorwohl 1979; Roubik 1989; Wilms et al. 1996; Lorenzon et al. 2003). Many other species, however, appear to have a narrower diet than their Apine cousins (Sommeijer et al. 1983; Imperatriz-Fonseca et al. 1989; Wilms et al. 1996).

Despite the large number of pollen types that can be found in samples, most pollen found in a stingless bee colony usually originates from less than ten different plant species (e.g. Ramalho et al. 1985; Kleinert-Giovannini and Imperatriz-Fonseca 1987; Ramalho 1990; Teixeira et al. 2007; Ramírez-Arriaga and Martínez-Hernández 2007; Vossler et al. 2010; Obregon and Nates-Parra 2014; Roubik and Patiño 2018; Villanueva-Gutiérrez et al. 2018; Vossler 2018). This could be explained, for example, by colonies having preferences for particular plant species at a given moment (Wilms and Wiechers 1997; Maia-Silva et al. 2015).

The numbers of visited plant species reported in different studies are often difficult to compare because of the different methods used to harvest pollen grains. Two factors that play important roles in determining the niche breadth of colonies are the number of colonies that were sampled and the sampling duration (Fig. 9.2). Data from 59 different stingless bee species show that studies with a sampling duration of 2 months or less find on average fewer than 20 pollen types, whereas studies that sampled colonies during an entire year or more found on average about 70 pollen types (Fig. 9.2). Likewise, studies that sample only 1–2 colonies find fewer

Table 9.1 Number of visited plant species, based on pollen and honey samples or flower visits. Only Neotropical species and studies with a minimum sampling period of 12 months were included

Species	Plant species	Sampling period	Sample type	Sampling location	Reference
Frieseomelitta varia	77	12 months	Pollen foragers	São Paulo state, Brazil	Aleixo et al. (2013)
Melipona beecheii	68	240 months	Pollen pots	Yucatán, Mexico	Villanueva-Gutiérrez et al. (2018)
Melipona bicolor	57	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)
Melipona eburnea	92	12 months	Honey & pollen pots	Cundinamarca, Colombia	Obregon and Nates-Parra (2014)
Melipona marginata	43	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)
Melipona quadrifasciata	31	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)
Melipona rufiventris	51	12 months	Nectar foragers	Amazonas state, Brazil	Absy et al. (1980)
Melipona seminigra	48	12 months	Nectar foragers	Amazonas state, Brazil	Absy et al. (1980)
Melipona subnitida	19	12 months	Foragers and food pots	Caatinga dry forest, RN, Brazil	Maia-Silva et al. (2018)
Paratrigona subnuda	95	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)
Partamona helleri	77	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)
Plebeia droryana	103	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)
Plebeia remota	97	12 months	Honey stores	São Paulo state, Brazil	Ramalho et al. (1985)
Scaptotrigona depilis	66	12 months	Pollen stores	São Paulo state, Brazil	Aleixo et al. (2017)
Scaptotrigona bipunctata	51	30 months	Bees on flowers	São Paulo state, Brazil	Wilms et al. (1996)
Scaptotrigona fulvicutis	97	12 months	Pollen foragers	Amazonas state, Brazil	Marques-Souza et al. (2007)
Scaptotrigona hellwegeri	165	12 months	Pollen stores	State of Jalisco, Mexico	Quiroz-García et al. (2011)
Schwarziana quadripunctata	61	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)

(continued)

Table 9.1 (continued)

Species	Plant species	Sampling period	Sample type	Sampling location	Reference
Tetragonisca angustula	73	17 months	Honey stores	Pará state, Brazil	de Novais and Absy (2015)
Trigona spinipes	89	30 months	Bees on flowers	Atlantic rain forest, SP, Brazil	Wilms et al. (1996)
Trigona williana	56	12 months	Pollen loads	Amazonas state, Brazil	Marques-Souza et al. (1996)

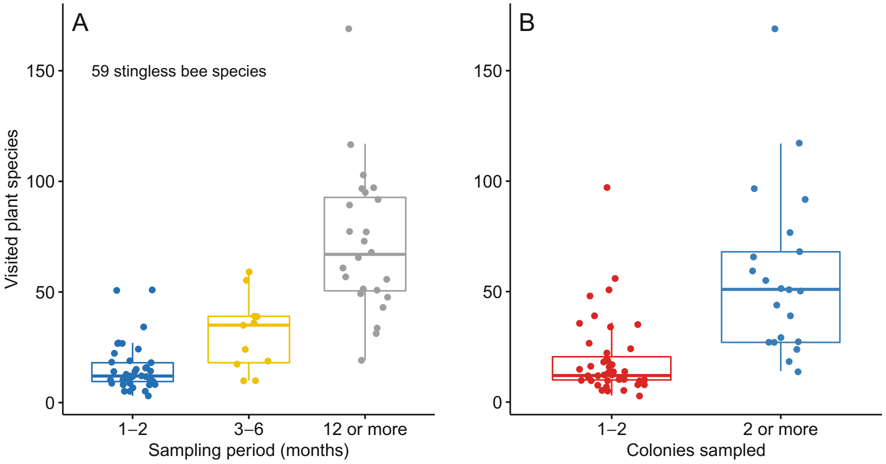

Fig. 9.2 The number of different pollen types (plant species) identified from stingless bee foragers and food stores depending on the sampling duration (**a**) and the number of sampled colonies (**b**). Eleven species were studied more than once and are, thus, represented by more than one data point. Sampling duration and the number of sampled colonies both have a significant positive effect on the number of pollen types found (Poisson GLM with two predictors: sampling period, z-value = 24.4, $p < 0.0001$; colony number, z-value = 12.1, $p < 0.0001$). Data taken from Koeniger and Vorwohl (1979), Absy et al. (1980, 1984), Ramalho et al. (1985, 1989), Imperatriz-Fonseca et al. (1989), Wilms et al. (1996), Marques-Souza et al. (1996, 2007), Nagamitsu and Inoue (2002), Vossler et al. (2010), Quiroz-García et al. 2011, Rech and Absy (2011), Obregon and Nates-Parra (2014), de Novais and Absy (2015), Aleixo et al. (2013, 2017), Ferreira and Absy (2018), Ghazi et al. (2018), Layek and Karmakar (2018), Maia-Silva et al. (2018), Saravia-Nava et al. (2018), Ramírez-Arriaga et al. (2018) and Villanueva-Gutiérrez et al. (2018) (Excel file can be downloaded here: www.socialinsect-research.com/book.php)

than 20 pollen types on average, whereas studies that include at least 3 colonies identify 57 different types on average (Fig. 9.2). This is because colonies of the same species and location differ somewhat in their diet (see e.g. Rech and Absy 2011; Ramírez-Arriaga et al. 2018; Saravia-Nava et al. 2018). One pattern that emerges from the data presented in Table 9.1 is that the eight *Melipona* species collected fewer pollen types than the 13 non-*Melipona* species (51.1 ± 22.4 vs. 85.2 ± 29.3).

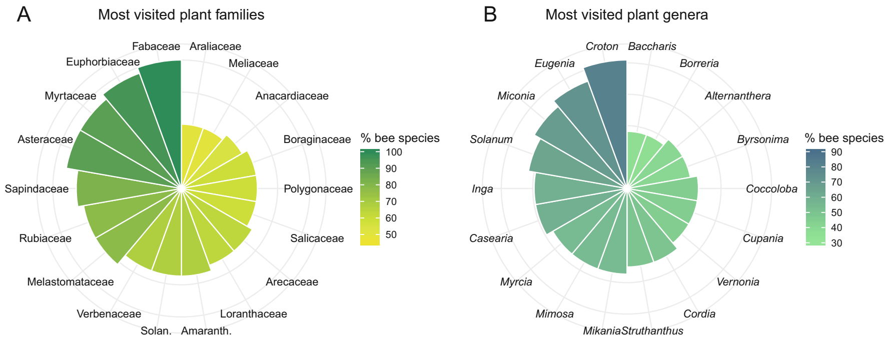

Fig. 9.3 The plant families (**a**) and genera (**b**) that were most visited by the 21 stingless bee species listed in Table 9.1. Fabaceae were visited by all of the 21 stingless bee species, followed by Euphorbiaceae, etc. (Solan. = Solanaceae, Amaranth. = Amaranthaceae). *Croton* plant species were visited by 18 of 21 stingless bee species (Excel file can be downloaded here: www.socialinsect-research.com/book.php)

This is an indication that *Melipona* colonies might have a narrower, more specialised diet than non-*Melipona* species (but see *Melipona eburnea*, Obregon and Nates-Parra 2014).

Given that individual stingless bee species collect pollen from many plant species, one might ask how many flowering plants are visited by all stingless bee species combined. Information about stingless bee flower visiting behaviour is still very limited, particularly for Asian and African species, but we can use the existing data from Neotropical species to obtain preliminary estimates. I used the data from 21 Neotropical stingless bees, sampled during at least 12 months, to assess the number of plant families, genera and species that they visited in their habitats (see Table 9.1). Overall, the 21 species visited ~919 different plant species belonging to 407–477[1] genera and 113 plant families. The most commonly visited plant families were the Fabaceae (pea family), Euphorbiaceae (spurge family), Asteraceae (aster family) and Myrtaceae (myrtle family), which were visited by at least 19 of the 21 species (Fig. 9.3) (see also Absy et al. 2018). The most commonly visited genera were *Croton* (Euphorbiaceae), *Eugenia* (Myrtaceae) and *Miconia* (Melastomataceae), which were visited by at least 15 of the 21 stingless bee species (Fig. 9.3). Rarefaction and extrapolation can be used to estimate the number of plant taxa that would have been found in samples obtained from a larger number of stingless bee species (Chao et al. 2014). For instance, if we had collected samples

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[1] The exact number of species and genera depends on how the unidentified species and genera in the different studies are considered. Unidentified genera, e.g. in the Fabaceae, can be considered to be the same or different if they are reported in different studies and locations. For the rarefaction, I assumed that unidentified genera and species represented unique genera and species if they were reported in different studies performed in different areas. It should also be noted that the number of species will be slightly lower because taxonomists occasionally give different names to the same species (Joppa et al. 2011).

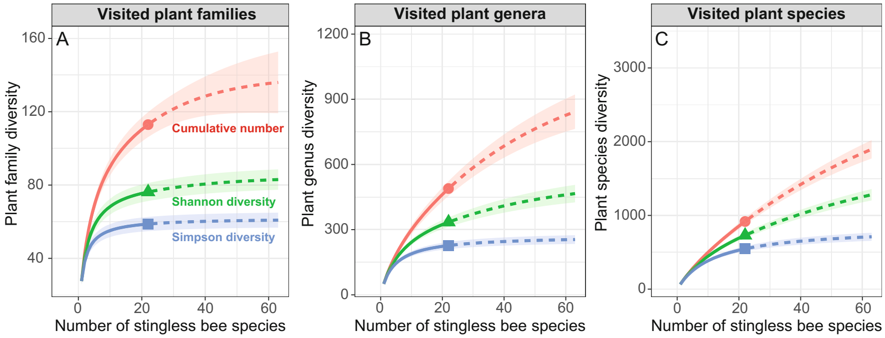

Fig. 9.4 Rarefaction and extrapolation curves to estimate the number of plant families, genera and species visited by Neotropical stingless bees. Pollen identification was based on studies conducted in different areas in Brazil, Mexico and Colombia. The package iNEXT and the software R 3.6 were used for this analysis (R Development Core Team 2013) (Excel file can be downloaded here: www.socialinsect-research.com/book.php)

from 60 stingless bee species, approximately the number of stingless bees found in Costa Rica (Aguilar et al. 2013), we would have expected to record pollen from ~140 families, ~850 genera and ~2000 plant species in samples. The slopes and error ranges of the curves shown in Fig. 9.4 suggest that many more stingless bee species have to be sampled to reliably predict the number of Neotropical plants that are exploited by the ~426 currently known Neotropical stingless bee species. Thus, these extrapolations should be interpreted carefully as they become unreliable if the extrapolated species range is large (Chao et al. 2014). These estimates are likely to considerably underestimate the diet breadth of stingless bee species because the data are based on a small number of colonies in a small number of habitats for each species.

Another approach to project the number of plants visited by stingless bees is to estimate the proportion of flowering plants that are visited by stingless bees in a particular environment. Roubik (1989, pp. 153) estimated that stingless bees visit 15–20% percent of the local angiosperm fauna, which is slightly lower than the ~25% reported in a Malaysian habitat (Sakai et al. 1999). This suggests that about 30,000–50,000 tropical plant species are visited by stingless bees worldwide.[2]

Diet diversity not only depends on the total number of plant taxa that are exploited, but also on the abundance of a particular taxon in the samples (Chao et al. 2014). The Simpson diversity takes into account both the number of types and their abundance in collected samples. It decreases if there are dominant families, genera and species in the samples. Shannon diversity is another commonly used diversity measure that also takes into account visited plant taxa number and their abundance, but which weighs the dominant plant taxa a bit less than the Simpson

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[2] There are 300,000–400,000 flowering plants (Ollerton et al. 2011; Joppa et al. 2011; Ollerton 2017) and more than half are tropical (Antonelli et al. 2015).

diversity. The estimates for Simpson and Shannon diversity shown in Fig. 9.4 indicate that, as a group, Neotropical stingless bees do not visit flowering plant species evenly, but rely more on certain plant groups and species. This is probably not surprising as we have already seen that some plant families and genera are particularly popular with stingless bees (Fig. 9.3).

9.1 Pollination of Crops

Recent worries about global bee pollination services are driven by the fact that many crops depend on or benefit from bee pollination (e.g. Klein et al. 2007; Potts et al. 2010, 2016; Garibaldi et al. 2013).[3] In the tropics alone, about 1300 plant species are estimated to be cultivated, but information about the pollination needs of these plants and which bee species pollinate them is usually absent (Roubik 1995). Stingless bees are known to visit many of these tropical crops (Roubik 1995; Heard 1999; Giannini et al. 2015, 2020). For example, 60% (23 of 38) of the most commonly commercialised plants in markets in Manaus (Amazonas state, Brazil) were found to be visited by stingless bees as evidenced by the presence of pollen in their nests (Absy et al. 2018). Especially crops planted in proximity to intact natural habitat benefit from pollination by stingless bees (Slaa et al. 2006). For example, stingless bee visitation and fruit set of Rambutan (*Nephelium lappaceum*) in orchards in Thailand are significantly higher in the vicinity of forest patches (Sritongchuay et al. 2016). Commercial pollination is further facilitated by the possibility to keep colonies of many species (mainly those nesting in cavities) in artificial hives (Nogueira-Neto 1997) and a number of species can be used for pollination in greenhouses (Table 9.2) (Heard 1999; Slaa et al. 2006; Greco et al. 2011; Nunes-Silva et al. 2013; Kishan et al. 2017; Azmi et al. 2019; Meléndez Ramírez et al. 2018; see Slaa et al. (2006) and Meléndez Ramírez et al. (2018) for more detailed discussions of the use of stingless bees in greenhouses). *Melipona fasciculata*, for example, has been employed to increase the fruit weight of aubergine (*Solanum melongena*) in greenhouses (Fig. 9.5). Since species differ in body size, colony size, foraging strategy and pollination behaviour (including buzz collection in *Melipona*), they can potentially be used for a variety of crops, including crops for which honey bees are not suited as pollinators (Slaa et al. 2006; Meléndez Ramírez et al. 2018). Despite the potential of stingless bees for crop pollination, there are also disadvantages compared to honey bees. Large numbers of colonies are often not available and colony growth and reproduction are relatively slow (see Chaps. 1 and 4) (Heard 1999; Slaa et al. 2006).

The importance of stingless bees for pollination depends on the crop and, for many crops, remains unclear (Heard 1999; Slaa et al. 2006). In the case of mango (*Mangifera indica*), stingless bees are not only among the most frequent visitors

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[3] Gallai et al. (2009) estimated a total value of crop pollination to be ~ €150 billion worldwide, with vegetables and fruits accounting for ~€50 billion each.

Table 9.2 Twenty crops that benefit substantially from stingless bee pollination

Common name	Scientific name	Family	Pollinator genus	Reference
Annatto, Achiote	*Bixa orellana*	Bixaceae	*Melipona*	Heard (1999)
Aubergine (eggplant)[a]	*Solanum melongena*	Solanaceae	*Melipona*	Nunes-Silva et al. (2013)
Avocado	*Persea americana*	Lauraceae	*Nannotrigona, Trigona*	Heard (1999); Slaa et al. (2006)
Camu-Camu	*Myrciaria dubia*	Myrtaceae	*Melipona, Scaptotrigona, etc.*	Heard (1999)
Carambola	*Averrhoa carambola*	Oxalidaceae	*Trigona*	Heard (1999)
Chayote, Choko	*Sechium edule*	Cucurbitaceae	*Trigona, Partamona*	Heard (1999)
Coconut	*Cocos nucifera*	Arecaceae	various genera	Heard (1999)
Coffee 1	*Coffea arabica*	Rubiaceae	*Lepidotrigona, Trigona*	Heard (1999), Slaa et al. (2006)
Coffee 2	*Coffea canephora*	Rubiaceae	*Lepidotrigona, Trigona*	Slaa et al. (2006)
Cucumber[a]	*Cucumis sativus*	Cucurbitaceae	*Nannotrigona, Scaptotrigona*	Heard (1999), Slaa et al. (2006)
Cupuaçu	*Theobroma grandiflorum*	Sterculiaceae	*Trigona*	Heard (1999)
Macadamia	*Macadamia integrifolia*	Proteaceae	*Trigona*	Heard (1999)
Mango	*Mangifera indica*	Anacardiaceae	*Trigona*	Heard (1999)
Mapati, Uvilla, Amazon Tree Grape	*Pourouma cecropiifolia*	Moraceae	*Oxitrigona, Trigona*	Heard (1999)
Mealy sage[a]	*Salvia farinaceae*	Lamiaceae	*Nannotrigona, Tetragonisca*	Slaa et al. (2006)
Rambutan[a]	*Nephelium lappaceum*	Sapindaceae	*Scaptotrigona*	Heard (1999), Slaa et al. (2006)
Rockmelon[a]	*Cucumis melo*	Cucurbitaceae	*Heterotrigona*	Azmi et al. (2019)
Strawberry[a]	*Fragaria sp.*	Rosaceae	various genera	Heard (1999), Slaa et al. (2006)
Sweet Pepper, Chili[a]	*Capsicum annuum* varieties	Solanaceae	*Austroplebeia, Melipona, Tetragonula*	Heard (1999), Slaa et al. (2006), Putra et al. (2017)
Tomato[a]	*Lycopersicon esculentum*	Solanaceae	*Melipona, Nannotrigona*	Slaa et al. (2006)

[a] Pollination in greenhouses

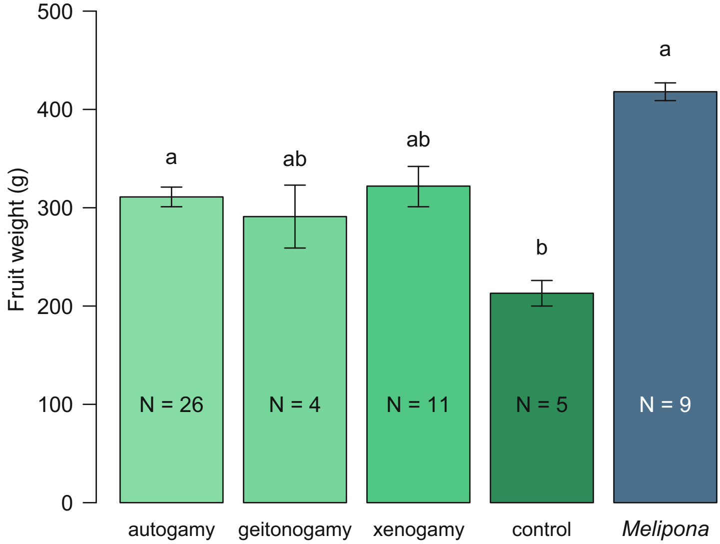

Fig. 9.5 Weight (mean and SE) of aubergine fruits (*Solanum melongena*) produced in a greenhouse by autogamy (by hand with pollen from same flower), geitonogamy (by hand with pollen from different flower but same plant), xenogamy (by hand with pollen from a different plant), without pollination (control) and by *Melipona fasciculata*. Letters above bars indicate statistically significant differences at $p < 0.05$ (from Nunes-Silva et al. 2013)

(Heard 1999), but also among the most effective pollinators (Anderson et al. 1982). Another crop that depends strongly on stingless bee pollination is the Achiote or Annatto tree. The seeds are used in traditional medicine and in dishes in Mexico, the Caribbean, Central and South America, both as food colouring and for flavouring. The flowers are particularly suitable for buzz pollination, which is why the buzz-pollinating *Melipona beecheii* is a more effective pollinator than *Apis mellifera* (Caro et al. 2017). Passion fruit (*Passiflora edulis*), on the other hand, is visited by stingless bees (e.g. *Trigona spinipes*), but observations suggest that these visits are often not beneficial to the plant and can even have a negative impact if stingless bees (e.g. the aggressive *Trigona*) deter more effective pollinators (e.g. *Xylocopa* carpenter bees) (Sazima and Sazima 1989). Table 9.2 provides a list of 20 crops that substantially benefit from stingless bee pollination.[4] Many other crops exist that potentially or occasionally benefit from stingless bee pollination (Table 9.3 lists 75 crops). For example, Roopa et al. (2017) found that numerous crops or plants of medicinal importance were visited by *Tetragonula iridipennis* in India, but more research is needed to assess the importance of stingless bee for pollination in these plants.

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[4] It is often stated that *Melipona* bees are the natural pollinators of *Vanilla* orchids in Mexico and Central America. However, there is little evidence that *Melipona* bees pollinate *Vanilla* flowers. Instead, *Eulaema* orchid bees are more likely to be the effective pollinators of *Vanilla* (Roubik 1995; Heard 1999; Lubinsky et al. 2006).

Table 9.3 Seventy-five crops that are at least occasionally or potentially pollinated by stingless bees

Common name	Scientific name	Family	Reference
Açai Palm	*Euterpe oleracea*	Arecaceae	Campbell et al. (2018)
Acapú	*Vouacapoua americana*	Fabaceae	Giannini et al. (2015)
Acerola	*Malpighia punicifolia*	Malpighiaceae	Heard (1999)
Ackee	*Blighia sapida*	Sapindaceae	Heard (1999)
Apple	*Malus domestica*	Rosaceae	Viana et al. (2014)
Bacaba (oily bacaba)	*Oenocarpus distichus*	Arecaceae	Giannini et al. (2015)
Bacaba Tree	*Oenocarpus mapora*	Arecaceae	Giannini et al. (2015)
Bellyache Bush	*Jatropha gossypifolia*	Euphorbiaceae	Heard (1999)
Bitter Gourd	*Momordica charantia*	Cucurbitaceae	Heard (1999)
Brazil Nut Tree	*Bertholletia excelsa*	Lecythidaceae	Giannini et al. (2015)
Breadfruit	*Artocarpus altilis*	Moraceae	Heard (1999)
Canola	*Brassica napus*	Brassicaceae	Giannini et al. (2015)
Cardamom	*Elettaria cardamomum*	Zingiberaceae	Heard (1999)
Carrot	*Daucus carota* Cultivar Brasilia	Apiaceae	Giannini et al. (2015)
Cassava/mandioca	*Manihot esculenta*	Euphorbiaceae	Giannini et al. (2015)
Castor Oil	*Ricinus communis*	Euphorbiaceae	Heard (1999)
Citrus Trees (e.g. Orange, Tangerine)	*Citrus spp.*	Rutaceae	Heard (1999)
Coriander	*Coriander sativum*	Apiaceae	Heard (1999)
Cotton	*Gossypium spp.*	Malvaceae	Giannini et al. (2015)
Fennel	*Foeniculum vulgare*	Apiaceae	Heard (1999)
Field Mustard	*Brassica campestris*	Brassicaceae	Heard (1999)
Guaraná	*Paullinia cupana*	Sapindaceae	Heard (1999)
Guava	*Psidium guajava*	Myrtaceae	Heard (1999)
Hogplum, Caja Tree	*Spondias mombin*	Anacardiaceae	Heard (1999)
Ice Cream Bean, Sipo, Guamo	*Inga edulis*	Mimosoideae	Heard (1999)
Indian Jujube	*Ziziphus mauritiana*	Rhamnaceae	Heard (1999)
Indian Shot	*Canna indica*	Cannaceae	Heard (1999)
Indigofera	*Indigofera endocaphylla*	Papilionoideae	Heard (1999)
Jackfruit	*Artocarpus heterophyllus*	Moraceae	Heard (1999)
Jambolan	*Syzygium cumini*	Myrtaceae	Heard (1999)

(continued)

Table 9.3 (continued)

Common name	Scientific name	Family	Reference
Jatropha	*Jatropha curcas*	Euphorbiaceae	Giannini et al. (2015)
Jaboticaba	*Myrciaria cauliflora*	Myrtaceae	Heard (1999)
Kapok	*Ceiba pentandra*	Bombacaceae	Heard (1999)
Leucaena	*Leucaena leucocephala*	Mimosoideae	Heard (1999)
Litchi, Lychee	*Litchi chinensis*	Sapindaceae	Heard (1999)
Longan	*Euphoria longan*	Sapindaceae	Heard (1999)
Loquat	*Eriobotrya japonica*	Rosaceae	Heard (1999)
Luffa	*Luffa acutangula*	Cucurbitaceae	Heard (1999)
Membrillo	*Gustavia superba*	Lecythidaceae	Heard (1999)
Mkani, Msambo	*Allanblackia stuhlmannii*	Clusiaceae	Mrema and Nyundo (2016)
Monsterio	*Monstera deliciosa*	Araceae	Heard (1999)
Mulberry	*Rubus sp.*	Rosaceae	Giannini et al. (2015)
Murici cherry	*Byrsonima chrysophylla*	Malpighiaceae	Giannini et al. (2015)
Nanche, Murici	*Byrsonima crassifolia*	Malpighiaceae	Heard (1999)
Niger	*Guizotia abyssinica*	Asteraceae	Heard (1999)
Okra Tree	*Abelmoschus esculentus*	Malvaceae	Giannini et al. (2015)
Onion	*Allium cepa*	Alliaceae	Heard (1999)
Orange	*Citrus spp.*	Rutaceae	Giannini et al. (2015)
Panama Hat Plant	*Carludovica palmata*	Cyclanthaceae	Heard (1999)
Peach	*Prunus persica*	Rosaceae	Heard (1999)
Peach Palm	*Bactris gasipeas*	Arecaceae	Heard (1999)
Pear	*Pyrus communis*	Rosaceae	Heard (1999)
Pigeon Pea	*Cajanus cajan*	Papilionoideae	Heard (1999)
Pitomba	*Talisia esculenta*	Sapindaceae	Giannini et al. (2015)
Pomegranate	*Punica granatum*	Lythraceae	Giannini et al. (2015)
Plum	*Prunus domestica*	Rosaceae	Heard (1999)
Pumpkin	*Cucurbita moschata*	Cucurbitaceae	Slaa et al. (2006)
Radish	*Raphanus sativus*	Cruciferae	Slaa et al. (2006)
Rattan	*Calamus spp.*	Arecaceae	Heard (1999)
Red jambo	*Syzygium malaccense*	Myrtaceae	Giannini et al. (2015)
Rose apple	*Syzygium jambos*	Myrtaceae	Heard (1999)
Rubber	*Hevea brasiliensis*	Euphorbiaceae	Heard (1999)
Sago palm	*Metroxylon sagu*	Arecaceae	Heard (1999)
Saren	*Amomum villosum*	Zingiberaceae	Heard (1999)

(continued)

Table 9.3 (continued)

Common name	Scientific name	Family	Reference
Sesame	*Sesamum indicum*	Pedaliaceae	Heard (1999)
Sisal	*Agave sisalana*	Agavaceae	Heard (1999)
Soap-Nut	*Sapindus emarginatus*	Sapindaceae	Heard (1999)
Squash	*Cucurbita pepo*	Cucurbitaceae	Heard (1999)
Stylo, Brazilian Lucerne	*Stylosanthes guianensis*	Papilionoideae	Heard (1999)
Sunflower	*Helianthus annuus*	Asteraceae	Heard (1999)
Tamarind	*Tamarindus indica*	Caesalpinioideae	Heard (1999)
Umbu	*Spondias tuberosa*	Anacardiaceae	Giannini et al. (2015)
Watermelon	*Citrullus lanatus*	Cucurbitaceae	Heard (1999)
White Jute	*Corchorus capsularis*	Tiliaceae	Heard (1999)
Wild chili pepper	*Capsicum frutescens*	Solanaceae	Giannini et al. (2015)

Further references can be found in Heard (1999), Slaa et al. (2006) and Giannini et al. (2015)

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Recruitment and Communication in Foraging

10

Finding a good food source can be challenging for a bee, especially if she is on her own. This task can be greatly simplified if a nestmate tells her how and where to find a good food source. Information from nestmates is probably even more important during swarming when large numbers of bees need to find the way from their old to the new nest site (Chap. 4). How should this be achieved if not with the aid of some form of communication? Since communication and recruitment remain largely unstudied in the context of stingless bee swarming (Chap. 4), this chapter will focus on foraging (see Nieh 2004; Barth et al. 2008; Hrncir 2009; Jarau 2009; Hrncir and Barth 2014; Leonhardt 2017 for reviews on different aspects of stingless bee communication). Recruitment in social insects is often defined as communication that brings nestmates to some point in space where work is required (Wilson 1971; Hölldobler and Wilson 1990).[1] This communication process can involve social information that is unspecific in terms of the location, e.g. when a recruiting individual motivates nestmates to search for a particular type of food or it can be location-specific and guide nestmates to the location where the recruiting individual has found food.

The first studies on recruitment communication in stingless bees were performed by Lindauer (1956, 1957) and Lindauer and Kerr (1958, 1960). Lindauer and Kerr (1958, 1960) performed their pioneering experiments on recruitment communication in Brazil, where they explored the question whether foragers that have discovered a food source are able to bring nestmates to the same resource. The basic methodological principle of their and most subsequent studies was originally developed by Karl von Frisch to study honey bee foraging (von Frisch 1919, 1923, 1967). This method involves the training of foragers to collect sucrose solution from an artificial feeder. The training usually starts by placing a high-quality sucrose solution (>1 M) very close (a few cm) to the nest entrance of a colony. Some foragers leaving the nest will

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[1] This should not imply, however, that colonies always benefit from recruitment communication. Recruitment could be detrimental to colony success and increase mortality, energy or time costs (Johnson 1987; Dechaume-Moncharmont et al. 2005; I'Anson Price et al. 2019).

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Fig. 10.1 *Plebeia droryana* foragers collecting sucrose solution from an artificial feeder on the University of São Paulo campus in Ribeirão Preto, Brazil. One forager has been marked with acrylic paint (Photo: C. Grüter)

find this food source and return to it after unloading the food inside their nest. Once the number of foragers collecting food from the artificial feeder is sufficient for the particular experimental question to be addressed, the feeder is moved to a new location several metres from the original location while the bees are still drinking. The bees will then learn this new location and return to it. This procedure is repeated until the desired feeder distance is reached. Foragers are often marked with paint or number plates while they are drinking (Fig. 10.1). This training procedure is seemingly simple, but has its challenges (see also Nieh 2004 for a discussion). For example, foragers of some stingless bee species can be very difficult to train, training might not be possible for long periods of the year (e.g. when natural food sources are abundant) or foragers from non-focal conspecific nests discover the feeder (this is sometimes noticed by the observer due to fights at the feeder). Another potential problem is that bees can lose their marks and what appears to be a naïve newcomer is, in fact, an experienced recruiter.

In their experiments, Lindauer and Kerr (1958, 1960) trained five foragers from a focal colony to an artificial feeder offering 2 M sucrose solution for 1 h. Overall, 10 species were tested in this way and compared with *Apis mellifera*. Feeders were placed at various distances from the nest, ranging from 10 m in the case of *Tetragonisca angustula* to 180 m in *Melipona scutellaris* (Fig. 10.2) (Lindauer and Kerr 1958, 1960).[2] *Frieseomelitta silvestrii* and *Tetragonisca angustula*

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[2] The authors acknowledged that these differences in feeder distance somewhat undermined their attempts to create a standardized protocol that would allow comparison between species, but this was done because the terrain did not always allow for similar feeder distances for all species and because it was not possible to train all species to the same distances. Training was particularly challenging with *Frieseomelitta silvestrii* and *Tetragonisca angustula* (Fig. 10.2). It should also be noted that the tested species differ considerably in body and colony size (Chap. 1), which is likely to affect their preferred foraging distances (see Chap. 8) and the size of the recruit pool.

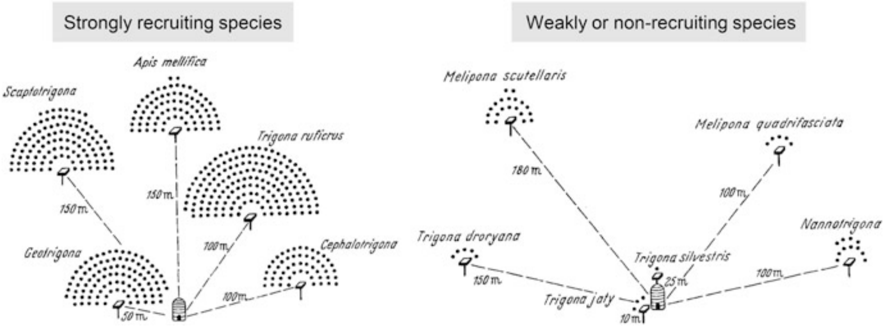

Fig. 10.2 Original figure from Lindauer and Kerr (1958) showing the recruitment success of 10 stingless bees and *Apis mellifera* to a 2 M sucrose feeder that was offered during 1 h. Each dot represents an unmarked recruit. Scientific names of some species have changed, *Trigona ruficrus* = *T. spinipes*, *Trigona droryana* = *Plebeia droryana*, *T. jaty* = *Tetragonisca angustula* and *T. silvestris* = *Frieseomelitta silvestrii*

recruited only a small number of nestmates to the feeder, even though the food source was placed very close to the nest (Fig. 10.2). Recruitment was strongest in *Cephalotrigona capitata*, *Geotrigona mombuca*, *Scaptotrigona postica* and *Trigona spinipes*. These species recruited as many as or more nestmates than honey bee foragers and could be considered mass recruiters. When Lindauer and Kerr (1958, 1960) studied the behaviour of foragers inside artificial hives, they found no sign of a dance-like behaviour as in honey bees, but they noticed a general state of excitement during periods when foragers returned with food from artificial feeders.

10.1 Nest-Based Recruitment Communication

10.1.1 Zigzag or Jostling Runs

The excitement observed by Lindauer and Kerr originated from the returning foragers, which performed "zigzag" or "jostling" runs during which they jostled other bees in their way (Lindauer and Kerr 1958, 1960; Hrncir et al. 2000).[3] During these runs, which are usually performed close to the nest entrance (Nieh 1998a; Hrncir 2009), foragers irregularly run in all directions and occasionally perform sharp turns in a semicircle, which often end while the bee is facing the entrance (Lindauer and Kerr 1960; Nieh 1998a, b; Hrncir 2009). Under natural circumstances, foragers perform these behaviours in narrow entrance structures that provide little space for "dancing" (unlike honey bee dances, which occur on plane surfaces). Nieh

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[3] Similar alerting runs are performed by successful bumble bee foragers (Dornhaus and Chittka 1999).

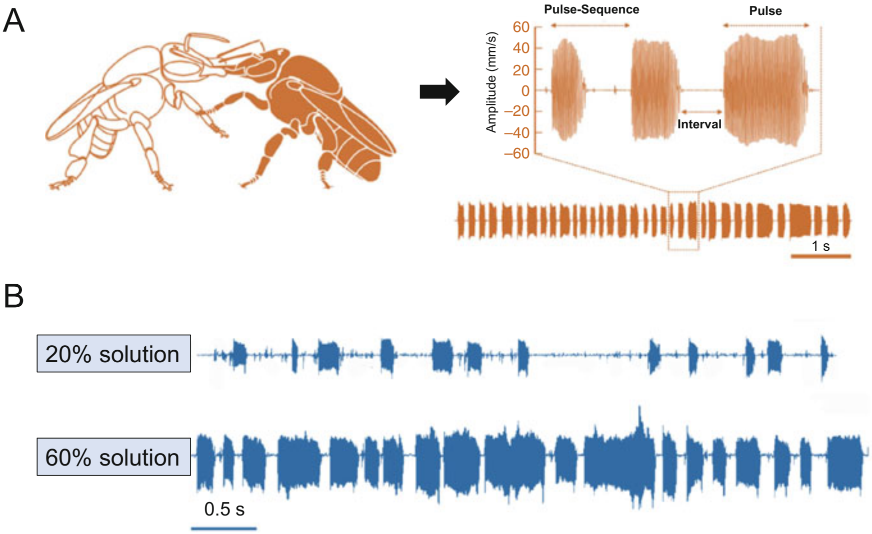

Fig. 10.3 Trophallaxis between two *Melipona* workers. Foragers often produce thoracic vibration pulses during trophallaxis. The bee on the left side (open mandibles) offers food to the bee on the right side (extended proboscis) (**a**) (from Hrncir 2009). Thorax vibrations recorded with a laser-Doppler vibrometer from a *Melipona seminigra* forager after returning from a food source offering 20% or 60% sucrose solution (**b**) (from Hrncir et al. 2006)

(1998a, b) tested in *Melipona panamica* whether foragers might provide directional information during these runs, but found no indication that this is the case. The zigzag or jostling runs are occasionally interrupted by trophallactic contacts during which the foragers transfer food samples to their nestmates (Fig. 10.3). This behavioural pattern seems to be widespread in both Neotropical and Asian species (Lindauer 1956; Lindauer and Kerr 1958, 1960; Hrncir et al. 2000) and might be shared by all stingless bees. Jostling runs play an important role in the activation of foragers. The number of jostles (collisions) during hive stays predicts the number of bees recruited to a food source (Hrncir et al. 2000) and in *Melipona seminigra*, inactive foragers start to bump into other bees themselves after they were jostled by a recruiting bee (Hrncir 2009).

Whether active foragers perform excitatory runs partly depends on the profitability of the food source and the general availability of food (Lindauer and Kerr 1958; Schmidt et al. 2008; but see Schmidt et al. 2006b). How exactly food source profitability is assessed by stingless bee foragers is not well understood, but it seems to depend on various factors, such as the sugar concentration, the distance of the food source, the time that is required to collect a load, the presence of other bees and the previous foraging experience (see also Chap. 8) (reviewed in Hrncir 2009). Additionally, the time of year and even the time of day affect whether a food source is considered attractive and worth recruiting to (Hrncir 2009). One physiological reason for this is that the responsiveness of workers towards sugar rewards is not fixed (honey bees: Lindauer 1948; Seeley 1995; Martinez and Farina 2008).[4] In honey bees, foragers are also more likely to recruit if the time to find an unloading partner inside the nest is short and the number of unloading partners is high (Lindauer 1948; Seeley 1986; Anderson and Ratnieks 1999; Farina 2000; De Marco 2006; Grüter and Farina 2009a). The availability of unloading partners also depends on the foraging conditions since this affects the need for food unloaders (Seeley 1995). However, it remains to be studied whether the waiting time and the number of food receivers affect the motivation to recruit in stingless bees (Hrncir 2009).

10.1.2 Thoracic Vibrations

During the jostling runs, foragers use their thoracic muscles to produce vibration pulses of variable duration, which can be perceived as buzzing sounds (Fig. 10.3) (Esch et al. 1965; Esch 1967; Nieh 1998a, b; Hrncir et al. 2000, 2006, 2008a, b; Aguilar and Briceño 2002; Nieh et al. 2003b; Hrncir and Barth 2014; Krausa et al. 2017a).[5] The strength and frequency of these pulses vary among species, with frequencies ranging from 200 to 600 pulses per second (Hz) (Lindauer and Kerr 1958, 1960; Aguilar and Briceño 2002; Hrncir and Barth 2014; Krausa et al. 2017a, b).

Vibration pulses are often produced during trophallaxis, i.e. food transfer, possibly in order to further stimulate the vibrated receiver bee to initiate foraging (Hrncir et al. 2006, 2008b; Hrncir and Barth 2014; Krausa et al. 2017b). Interestingly, *Melipona quadrifasciata* bees that experienced more vibrations while receiving food showed an increased learning performance during olfactory conditioning (Mc Cabe et al. 2015). This supports the hypothesis that vibrations alter the motivational state of bees and their responsiveness towards foraging-related stimuli (Mc Cabe et al. 2015). How nestmates perceive these vibration pulses is not yet clear, but direct contact during trophallaxis, substrate vibrations and airborne sounds (air particle movement) are all potential routes of information transfer (Hrncir et al. 2008b). While substrate vibrations can reach bees at distances corresponding to several bee lengths, direct contact and air particle oscillations would be modes of short-range transmission (Hrncir and Barth 2014). Given the importance of the subgenual organ for vibration perception in insects (Barth et al. 2008), this organ, located just below the knee in the tibia of all legs, is a potential candidate, but other vibration-detecting organs, including the Johnston's organ of the antennae, might be involved (Hrncir et al. 2006, 2008b).

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[4] If a honey bee colony collects large amounts of very sweet nectar, the workers in this colony experience an increase in their sucrose response threshold and they become more demanding (Pankiw et al. 2004; Martinez and Farina 2008).

[5] Occasionally, buzzing sounds are also produced at food sources (Lindauer and Kerr 1960; Nieh 1998b), but it is not yet known whether buzzing at food sources has a signalling function.

As with the jostling runs, thoracic vibrations become less likely if the food source is of low profitability (Lindauer and Kerr 1958; Nieh et al. 2003a, b; Hrncir 2009; Hrncir and Barth 2014).[6] For example, in *Melipona scutellaris* and *M. quadrifasciata*, sound production did not occur when foragers were offered a 0.75 M sucrose solution, whereas sound production was noted when the sugar solution was 1.5 M (Jarau et al. 2000). Also the amplitude, the duration during which sound pulses are produced and the duration of the sound pulses themselves are positively correlated with the quality of a food source (Fig. 10.3) (Aguilar and Briceño 2002; Nieh et al. 2003a, b; Hrncir et al. 2006; Schmidt et al. 2008; Krausa et al. 2017b). The time between sound pulses, i.e. the interpulse duration, decreases with increasing food source profitability in some species (Aguilar and Briceño 2002; Nieh et al. 2003a, b; Hrncir et al. 2006; Schmidt et al. 2008). Food source distance, on the other hand, has a negative effect on sound production, possibly because more distant food sources are more costly to exploit and, thus, less profitable (Aguilar and Briceño 2002; Hrncir 2009). Lower food source profitability leads to fewer newcomers arriving at food sources (e.g. Nieh et al. 2003a, b; Schorkopf et al. 2016). Interestingly, vibration pulses during trophallaxis were also longer and more frequent if the food source was scented (Mc Cabe et al. 2015). This suggests that more strongly scented food sources might be perceived as more profitable (Mc Cabe et al. 2015; see Kaschef 1957 for similar effects in honey bees), possibly because they are easier to locate for recruits and, thus, save time costs.

In *Melipona scutellaris*, the pulsed vibrations are sufficient to induce foragers to leave the colony and search for the advertised food source (Lindauer and Kerr 1958, 1960; Hrncir et al. 2000), but vibration pulses alone appear to be less successful in activating foragers than when they are produced in combination with zigzag runs and jostling (Lindauer and Kerr 1958, 1960). An interesting collective behaviour was observed in the African species *Axestotrigona ferruginea*. Here, buzzing by a successful forager seems to be contagious and is soon adopted by many other bees in the nest (Kerr et al. 1963; Esch et al. 1965). Shortly afterwards, large numbers of foragers leave the hive to search for the food source.

Do Vibration Pulses Provide Information About Food Source Distance?

There is evidence that the duration of the sound pulses produced during the hive stays is related to the food source distance in *Melipona bicolor*, *M. quadrifasciata*, *M. mandacaia*, *M. panamica* and *M. seminigra* (Fig. 10.4) (Kerr et al. 1963; Esch et al. 1965; Nieh and Roubik 1998; Aguilar and Briceño 2002; Nieh et al. 2003b). Hrncir et al. (2000), on the other hand, found no positive correlations between distance and pulse duration in *Melipona quadrifasciata* and *M. scutellaris*. Likewise, in *M. costaricensis*, pulse duration does not appear to correlate with food source

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[6] Nieh and Sánchez (2005) found that the thoracic temperature of *Melipona panamica* is positively correlated with food source profitability. This phenomenon seems to be quite widespread in social insects (see references in Nieh and Sánchez 2005). Whether this is linked to sound production in *M. panamica* is not known.

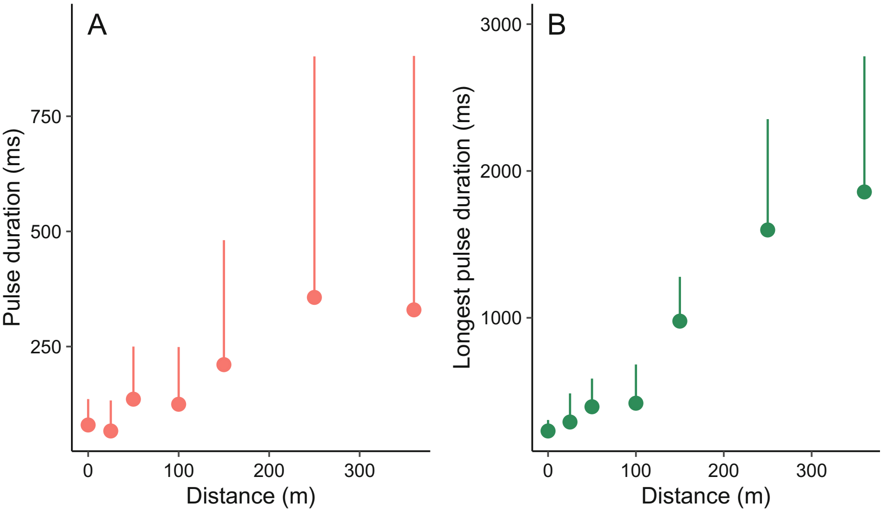

Fig. 10.4 Average (**a**) and longest (**b**) duration of vibration pulses produced during the zig-zag runs of *Melipona panamica* foragers. The dots represent the mean and the lines represent one standard deviation (data from Nieh and Roubik 1998)

distance (Aguilar and Briceño 2002). In *Melipona panamica*, sound pulses produced during unloading were longer when food sources were close to the ground compared to food sources in the canopy (Nieh and Roubik 1998).

These correlations raise the question whether distance (and height) information could be transmitted by means of sound pulses and subsequently be used by nestmates to locate food sources. Attempts to demonstrate that recruits use this information have so far remained inconclusive (Nieh and Roubik 1998; Hrncir et al. 2000; Nieh 2004) and a positive correlation between pulse duration and food source distance could simply be a by-product of physiological processes without any communicative function (Esch 1967). Indeed, the variation in average and maximum pulse duration for a given distance is considerable (Fig. 10.4) (Nieh and Roubik 1998; Hrncir et al. 2000; Aguilar and Briceño 2002; Schmidt et al. 2008; Hrncir and Barth 2014), which would make any spatial information encoded in sound and vibrational signals very imprecise (Hrncir et al. 2000; Barth et al. 2008). Foragers could attempt to average several sound pulses to acquire more accurate spatial information. Averaging of multiple waggle runs is a strategy used by honey bee dance followers to acquire more accurate information from variable waggle runs (von Frisch and Jander 1957; Tanner and Visscher 2008). However, this strategy is likely to fail if pulse duration also depends on food quality as is the case in several species (Aguilar and Briceño 2002; Hrncir and Barth 2014). On the other hand, recruits might be able to distinguish sounds produced for low- or high-quality food sources in other ways and, thus, might still be able to use sounds to determine the approximate distance (Nieh 2004). In other, non-*Melipona* species, the duration of the sound pulses does not correlate with food source distance (Esch et al. 1965).

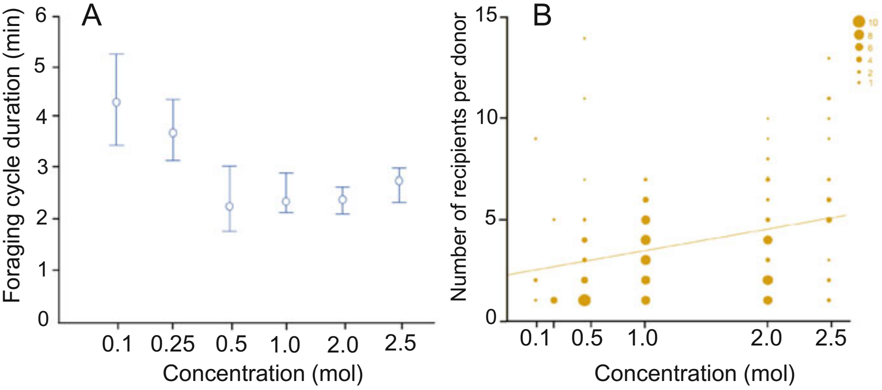

Fig. 10.5 The effect of the sucrose concentration on foraging-related behaviours in the African *Plebeina armata.* (**a**) The duration of foraging cycles decreased with increasing sugar concentrations. (**b**) The number of receivers of food samples increases with increasing sugar concentrations (from Krausa et al. 2017b)

Thus, positive correlations between the sound pulses and food source distance may not be a widespread phenomenon. It remains an intriguing phenomenon and deserves further study as it could represent an example of the "raw material" that could lead to the evolution of complex signalling, such as the honey bee waggle dance.

10.1.3 Social Learning of Food Odours

Foragers returning with nectar or fruit juice not only transfer food to other bees during trophallaxis (see also Chap. 6) (Hart and Ratnieks 2002) but also distribute small taste samples to potential recruits (Hrncir 2009). This allows the recipients of the "amuse-bouche" to assess their quality and, potentially, learn the odour (see also Chap. 8) (Lindauer and Kerr 1958, 1960; Jarau 2009). In *Apis mellifera*, receiving samples through trophallaxis leads to the establishment of stable associative olfactory memories (Farina et al. 2005; Gil and De Marco 2005; Grüter et al. 2006, 2009). Trophallaxes between active foragers and nestmates usually happen close to the entrance inside the nest and, occasionally, they can be seen outside the nest near the entrance hole when foraging activity is high (Kerr 1994; Krausa et al. 2017b). Receiving food samples seems to be an important aspect of recruitment. In *Melipona quadrifasciata* and *M. seminigra,* all bees that successfully located an advertised food source received at least one sample from the recruiting bee (Hrncir 2009). Strikingly, food with higher sucrose content is shared with a larger number of receiver bees (Fig. 10.5) (in *Plebeina armata*, Krausa et al. 2017b). This suggests that information about high-quality food sources is shared more widely among nestmates. In species without or with poor site-specific recruitment, foragers could use this information and search for the food source that emits the same odour (Nieh et al. 2000; Aguilar et al. 2005; Jarau 2009). The assumption that bees indeed learn food odours during recruitment was confirmed in the Neotropical species *Plebeia tica* and the Asian *Tetragonula iridipennis*: recruits that left the nest after interacting with a successful forager showed a strong preference for food sources emitting the odour that was carried back by the recruiting bee (Lindauer 1956, 1957; Aguilar et al. 2005). In *Tetragonula carbonaria*, recruitment was much more successful if food sources were scented and if feeders were located upwind from the nest (Nieh et al. 2000).

10.1.4 Reactivation of Experienced Foragers

One important function of nest-based communication is the reactivation of experienced foragers. Reactivation refers to the process whereby temporarily inactive, but experienced foragers are socially stimulated to inspect food source locations where food was found in the past (von Frisch 1967; Biesmeijer and de Vries 2001; Grüter and Farina 2009b; Hrncir and Maia-Silva 2013). The job of reactivating experienced foragers most likely falls to scouts and inspector bees that have discovered a profitable food source. Scouts are bees that search for new food sources proactively and without waiting for information provided by recruiting bees, whereas inspector bees are experienced foragers that occasionally inspect foraging sites that were profitable in the past, but may have become temporarily unrewarding, e.g. due to unfavourable weather conditions or time of day (von Frisch 1967; Biesmeijer and de Vries 2001; Al Toufailia et al. 2013; Hrncir and Maia-Silva 2013; Jesus et al. 2014). Figure 10.6 summarises the main foraging strategies and states.

In honey bees, reactivation is often triggered by the perception of familiar food odours inside the nest (e.g. present on the body of a returning bee or in food), cuticular hydrocarbons of dancing bees or the dancing itself (von Frisch 1923; Johnson 1967; Reinhard et al. 2004; Grüter et al. 2008; Grüter and Farina 2009b; Gilley et al. 2012). The perception of familiar food odours also seems to be sufficient for reactivation in stingless bees (Biesmeijer et al. 1998). Lindauer and Kerr (1958) suggest that reactivation (in *Melipona scutellaris*) does not require direct physical contact between bees, but that the thoracic vibration pulses perceived from a distance might be enough for reactivation (Hrncir and Barth 2014). In this species, playback of sound was sufficient to reactivate experienced foragers, but only if the food source was nearby (Esch et al. 1965; Esch 1967). On the other hand, reactivation might be more efficient in the presence of physical contacts and jostling runs. Reactivation is a crucial process because it allows colonies to rapidly activate their experienced foraging force when foraging conditions improve (von Frisch 1967; Sánchez et al. 2004; Granovskiy et al. 2012; Hrncir and Maia-Silva 2013).

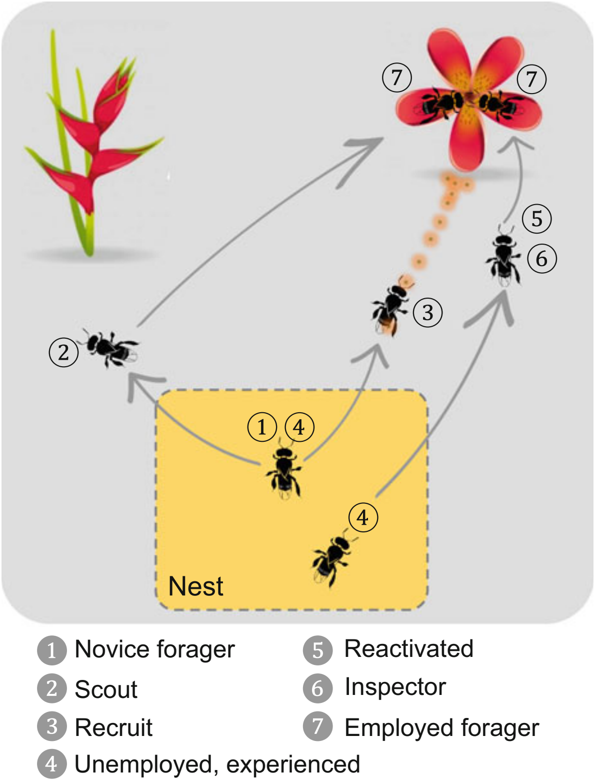

Fig. 10.6 The main foraging strategies and states (see also Biesmeijer and de Vries 2001; Grüter and Farina 2009b; Hrncir and Maia-Silva 2013). Scouts (2) search for food sources without the instructions of recruiters. They may, however, rely on food odour information learned inside the nest when they were younger. Recruits (3) use social information to find a food source location, e.g. a pheromone trail (dotted trail). Some recruits might discover new food sources by "mistake", e.g. after failing to follow a pheromone trail. Foragers that are temporarily unemployed (4) have various options to continue foraging, including reactivation (5) through social interactions or (6) inspection of known food sources. Employed foragers (7) exploit a food source and, depending on the quality of the food source and the species, recruit nestmates to this food source or exploit it solitarily (illustration of *Scaptotrigona bipunctata* bee by Rodolfo Guimarães)

10.2 Location-Specific Recruitment

Given how efficiently foragers of some stingless bee species recruit nestmates to food sources (Fig. 10.1), it is evident that these foragers can transmit information about the location of food sources to their sisters (Lindauer and Kerr 1958, 1960; Hubbell and Johnson 1977, 1978). For example, while *Melipona scutellaris* seemed unable to direct nestmates to a particular food source in the experiments performed

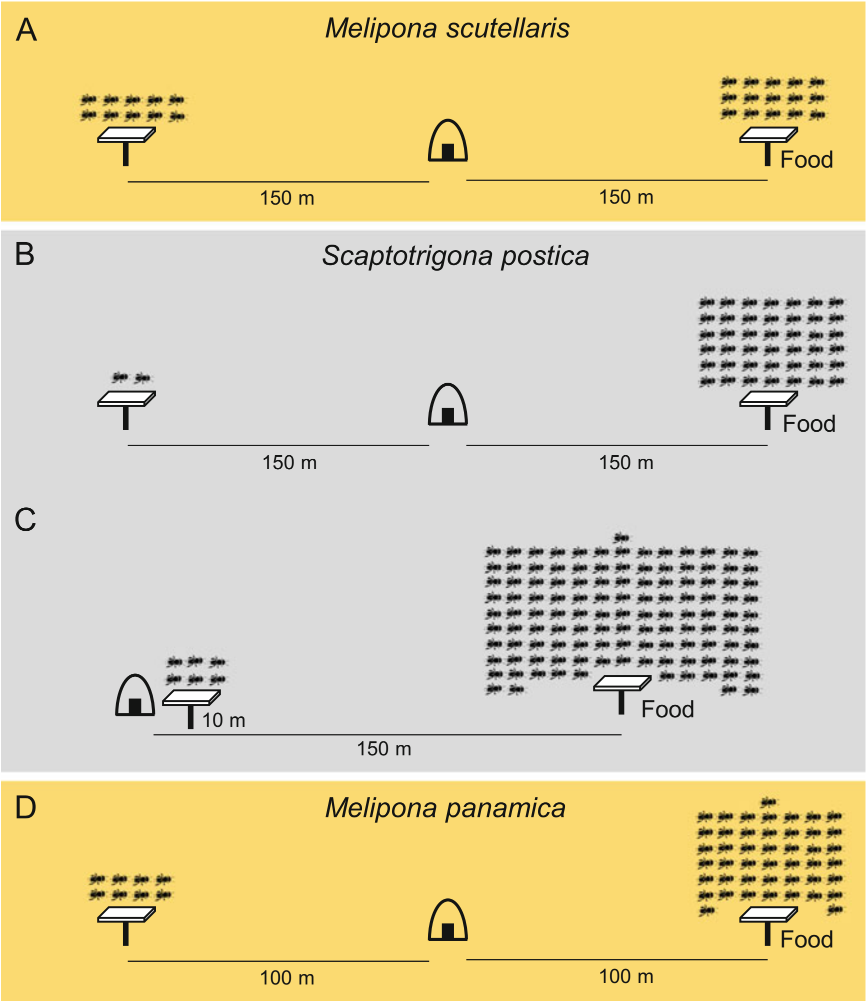

Fig. 10.7 The recruitment success of *Melipona scutellaris* (**a**), *Scaptotrigona postica* (**b**, **c**) and *M. panamica* (**d**). Two feeders were used simultaneously but only one offered food. The bee symbols indicate the number of newly arrived bees (modified from Lindauer and Kerr 1958; Nieh and Roubik 1995)

by Lindauer and Kerr (1958, 1960)[7] (Fig. 10.7a) (but see below), *Scaptotrigona postica* foragers provided site-specific information during recruitment (Fig. 10.7b, c) (see also Table 10.1). In the latter species, 42 recruits arrived at a feeder that was

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[7] Lindauer and Kerr (1958, 1960) argue that the tested *Melipona* species do show some directionality in recruitment and propose guidance flights as a likely method. However, the data presented in their studies, e.g. Table 3 in Lindauer and Kerr (1960), do not provide convincing support for this claim.

Table 10.1 List of species with information about whether they recruit to food sources or not

Species	Clade	Scent trails confirmed	Site specific recruitment	Notes	References
Apotrigona nebulata	AT	No	No		Darchen (1969)
Axestotrigona ferruginea	AT	No	No		Esch et al. (1965)
Tetragonula carbonaria	IM/AA	No	Partly	Only direction	Nieh et al. (2000, 2004)
Tetragonula iridipennis	IM/AA	No	No		Lindauer (1956)
Cephalotrigona capitata	NE	Yes	Yes		Lindauer and Kerr (1960)
Frieseomelitta silvestrii	NE	No	No		Lindauer and Kerr (1958, 1960)
Frieseomelitta varia	NE	No	No		Esch et al. (1965), Jarau et al. (2003)
Geotrigona mombuca	NE	Yes	Yes		Lindauer and Kerr (1958, 1960), Stangler et al. (2009)
Lestrimelitta limao	NE	No	Yes		Chap. 7
Melipona bicolor	NE	No	Yes		Nieh et al. (2003b)
Melipona mandacaia	NE	No	Yes		Nieh et al. (2003b)
Melipona panamica	NE	No	Yes		Nieh and Roubik (1995)
Melipona quadrifasciata	NE	No	Partly	Only direction	Jarau et al. (2000)
Melipona rufiventris	NE	No	Yes		Kerr and Rocha (1988)
Melipona scutellaris	NE	No	Partly	Only direction	Jarau et al. (2000)
Nannotrigona testaceicornis	NE	No	No		Lindauer and Kerr (1958, 1960)
Partamona helleri	NE	No	No		Esch et al. (1965)
Partamona orizabaensis	NE	No	Yes		Flaig et al. (2016)
Plebeia droryana	NE	No	Partly	Only direction	Lindauer and Kerr (1958, 1960), Peng et al. (in press)
Plebeia tica	NE	No	Partly	Only direction	Aguilar et al. (2005)
Scaptotrigona bipunctata	NE	Yes	Yes		Kerr et al. (1963)

(continued)

Table 10.1 (continued)

Species	Clade	Scent trails confirmed	Site specific recruitment	Notes	References
Scaptotrigona mexicana	NE	Yes	Yes		Sánchez et al. (2004, 2007)
Scaptotrigona pectoralis	NE	Yes	Yes		Hubbell and Johnson (1977)
Scaptotrigona postica	NE	Yes	Yes		Lindauer and Kerr (1958, 1960)
Scaptotrigona depilis	NE	Yes	Yes		Schorkopf et al. (2011)
Scaptotrigona xanthotricha	NE	Yes	Yes		Kerr et al. (1963)
Tetragona clavipes	NE	No	No		Jarau et al. (2003)
Tetragonisca angustula	NE	No	Partly	Only direction	Lindauer and Kerr (1958, 1960), Aguilar et al. (2005)
Trigona amalthea	NE	Yes	Yes		Kerr et al. (1963)
Trigona corvina	NE	Probable	Yes		Aguilar et al. (2005)
Trigona fulviventris	NE	Yes	Yes		Johnson (1987), cited in Schmidt et al. (2003)
Trigona fuscipennis	NE	Yes	Yes		Hubbell and Johnson (1977)
Trigona hyalinata	NE	Yes	Yes		Nieh et al. (2003a, b, c)
Trigona hypogea group	NE	Yes	Yes		Roubik (1982), Noll (1997), Jarau et al. (2003)
Trigona recursa	NE	Yes	Yes		Jarau et al. (2003)
Trigona silvestriana	NE	Yes	Yes		Hubbell and Johnson (1977)
Trigona spinipes	NE	Yes	Yes		Lindauer and Kerr (1958, 1960), Nieh et al. (2004a, b)

Species with confirmed use of scent trails are indicated. *AT* Afrotropical, *IM/AA* Indo-Malay/Australasia, *NE* Neotropics

visited by only a handful of marked foragers, whereas only 2 bees were captured at an equidistant, unvisited control feeder during the same time period (Fig. 10.7b). The distance and direction information provided by recruiting *S. postica* foragers was remarkably persuasive: many more recruits arrived at a distant (150 m) feeder that was visited by marked bees than at a control feeder in the same direction, placed only 10 m from the colony (Fig. 10.7c). As mentioned earlier, *Melipona panamica* was not only able to guide nestmates towards food in a specific direction and distance but even seemed to provide information about food source height (Nieh and Roubik 1995).

Unlike the preliminary trials performed by Lindauer and Kerr (1958, 1960), more recent work on *Melipona scutellaris* has demonstrated that foragers of this species can provide some spatial information to nestmates. However, nestmates seem to acquire information about the direction only, but not about the food source distance (Jarau et al. 2000).[8] This is similar to several other species where foragers are able to recruit nestmates to food sources in a particular direction, without providing accurate distance information (Table 10.1) (Jarau et al. 2000; Nieh et al. 2000; Aguilar et al. 2005; Peng et al. in press). In *Plebeia tica*, for example, newcomers mostly landed on the feeder closest to the nest in the direction of the advertised feeder (Aguilar et al. 2005). Thus, in these species, recruits seem to fly in the direction of the food source and then home-in on food sources that smell similar to the one that was advertised inside their nest. Yet, how the foragers in these species are able to send nestmates in the general direction of a food source remains a mystery.

10.2.1 Pheromone Trails

When Lindauer and Kerr (1958, 1960) studied *Scaptotrigona postica*, they observed that a forager, upon leaving the food source, landed on the vegetation, a stone or a piece of earth every couple of metres to deposit a scent mark. In one case, they observed 32 scent marking events from a food source that was 50 m from the nest (Fig. 10.8). The scent depositing foragers would often not even enter the nest, but return to the food source right away after depositing a scent mark a few metres from the nest entrance. Interestingly, *S. postica* foragers were never observed depositing scent marks very close to the nest and the closest scent marks were always several metres away. This could help prevent the crossing of multiple scent paths near the entrance, which could disorientate recruits (Lindauer and Kerr 1958, 1960). Scent marking was also only seen when food sources were at least 10–20 m from the nest (species differ in the minimum distance that is required to elicit scent marking, from ~3 m in *Geotrigona mombuca* to ~35 m in *Trigona spinipes*) (Lindauer and Kerr 1960; Kerr et al. 1963). The first scent marks are often left on or very close to the food source and the frequency of scent marking decreases with increasing distance from the food source (Lindauer and Kerr 1960; Kerr et al. 1963; Noll 1997; Nieh et al. 2003a; Stangler et al. 2009; Schorkopf et al. 2011). The length of scent trails also differs greatly: while *Trigona amalthea* has been observed to lay trails of up to 900 m length (Kerr 1960, there as *T. trinidadensis*), odour trails in *T. hyalinata* and *T. spinipes* are relatively short. In the latter species, scent marking by foragers is highest at the food source and the frequency gradually decreased to a maximum distance of ~30 m from the food source, even if the food source is more than 150 m

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[8] In *M. quadrifasciata*, direction information was only effective when food sources were relatively close to the nest (<30 m) (Jarau et al. 2000).

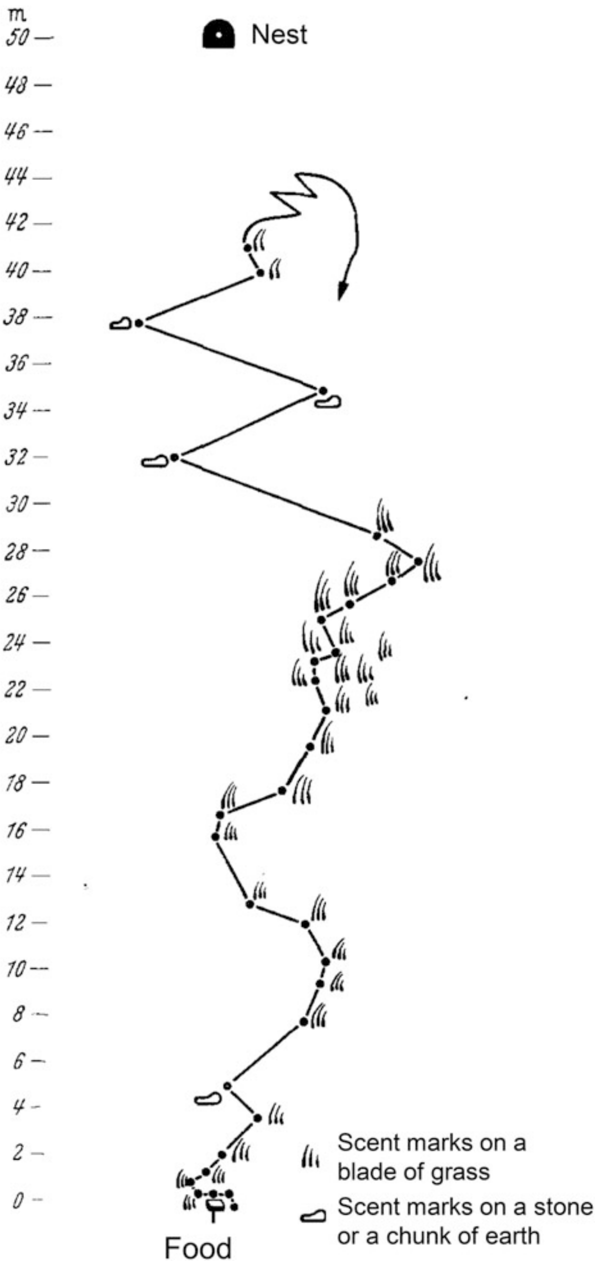

Fig. 10.8 Scent marks (dots) left by a *Scaptotrigona postica* forager when returning from a food source to the nest. Most scent marks were deposited on blades of grass, stones or pieces of earth. The forager returned to the food source without entering the colony. The last scent mark was deposited a few metres from the nest (modified from Lindauer and Kerr 1958)

from the nest (Nieh et al. 2003a, 2004b; see also Stangler et al. 2009). This pattern suggests that scent trails can have a polarity that allows recruits to determine the direction to and proximity of a food source (Kerr et al. 1963; Nieh et al. 2003a, 2004a, b). However, it also raises the question how recruits find the starting point of the pheromone trail.

In *Scaptotrigona postica*, scent marks were left when flying towards the nest, but not when flying towards the food source and their effect lasted only about 15 min (Lindauer and Kerr 1958, 1960). In other species, scent marks were behaviourally active for ~14 min (*S. bipunctata)*, ~9–20 min (*Trigona spinipes*, Kerr et al. 1963; Nieh et al. 2004a, b) and ~ 15 min (*T. hyalinata*, Nieh et al. 2003a). This suggests that stingless bee trail pheromones are highly volatile and short-lived (see also Jarau 2009). Kerr et al. (1981), for example, reported that even a human can smell the *T. spinipes* trail pheromone from a 1 m distance. This high volatility will help bees detect the pheromone markings from further away, but could also attract competitors (see below).

Identity and Glandular Source of Trail Pheromones

Scent marking involves the rubbing of the mouth parts (open mandibles and extended proboscis; Jarau et al. 2004b; Nieh et al. 2004a, b; Jarau 2009) on the substrate at species-specific intervals (every ~5 m in *Cephalotrigona capitata* and every 1–2 m in *Scaptotrigona postica*), suggesting that the chemicals are released from glands in the head (i.e. cephalic glands) (Lindauer and Kerr 1958, 1960).[9] Other scent marking species (e.g. *Trigona hypogea*) also rub their posterior legs and the abdomen against the substrate (Noll 1997). Early research led to the hypothesis that the cephalic gland in question is the mandibular gland (Lindauer and Kerr 1958, 1960; Blum et al. 1970; Nieh et al. 2003a). More recent research, however, has convincingly demonstrated that trail pheromones originate from the labial glands and are deposited via the extended proboscis (Fig. 10.9) (*Geotrigona mombuca*: Stangler et al. 2009; *Scaptotrigona pectoralis*: Jarau 2009; *Trigona corvina*: Jarau et al. 2010; *T. hyalinata*: Lichtenberg et al. 2011; *T. recursa*: Jarau et al. 2004a, b, 2006; *T. spinipes*: Schorkopf et al. 2007; reviewed in Jarau 2009). Mandibular gland secretions, on the other hand, mainly seem to function as alarm pheromones (see also Chap. 7). In *T. spinipes*, the recruitment pheromone consists of a single compound, the ester octyl octanoate (Schorkopf et al. 2007). The same compound is the main constituent of the trail pheromone of *T. hyalinata*, in combination with other esters (Lichtenberg et al. 2011). In *T. recursa*, the trail pheromone is more complex but its main component is again an ester (hexyl decanoate, accounting for ~72% of all volatiles) (Fig. 10.9) (Jarau et al. 2006). In *Geotrigona mombuca*, farnesyl butanoate plays a key role in combination with other esters (Stangler et al. 2009).

Colonies of some species show considerable differences in the quantitative composition of their trail pheromone and foragers are more likely to follow their own pheromone blend than that of a conspecific colony (Jarau et al. 2010; Reichle et al. 2011; John et al. 2012). This could help colonies avoid competition or

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[9] These intervals also mean that water bodies, e.g. rivers, of >10 m width might seriously inhibit recruitment and, thus, efficient exploitation by mass-recruiting species (Kerr 1960; but see Schorkopf et al. 2011).

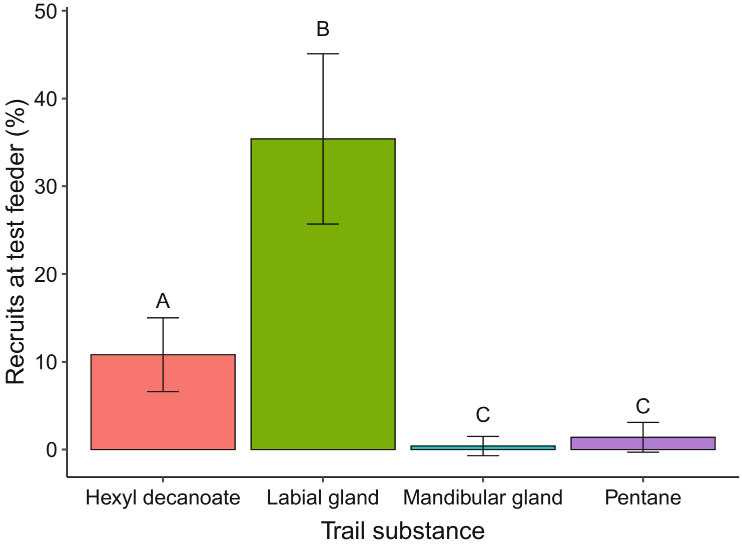

Fig. 10.9 The proportion of *Trigona recursa* recruits arriving at a test feeder at the end of a 10 m artificial scent trail that was made with either hexyl decanoate (see text), labial gland extract, mandibular gland extract or the solvent pentane (modified from Jarau et al. 2006). The artificial trails competed with a natural trail leading to a competing feeder 10 m from the test feeder (see Jarau et al. 2006, their Fig. 1 for details), which explains why the percentage of recruits at the test feeder was always less than 50%

aggressive encounters at food sources (see Sect. 10.3). In *Scaptotrigona pectoralis* and *S. subobscuripennis*, foragers seem to learn the chemical composition of their nest-specific trail pheromone (Reichle et al. 2011, 2013). Bees raised in heterospecific foster colonies can learn to respond to the trail pheromones of their heterospecific nestmates (Reichle et al. 2013). Learning of pheromone identity is striking as it is usually assumed that the response to pheromones is innate in social insects (Wyatt 2010; Grüter and Czaczkes 2019), but it could be beneficial if the chemical composition of pheromones is not fixed, but changes as a result of changing internal (genetic structure, e.g. due to queen replacement) and external conditions (changes in food sources and climatic conditions) (Reichle et al. 2011). Whether the pheromone composition indeed changes in *Scaptotrigona*, e.g. with seasons, remains to be investigated.

It is not well known if and how scent trail laying is affected by food source characteristics (i.e. food source distance, quality and quantity), but Schmidt et al. (2006a) found that the scent trail laying *Trigona recursa* recruited a larger number of workers to a 40% sucrose solution than to a 20% sucrose solution. This suggests that foragers mark trails with greater intensity if food source profitability is higher, but further research is needed to confirm this (Schmidt et al. 2006a). In *Scaptotrigona depilis*, on the other hand, sucrose concentration *per se* did not seem to greatly affect recruitment (Schmidt et al. 2006b), suggesting no effect on pheromone deposition rate. However, if the quality of the food source was lowered from 40% to 20%, significantly fewer recruits arrived at an artificial food source. Thus, recruitment decisions by experienced foragers might be based on comparisons with other food sources or previous experience.

Because pheromone trails can attract a large number of nestmates, they create a strong positive feedback during recruitment. In one study, *Scaptotrigona depilis* foragers recruited more than 1500 nestmates to a feeder during a 4-h period (Jarau et al. 2003) (see also Fig. 10.7c). This could have the disadvantage that colonies are unable to switch to other, potentially better food sources that appear later (in ants: Beckers et al. 1990; Camazine et al. 2001; Czaczkes et al. 2016). In *Trigona recursa*, the only mass-recruiting species where the flexibility of forager allocation at colony level has been studied so far, colonies continued to send more foragers to a 20% sucrose solution if it was discovered before a 40% solution, most likely because the pheromone trail to the inferior food source remained stronger due to the continuous deposition of pheromone by a large number of foragers (Schmidt et al. 2006a; see also Noll 1997).

One advantage of pheromone trails compared to the vector information conveyed by the honey bee waggle dance is that scent marking adds further specificity in a 3-dimensional environment. This could be particularly useful in tropical forests where food sources often exist in different vertical strata (Chap. 8) (Nieh and Roubik 1995; Nieh 2004). *Scaptotrigona postica*, for example, was much more successful in recruiting nestmates to a food source on a water tower than *Apis mellifera* (Lindauer and Kerr 1958, 1960).

Pheromone Trails and Colony Size

Pheromone trails that guide foragers to a food source have been confirmed in several species and yet, there are also many species that do not seem to use trail pheromones in foraging (Table 10.1).[10] Differences in colony size, for example, could affect whether scent trails are effective and beneficial. Due to the evaporation of scent marks, scent trails require a critical number of individuals to deposit pheromone in order to maintain a trail (Beekman et al. 2001; Beekman and Dussutour 2009). Furthermore, larger colonies are better able to monopolise a food source, which also makes recruitment more useful (Aguilar et al. 2005). Accordingly, stingless bee species with a confirmed use of pheromone trails (Table 10.1) have larger colony sizes than species without a confirmed use of trails (Fig. 10.10).

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[10] Recruitment based on pheromone marking might be more common during swarming (Chap. 4) (Roubik 1989, p. 205). This is similar to some ant species that communicate the location of new nest-sites during house hunting, e.g. by tandem running, but do not use tandem running when foraging (e.g. Franklin 2014; Grüter 2018). Whether communication is beneficial for colony success is likely to depend on the spatio-temporal distribution of food sources and competition (Sherman and Visscher 2002; Dornhaus and Chittka 2004; I’Anson Price and Grüter 2015).

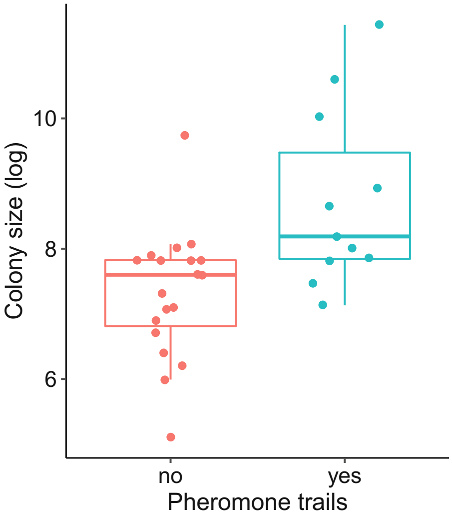

Fig. 10.10 Colony sizes (transformed with the natural logarithm) of species with and without confirmed pheromone trails (Table 10.1). Colony size data were taken from Table 1.3. A generalised least squares (GLS) model that incorporated the phylogenetic relationships among the species (a tree based on the relationships and chronogram provided by Rasmussen and Cameron 2010) and based on the Ornstein–Uhlenbeck model approach (see Paradis 2011 for methods) found a marginally significant colony size difference when comparing species with and without confirmed pheromone trail-based recruitment (t-value = 2.1, p = 0.045) (Excel file and tree can be downloaded here: www.socialinsect-research.com/book.php)

10.2.2 Chemicals Deposited on Food Sources

Foragers of many species, even those that do not lay pheromone trails, often leave scent marks on or near food sources during foraging (Kerr 1994; Nieh 1998b; Aguilar and Sommeijer 2001; Schmidt et al. 2003, 2005; Hrncir et al. 2004; Jarau et al. 2004a; Boogert et al. 2006; Contrera and Nieh 2007; Alavez-Rosas et al. 2017; Koethe et al. 2020; reviewed in Jarau 2009). These chemical “signposts” are not only helping nestmates to locate a food source but can also be used by the marking bee herself to return to her foraging site (Boogert et al. 2006). Chemical compounds could be left deliberately to signal the location of a food source, i.e. as pheromones (Kerr 1994; Schmidt et al. 2003; Jarau et al. 2004a), or passively in the form of footprints (e.g. in *Nannotrigona testaceicornis*, Schmidt et al. 2005). In the latter case, they represent information cues, rather than evolved signals (Seeley 1995; Danchin et al. 2004; Dall et al. 2005; Schmidt et al. 2005; Jarau 2009).[11] In *Melipona seminigra*, these scent marks have an attractive range of about 1 m and their effect

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[11] Only information transfer involving signals (i.e. traits shaped by natural selection to convey information) is considered communication (Seeley 1995; Danchin et al. 2004; Dall et al. 2005). Following this definition, any use of information cues, e.g. hydrocarbons left passively as food prints during foraging (Chap. 7), does not represent an example of communication.

lasts about ~2 h (Hrncir et al. 2004). In *M. panamica*, this attraction range reaches several metres (Nieh 1998b) and in *Scaptotrigona depilis*, scent marks left near food sources were perceived by other bees that were ~20 m away (Schmidt et al. 2003).

In some *Melipona* species, foragers were observed to leave anal or abdominal fluids on and near artificial feeders, which were attractive to other foragers (Aguilar and Sommeijer 2001; Nieh et al. 2003c). Foragers produce more abdominal fluids when collecting more dilute (i.e. less profitable) artificial sucrose solutions, which could indicate that the fluids represent excess liquid released by foragers (Nieh 1998b; Nieh et al. 2003b). Whether abdominal droplets are also produced by foragers that visit natural food sources, instead of *ad libitum* feeders, is not currently known. Nieh et al. (2003c) provide evidence that *M. mandacaia* marks food sources with a ventro-abdominal odour. Foragers of some *Melipona* species were also observed rubbing their mouth parts on vegetation near a food source (Aguilar and Sommeijer 2001; see also Kerr 1994), but it remains unclear whether bees were cleaning themselves or whether this behaviour has communicative function.

The source of attractive chemical marks requires further study and while abdominal droplets may be used to mark food sources in *M. favosa* and *M. mandacaia*, they have been excluded in other *Melipona* species (Nieh 1998b; Hrncir et al. 2004). *Melipona solani* leaves hydrocarbons on feeders, most likely passively as footprints during food uptake. Additionally, workers of this species seem to actively mark higher quality food sources with a chemical blend that contains methyl oleate as a prominent component (Alavez-Rosas et al. 2017), possibly released from labial glands.

The most detailed studies on the origin of scent marks demonstrated that extracts from tarsomeres attracted nestmates in the same way as the scent marks left on food sources (in *Melipona seminigra*) (Hrncir et al. 2004; Jarau et al. 2004a). Hrncir et al. (2004) covered the tarsi and pre-tarsi of foragers with nail polish, which eliminated attraction (Hrncir et al. 2004). Histological and chemical analyses suggest that the scent marks mainly consist of hydrocarbons (mainly the alkanes pentacosane, heptacosane and the corresponding alkenes 7-(Z)-pentacosene and 7-(Z)-heptacosene). They originate from tendon glands in the legs (the glandular epithelium of the claw retractor tendons) and are released at the base of the legs (Jarau et al. 2004a).

Repellent Scent Marks

Scent marks can also be repellent, which could help bees avoid recently visited (and depleted) flowers (honey bees: e.g. Giurfa and Núñez 1992; bumble bees: e.g. Stout et al. 1998). *Trigona fulviventris* foraging on *Priva mexicana* was found to avoid previously visited flowers irrespective of whether they offered rewards or not, suggesting that they used scent marks as repellent cues (Goulson et al. 2001). On the other hand, scent marks did not seem to affect foragers robbing nectar from *Crotalaria cajanifolia*. The latter plant species has simpler flowers and more easily accessible nectar, which reduces flower handling time and, therefore, the costs of visiting depleted flowers (Goulson et al. 2001). This suggests that scent marks are used in a context-dependent way. Whether scent marks are attractive or repellent is likely to depend on the foraging experience of a forager. For example, *Scaptotrigona mexicana* foragers that experienced reward-predicting scent marks were attracted to them (Sánchez et al. 2008; from non-nestmates: Solórzano-Gordillo et al. 2018). If, on the other hand, scent marks indicated the absence of a reward, foragers learned to avoid them (see also Sect. 10.4) (Saleh and Chittka 2006; Sánchez et al. 2008; Jarau 2009; Slaa and Hughes 2009). Similar learning effects have been found in *Melipona scutellaris*: foragers that experienced a decrease in rewards in association with footprints subsequently preferred unvisited food sources, whereas foragers that experienced increasing rewards in association with footprints acquired a preference for feeders with footprints (Roselino et al. 2016). Additionally, scent marks can have dose-dependent effects. In *M. seminigra*, they are attractive when their quantity was equivalent to about 40 visits of bees at a food source, but they became repellent at higher concentrations (Hrncir et al. 2004; Jarau et al. 2004a). In natural situations, high concentrations of scent marks might indicate that the food source was visited repeatedly and is, therefore, more likely to be depleted. Whether scent marks are used by foragers also depends on whether the food source has a strong odour. Scent marks seem to be more important when food sources are unscented (Hrncir et al. 2004; Schmidt et al. 2005).

10.2.3 Guidance Flights (Piloting)

Lindauer and Kerr (1958) observed that *Scaptotrigona postica* recruits often arrived together with the scent marking bee. They interpreted this as evidence that additional information is used by recruits, such as visual guidance or the release of chemicals by the recruiting forager during flight. Similar observations have also been made in *Trigona corvina*, where recruits often would not land on a feeder before the recruiting bee has landed (see also Sect. 10.4) (Aguilar et al. 2005). Lindauer and Kerr (1958, 1960) speculated that the dark and shiny cuticle of many stingless bee species makes them visually conspicuous and, thus, easy to follow during flight.

Several *Melipona* species, e.g. *Melipona panamica, M. quadrifasciata*, *M. rufiventris* and *M. scutellaris*, are able to recruit nestmates to particular food source locations, without using scent trails (Nieh and Roubik 1995; Hrncir et al. 2000; Jarau et al. 2000, 2003).[12] For example, Hrncir et al. (2000) observed that in ~99.5% of all return flights, *M. quadrifasciata* and *M. scutellaris* foragers return to their nest without landing on vegetation. Despite the absence of obvious scent marking, when Nieh and Roubik (1995) trained *M. panamica* foragers to a food source 100 m from the hive, nearly all newcomers arrived at this feeder rather than at a similar equidistant control feeder in the opposite direction of the nest (Fig. 10.7d). Recruitment in *M. panamica* seems to be no less location specific than in species

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[12] Kerr and Rocha (1988) hypothesised that some *Melipona* species also deposit scent marks near the food source, but without providing experimental evidence (see Nieh and Roubik 1995 and Jarau 2009 for a discussion).

with pheromone trails. Guidance flights could explain this accuracy, but Nieh and Roubik (1995, 1998) remain cautious regarding the evidence for guidance flights in *Melipona*. One argument against guidance flights in *M. panamica* was the observation that newcomers often arrived minutes after the marked recruiters have been captured and arriving newcomers do often not arrive clustered in time (Nieh and Roubik 1995, 1998). On the other hand, the presence of actively recruiting bees seemed to be required. If the bees that were trained to a feeder were captured, recruits no longer showed a preference for the visited feeder over a control feeder placed in a different direction. Thus, some form of guidance by recruiters appears to be involved in *M. panamica* foraging, but the nature of the information used by recruits remains to be uncovered.

Melipona quadrifasciata foragers were observed performing a behaviour that was described as partial zigzag flights (Esch et al. 1965; Esch 1967), but Hrncir et al. (2000) found no indication that recruits follow zigzag flights performed by recruiting bees in their experiments (see also Jarau et al. 2003). Instead, recruiting foragers flew in a more or less straight line to the food source and recruits only rarely arrived together with the recruiting bee. No less puzzling is recruitment communication in *Partamona orizabaensis* (Flaig et al. 2016). Foragers of this mass-recruiting species are able to steer nestmates to the location of a food source without any signs of scent trails and guidance flights seem unlikely due to the dim light conditions of their habitat (Flaig et al. 2016). An alternative explanation is that foragers release odours during flight, thereby creating an aerial odour trail (Kerr 1960; Flaig et al. 2016). Detailed examinations of guidance flights and putative aerial odour trails are required to uncover whether these forms of recruitment exist and are widespread (see also Jarau 2009).

10.3 Eavesdropping

Marking the path between a food source and the nest or marking the food source itself could attract the attention of competitors that can then "listen in" on this communication, a process called eavesdropping.[13] For example, Lindauer and Kerr (1958) found that *Scaptotrigona postica* foragers followed scent trails of nearby conspecific colonies. *Agelaia vicina* wasps, known for their huge colony sizes of more than a million wasps (Zucchi et al. 1995), follow the scent trails of the meat eating *Trigona hypogea* (Noll 1997). Scent marks left by conspecifics from other colonies have also been shown to be attractive in *Nannotrigona testaceicornis* (Schmidt et al. 2005), *Tetragonisca angustula* (Villa and Weiss 1990) and *Trigona corvina* (Boogert et al. 2006), whereas scent marks left by heterospecifics were

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[13] I follow a broad definition of eavesdropping that includes both social cues and signals as sources of social information for eavesdroppers (as in Johnstone 2001; Danchin et al. 2004; Valone 2007). Others have restricted eavesdropping to the use of signals (e.g. Slaa et al. 2003; Slaa and Hughes 2009).

ignored by foragers of the latter species (Boogert et al. 2006). A different *Trigona* species, the mass-recruiting *T. spinipes*, on the other hand, is attracted to scent marks left by *Melipona rufiventris* and *T. spinipes* colonies can take over food sources by driving away the *Melipona* foragers (Nieh et al. 2004a).

As mentioned earlier, the reliance on scent marks is often context specific and can change as a result of learning (Nieh et al. 2004a; Solórzano-Gordillo et al. 2018). *Trigona spinipes* foragers prefer *M. rufiventris* scent marks over their own scent marks on newly discovered food sources, but they prefer their own scent marks over *M. rufiventris* scent marks on food sources that are already exploited by *T. spinipes* foragers (Nieh et al. 2004a). *Melipona rufiventris* foragers, on the other hand, avoid *T. spinipes* scent marks. *Trigona hyalinata* foragers avoid the recruitment pheromones of *T. spinipes*, even though they are considered to be dominant over *T. spinipes* at food sources (Lichtenberg et al. 2011). Since both species are relatively aggressive, this avoidance of recruitment pheromones of another aggressive species could reduce the risk of costly confrontations at food sources (Lichtenberg et al. 2011). On the other hand, *T. spinipes* foragers did not respond to the *T. hyalinata* recruitment pheromone (Lichtenberg et al. 2011). Eavesdropping can also depend on the strength of a pheromone trail. A weak trail could indicate a poorly defended resource, leading to lower potential costs of an attempted takeover of the food source. Accordingly, when *T. hyalinata* foragers encountered a weak *T. spinipes* trail, they switched from avoidance to attraction (Lichtenberg et al. 2014).

The costs imposed by eavesdroppers could select for different, species-specific signalling strategies (Lichtenberg et al. 2014). For example, it could explain why pheromone trails often do not reach far beyond the food source (see above) as this reduces the risk that the location of the food sources or the colonies themselves are discovered by competitors or predators following these trails (Nieh et al. 2004b; Lichtenberg et al. 2011). Interestingly, eavesdropping by competitors does not necessarily select for less conspicuous communication. If pheromones can signal costs to the eavesdroppers, such as aggressive confrontations, then conspicuous signalling could also be favoured by eavesdropping (Lichtenberg et al. 2014).

10.4 Local and Stimulus Enhancement

The presence of other bees (or their scents) near or on food sources can function as social cues that affect foraging decisions, e.g. by motivating bees to land on these flowers (see above). Foragers can then learn about the features of this food source, a form of social learning called *local enhancement* (Heyes 1994).[14] In *stimulus*

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[14] Local enhancement has been defined in different ways by different researchers (see Hoppitt and Laland 2008 for a discussion). Some authors do not include the requirement of learning, but focus primarily on the attraction component as the critical criteria for local enhancement (Slaa et al. 2003; Hoppitt and Laland 2008). I follow Heyes (1994) and define local enhancement as a process that involves learning. Given the propensity of bee foragers to learn food source-related cues (Chap. 8), it is unlikely that attraction to food sources occurs without subsequent learning about its features.

enhancement, the foragers do not only learn that this particular flower patch is rewarding but will acquire a general preference for flowers of the same type (Heyes 1994; Avargues-Weber and Chittka 2014). Local and stimulus enhancement consist of two sequential components: first the attraction component caused by a social cue, which then creates an opportunity for the forager to learn associations between food source features (e.g. colours, scents and landmarks) and a reward. The terms *eavesdropping* and *local enhancement* refer to related processes, but they differ in four aspects according to the way they are used here. First, eavesdropping refers to the exploitation of information provided by non-nestmates, whereas local enhancement does not make assumptions about the relationship between the copying and the copied individual (Heyes 1994). Second, the term eavesdropping focuses on ultimate processes explaining the use of social information (signals and cues) (e.g. Lichtenberg et al. 2014), whereas local enhancement puts emphasis on the proximate basis of the acquisition of new behaviour (Heyes 1994). Third, eavesdropping does not usually include a requirement of learning, whereas local enhancement is a process of social learning and, finally, eavesdropping can involve the exploitation of signals or social cues, whereas local enhancement is usually restricted to the use of social cues (Slaa and Hughes 2009).

Foragers can also be deterred from landing on flowers if they are already occupied by bees, a phenomenon that has been called *local inhibition* (Slaa et al. 2003). As was the case with repellent scent marks (one kind of local inhibition), avoiding occupied flowers can reduce sampling costs involving flowers that are likely to be depleted. Local enhancement and local inhibition based on visual cues have been found in a number of stingless bee species (reviewed in Slaa and Hughes 2009). Whether a forager is attracted or deterred by the presence of other bees is also likely to depend on prior experience (see also Sect. 10.2.2) (Slaa et al. 2003; Sánchez et al. 2008; Dawson et al. 2013; Avargues-Weber and Chittka 2014). In *Trigona amalthea*, for instance, foragers with little foraging experience showed local enhancement, whereas more experienced foragers were inhibited by nestmates (Slaa et al. 2003). The decision of how to react to other foragers at food sources is also likely to depend on whether these bees are aggressive or not. As would be expected, focal foragers avoided larger and more aggressive heterospecifics in flower choice experiments (Slaa et al. 2003). Thus, local enhancement involving heterospecifics is likely to depend on the competitive hierarchy of the involved species (Slaa and Hughes 2009).

These species-specific differences in the response to other bees at food sources affect the distribution of foragers at flower patches: local inhibition leads to more dispersed foragers, whereas local enhancement can facilitate the formation of foraging groups (see also Chap. 8) (Slaa et al. 2003; Slaa and Hughes 2009). Group formation, in turn, could increase protection against competitors and predators.

10.5 Evolution of Recruitment Communication

We still know very little about when and why different recruitment behaviours evolved. Running around inside the nest in an excited fashion (e.g. shaking, zigzagging and jostling runs) is also found in honey bees (von Frisch 1967) and bumble bees (Dornhaus and Chittka 1999). Thus, it is likely that agitated runs inside the nest represent an ancient and ancestral behaviour that alerts other foragers to the existence of a particular food type and motivates them to search for this food. In stingless bees and honey bees, foragers often interrupt their excited runs to perform trophallaxis, which allows other bees to taste the food and learn its odour (see above and Chap. 8). Curiously, bumble bees (Bombini) do not perform trophallaxis, possibly because their relatively small colony size makes finding trophallaxis partners too time-consuming (Anderson and Ratnieks 1999). These two concomitantly performed behaviours, agitated runs and trophallaxis, might represent an early stage in the evolution of recruitment communication in stingless bee ancestors (Kerr 1960; Nieh 2004). The production of buzzing sounds during agitated runs, which is common in most or all stingless bees (Kerr 1960), probably also evolved independently in stingless bees and honey bees (Hrncir and Barth 2014; I'Anson Price and Grüter 2015).

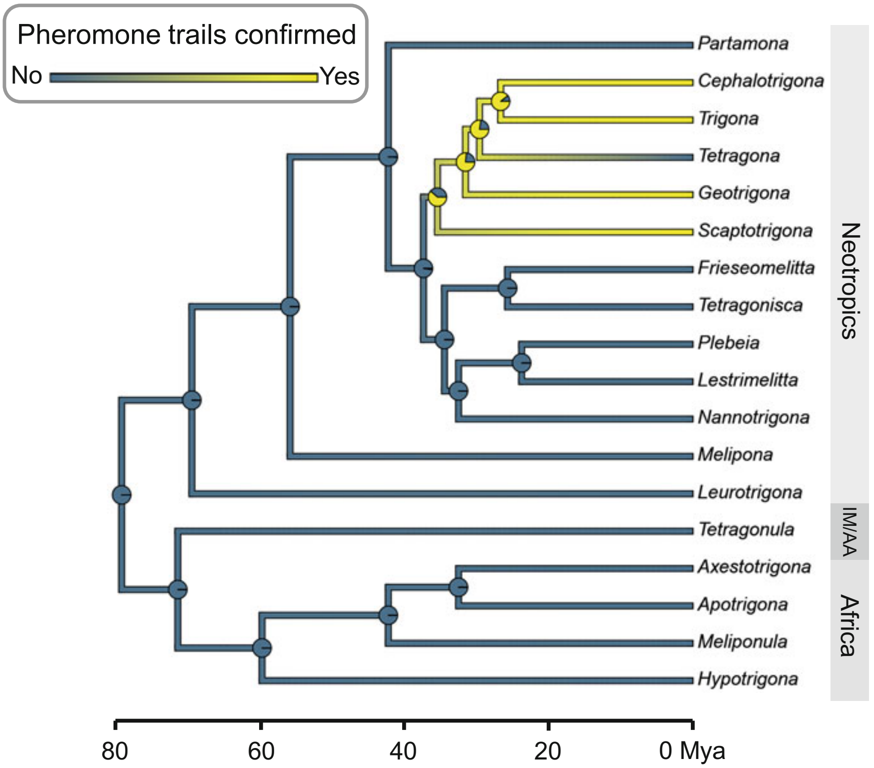

Fig. 10.11 Ancestral state estimation for the laying of trail pheromone to food sources (yes/no) (see Table 10.1 for more details). The colour of the branches is based on an MCMC approach (Paradis 2011). Pie charts provide estimates based on continuous-time Markov chain models (for more details on how this analysis was performed, see Grüter 2018). The chronogram is based on Rasmussen and Cameron (2010). Both methods suggest that the deposition of pheromones to food sources is a derived condition that appeared once approx. 30–35 mya

Jarau (2009) proposed that pheromone trails do not represent the ancestral condition in stingless bees. This was based on the observation that the genera *Scaptotrigona* and *Trigona*, which are known for their pheromone trails, are relatively derived genera (see also Fig. 2.2). An ancestral state reconstruction supports the hypothesis that pheromone trails represent a derived character in stingless bees (Fig. 10.11). The analysis suggests that trail laying species are relatively closely related and that pheromone trail laying appeared once, about 30–35 million years ago. Pheromone trail communication seems to have been lost in the ancestors of *Tetragona* (Fig. 10.11). Interestingly, Jarau et al. (2003) observed that *Tetragona clavipes* foragers performed a behaviour resembling scent marking at food sources, but they found no scent trail laying. Recruitment was weak in this species (Jarau et al. 2003) and foraging communication has not yet been studied in detail in *Tetragona*. The same is true for most other stingless bee genera.

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