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Assessing the impact of disease on pollinators

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1 Introduction

Spurred by the report of a sensational 75% loss of insect biomass over a period of 27 years around the city of Krefeld in northwest Germany (Hallmann et al., 2017), more recent analyses of insect trends strongly support a worldwide decline in terrestrial insect biodiversity as well as biomass (van Klink et al., 2020; Wagner et al., 2021). As insects, particularly bees, are the most important pollinators of the majority of crops requiring pollination (Klein et al., 2007) and as there is an ongoing agricultural expansion of pollinator-dependent crops (Aizen et al., 2019), the decline of insects has obvious negative implications for pollination and crop yields in agricultural settings.

Insect decline is often seen as a multifactorial problem, itself associated with agricultural expansion (Raven and Wagner, 2021). Chief among the factors causing the decline of insect pollinators are thought to be (i) lack of habitat and associated essential resources (flowers providing pollen and nectar, suitable nesting sites), (ii) pesticides and (iii) disease organisms (Brown and Paxton, 2009; Vanbergen and the Insect Pollinators Initiative, 2013; Goulson et al., 2015; Potts et al., 2016). The relative importance of these three likely varies from

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pollinator species to pollinator species, though pests and pathogens feature prominently (Dicks et al., 2021).

In agricultural settings, where managed bees such as the ubiquitous western honey bee (*Apis mellifera* L.) are typically employed for pollination (McGregor, 1976; Kevan et al., 1990; Free, 1993; Delaplane and Mayer, 2000), two factors may promote the impact of pathogens: host density and host dispersal. Firstly, elevated densities can facilitate pathogen transmission and theoretically result in higher disease prevalence (Anderson and May, 1981). Pollinators are often promoted in or near crops requiring pollination (Free, 1993), and their increased density clearly represents a threat in terms of enhanced disease transmission to conspecifics and, through pathogen spillover, to heterospecifics (Gisder and Genersch, 2017). Secondly, translocation has led to the dispersal and subsequent emergence of many pathogens in plants, animals and humans (Daszak et al., 2000). Pollinators are not exempt. The transport of managed pollinators, particularly *Apis mellifera* and the bumble bee *Bombus terrestris*, is likely to blame for the dispersal and emergence of infectious diseases that threaten conspecifics and heterospecifics (Cameron et al., 2011; Schmid-Hempel et al., 2014; Wilfert et al., 2016) alike, with knock-on consequences for the supply of pollination services to crops.

In this chapter we focus on diseases and their impact on insect pollinators; the direct impacts of habitat (degradation and loss) and pesticides are dealt with elsewhere in this book. We furthermore focus on bee pests and pathogens (disease-causing organisms) because of the importance of bees in crop pollination (Klein et al., 2007) and because most is known about their pests and pathogens.

2 A bestiary of honey bee diseases

Apis mellifera is arguably the best monitored of insects and numerically the most important commercial pollinator worldwide. The world stock of beehives (colonies) has grown consistently over the past 6 decades to around 90 million in the last 4 years (2016–2019; see Fig. 1a), likely driven by the agricultural expansion in pollinator-dependent crops (Aizen et al., 2008; Aizen and Harder, 2009). The logic of the argument is that farmers growing more, or a greater area of, pollinator-dependent crops like almonds and apples may pay for pollination services, encouraging beekeeping as an economic activity and thereby boosting the number of managed honey bee colonies.

Although these data might suggest that we should not be concerned about the world's population of honey bees, they belie the high annual colony losses suffered by beekeepers over the past 2 decades, particularly in Northern temperate regions of the world (Osterman et al., 2021). The massive die-off of US honey bees in the winter of 2006/2007 brought the issue of colony losses to

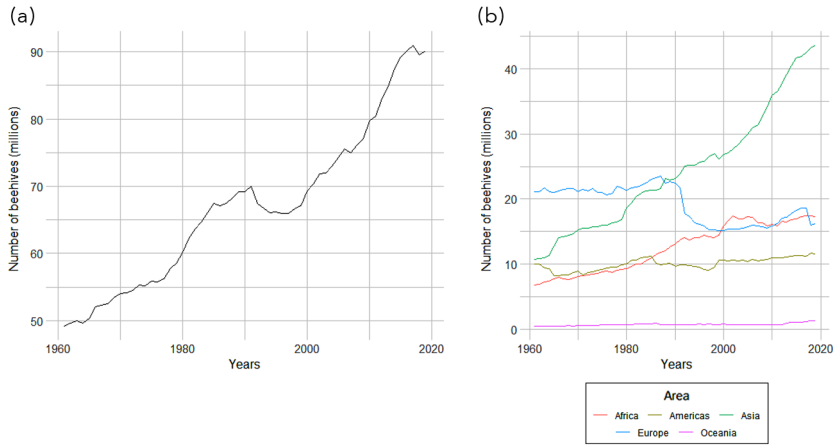

Figure 1 (a) The world stock of honey bee colonies (hives) from 1961 to 2019; (b) the number of colonies split by geographic regions (data from faostat: https://www.fao.org/faostat/en/#home).

world media and political attention (Oldroyd, 2007). Since 2007, North America has reported annual (overwinter) colony losses of 26%, including >40% losses in the last 3 years (2017/2018, 2018/2019, 2019/2020; https://beeinformed.org/citizen-science/loss-and-management-survey/) whilst those in Europe since 2007 have been around 16%, all well above 11% elsewhere in the world (Osterman et al., 2021) or the 'normal' expected mortality of ca. 10% (Chauzat et al., 2016). Part of the apparent discrepancy between the upward trend in the standing stock of honey bee colonies and high annual losses is because colony gains are in subtropical or tropical regions of the world (China, South America; Fig. 1b), whereas losses are in Northern temperate regions (Osterman et al., 2021), part is because beekeepers make up for colony losses by multiplying their surviving colonies.

Regardless of these arguments over global colony dynamics, there is widespread consensus that disease organisms (pests and pathogens) are a major cause of elevated honey bee colony mortality (Cox-Foster et al., 2007; Genersch et al., 2010; Johnson, 2010; Staveley et al., 2014; Barron, 2015; Chauzat et al., 2016). We now describe the major disease organisms of honey bees and their impact on hosts (for summary, see Table 1). Our list is not comprehensive; e.g. we have not addressed the havoc currently wreaked on honey bees by Asian hornet (*Vespa velutina*) predation in NW Europe (Keeling et al., 2017). Rather, we focus on pests and pathogens with a broad distribution or with the potential to cause considerable harm in the near future.

Table 1 Major parasites and pests of the western honey bee (*Apis mellifera*)

Disease/common name	Causative agent
Mites	
Varroosis	*Varroa destructor*
Tropilaelaps mite	*Tropilaelaps* spp.
Acarapisosis/tracheal mite	*Acarapis woodi*
Viruses	
Deformed wing virus	Deformed wing virus
Acute bee paralysis virus	Acute bee paralysis virus
Israeli acute paralysis virus	Israeli acute paralysis virus
Kashmir bee virus	Kashmir bee virus
Slow bee paralysis virus	Slow bee paralysis virus
Chronic bee paralysis virus	Chronic bee paralysis virus
Sacbrood virus	Sacbrood virus
Black queen cell virus	Black queen cell virus
Fungi	
Nosemosis type A	*Nosema apis*
Nosemosis type C	*Nosema ceranae*
Chalkbrood	*Ascophaera apis*
Bacteria	
American foulbrood	*Paenibacillus larvae*
European foulbrood	*Melisococcus plutonius*
Trypanosomatids	
Crithidia	*Crithidia mellificae*
Lotmaria	*Lotmaria passim*
Coleoptera	
Small hive beetle	*Aethina tumida*
Lepidoptera: Pyralidae	
Greater wax moth	*Galleria mellonella*
Lesser wax moth	*Achroia grisella*

2.1 Ectoparasitic mites

Social insects have long been known to harbour a diversity of parasitic mites, and the honey bee is no exception (Eickwort, 1990). We briefly detail three ectoparasitic mites of honey bees that are particularly well studied, in part because of their very serious impact on honey bee health.

2.1.1 Varroa destructor

The exotic mite *Varroa destructor* is a well-known ectoparasite of honey bees, present in almost every apiary worldwide, with the exception of Australia (Traynor et al., 2020). Successfully tolerated by its original host, the eastern honey

bee *Apis cerana*, after jumping over to *Apis mellifera* the mite has been held responsible – directly or indirectly – for the elevated colony losses of managed honey bees (Cox-Foster et al., 2007; Genersch et al., 2010; Rosenkranz et al., 2010; Nazzi and Le Conte, 2016).

A direct, detrimental effect of the mite is caused by its feeding behaviour. The parasite invades host pupae in brood cells as well as adult honey bees and feeds on their haemolymph and fat bodies (Ramsey et al., 2019), resulting in lowered body weight and water content. Heavy infestation of the brood cells may result in the death of pupae. Low body weight is associated with decreased sperm production in drones, which hampers their reproductive performance (Duay et al., 2002) and subsequently queen reproductivity. Feeding by mites on adult workers results in flight impairment of the host (Duay et al., 2002) and loss of orientation, thus severely affecting homing (Kralj and Fuchs, 2006). Besides the effects inflicted on individuals, varroa mites compromise the colony; the decreased number of emerging honey bees, coupled with the lower performance of foragers and reproductives (queens and drones), inexorably leads to colony decline (Neumann and Carreck, 2010).

Although varroa mites alone can potentially impair colony fitness, the major threat they pose arises from the pathogens – particularly viruses – they transmit whilst feeding on hosts (Yang and Cox-Foster, 2005; Neumann and Carreck, 2010). To date, a plethora of viruses is thought to be vectored by varroa mites (Levin et al., 2019; Beaurepaire et al., 2020; Yañez et al., 2020), some of which elicit severe disease. The presence of *V. destructor* has therefore shaped the current distribution of mite-transmitted viruses across the world, facilitating viral spread and, through a change in the route of viral transmission, leading to increased severity of viral disease (virulence) (Martin et al., 2012; Mondet et al., 2014).

Three other *Varroa* species, *jacobsoni*, *rindereri* and *underwoodi*, are known from south and east Asian honey bee species, though they are rarely associated with *Apis mellifera* (Chantawannakul et al., 2016). The exception is *V. jacobsoni* that parasitises *Apis mellifera* colonies in Papua New Guinea. Two *Euvarroa* species, *sinhai* and *wongsirii*, are hosted by Asian honey bees and occasionally by *Apis mellifera* imported into the region, though the biology of these mites is less well known (Chantawannakul et al., 2016) and their impacts on hosts are likely modest.

2.1.2 Tropilaelaps spp.

Tropilaelaps is a genus of ectoparasitic mite whose primary hosts are honey bee species native to tropical and subtropical south and east Asia, but which can now also be found on *Apis mellifera* imported into the region (de Guzman et al., 2017). Despite their high rates of reproduction than can exceed those of varroa mites, they have not (yet) been recorded widely outside of their native

range. Of four known species in the genus *Tropilaelaps*, namely *mercedesae*, *clerae*, *thaii* and *koenigerum*, the first two are the most prevalent and have successfully jumped over to *Apis mellifera* after its introduction to South and East Asia (Chantawannakul et al., 2016). The primary host of *T. mercedesae* is *Apis dorsata*, a widely distributed honey bee species of South and East Asia (Smith, 1991). Contrastingly, *T. clerae* and its primary hosts, *Apis dorsata binghami* and *Apis breviligula*, are restricted in distribution, and thus the mite is less prevalent than *T. mercedesae*. *Tropilaelaps thaii* and *T. koenigerum* are less well known (for further details, see Chantawannakul et al., 2016).

Tropilaelaps spp. and *V. destructor* share similarities in the life cycle, feeding behaviour and negative effects on honey bee hosts. An important distinction between these two mite genera is the inability of *Tropilaelaps* spp. to feed on adult honey bees due to their soft chelicerae (mouthparts) (Koeniger et al., 1988). This drastically shortens mite survivorship outside brood cells. This difference in biology means that *Tropilaelaps* spp. may not be able to spread to temperate regions of the world where honey bees enter a broodless period during the winter months.

Colonies infested with *Tropilaelaps* spp. mites suffer from a decreased number of emerging workers; those that do emerge are often crippled and weakened. The immunity of individual hosts is diminished due to direct damage by mite feeding and the lack of proteins that are harvested by the mite from pupal haemolymph (Khongphinitbunjong et al., 2015). The health status of a single, parasitised honey bee projects onto the general fitness of the colony and heavy infestation can lead to colony collapse. *Tropilaelaps* spp. mites, such as varroa mites, vector honey bee viruses (Dainat et al., 2009; Forsgren et al., 2009).

2.1.3 Acarapis woodi

Acarapis woodi is a microscopic parasitic mite inhabiting the tracheal tubes of young honey bees and the base of the wings of older honey bees, whose stiff tracheal hairs are too hard for the mite to penetrate into the host's tracheae. Initially discovered in the United Kingdom in the early 1900s, the mite has been reported across all continents (Matheson, 1993), although in many countries *Acarapis woodi* outbreaks occur only sporadically. Currently, it is not widely reported (Traynor et al., 2016), possibly because of widespread miticide use to treat colonies against varroa mites. The life cycle of *Acarapis woodi* is limited (exclusively for males and almost exclusively for females) to tracheal tubes, where mites pierce the tracheal tubes to feed on host haemolymph, with a short outside phoretic period reserved for post-mating host switching by females. Acarapisosis usually proceeds covertly and elicits symptoms only after heavy infestation. Affected honey bees are unable to fly and can be seen crawling

around the hive floor, the wings may be displaced and the abdomens are elongated.

2.2 Viruses

Honey bees host a rich diversity of viruses. To date, over 70 have been associated with them (McMenamin and Genersch, 2015; Grozinger and Flenniken, 2019; Beaurepaire et al., 2020), though only a small fraction of them has been studied in any depth. Most honey bee viruses are short (ca. 10 kb), positive sense, single-stranded RNA or (+)ssRNA viruses that produce a negative RNA strand using the machinery of the host cell to replicate. Because of their small genome size, they lack a good mechanism of RNA repair during replication and therefore have high mutation rates and likely a high capacity to evolve and adapt, e.g. to a change in transmission pathway or to novel hosts (Holmes, 2009).

2.2.1 Deformed wing virus

The best-studied (Martin and Brettell, 2019) and arguably the most important virus in terms of its impact, deformed wing virus (DWV), has long been reported in honey bees, even before varroa infestation (Bailey and Ball, 1991). The mite's presence changed DWV's main route of transmission from (presumed) faecal–oral to indirect via the varroa mite as a vector (Yañez et al., 2020). This change precipitated a dramatic increase in DWV's prevalence (number of infected colonies) and pathogen load (amount of virus per honey bee) (Martin et al., 2012; Mondet et al., 2014) as well as virulence (harm caused to the host) (Bowen-Walker et al., 1999; Nazzi et al., 2012; Di Prisco et al., 2016). DWV is notoriously found in collapsing colonies and is associated with high overwinter mortality rates in honey bees (Dainat et al., 2012; Francis et al., 2013; Natsopoulou et al., 2017). DWV and its transmission by varroa mites are therefore the leading causes of elevated honey bee colony mortality in the past 2 decades, at least in temperate regions of the world.

DWV, a (+)ssRNA virus, belongs to the family Iflaviridae (Picornavirales) and comprises three variants or genotypes: the first detected genotype A (Bailey and Ball, 1991), genotype B, previously known as *Varroa destructor virus 1* (VDV-1) (Ongus et al., 2004), and genotype C, the most recently discovered although rarely reported (Mordecai et al., 2016). Current opinion is that DWV-genotype A (DWV-A) is merely mechanically transmitted to honey bees, whereas DWV-genotype B (DWV-B) can also replicate inside *V. destructor* and therefore the mite acts as a biological vector for it (Gisder and Genersch, 2021). These subtleties aside, it is important to bear in mind that both DWV-A and DWV-B are virulent when vectored by varroa mites and can lead to colony collapse (McMahon et al., 2016).

In line with the nature of other RNA viruses, DWV is characterised by high rates of mutation and recombination, which potentially result in the development of new variants and recombinants (Moore et al., 2011; Ryabov et al., 2014). The comparative virulence among DWV genotypes is currently unclear. Whilst laboratory experiments demonstrate higher virulence of DWV-B over that of genotype A in adults (McMahon et al., 2016; for field experiments, see also Norton et al., 2021), there is no obvious fitness or mortality differences between genotypes in pupae (Tehel et al., 2019). Initial mild and covert infections transmitted via the oral route are aggravated when the virus is injected directly into the haemolymph/haemocoel of honey bee pupae or adults by the mite. The classic symptom of DWV is the presence of wing deformities in emerging honey bees when these have been infected as pupae (by varroa feeding), although the pathogenesis of DWV remains unclear; high viral titres can also be found in freshly emerging honey bees with normal wings (Tehel et al., 2019; Gusachenko et al., 2020). Honey bees infected as adults clearly cannot exhibit deformed wings because their wings were already fully formed during pupation. The lack of penetrance of the trait 'deformed wings' in freshly emerging honey bees and the lack of other clear DWV symptoms make it difficult to recognise that a colony of honey bees is suffering from a DWV infection. DWV prevalence is typically very high (Genersch et al., 2010; Traynor et al., 2016); potentially all colonies are infected when infested by varroa mites.

2.2.2 Acute bee paralysis virus, Israeli acute paralysis virus and Kashmir bee virus

Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV) and Kashmir bee virus (KBV) are a group of very closely related (+)ssRNA viruses in the family Dicistroviridae (Bailey et al., 1963; for review: de Miranda et al., 2010). All three may cause covert infections and maintain low viral titres. However, all three, like DWV, can be transmitted by varroa mites (Chen et al., 2004; Ball and Allen, 1988); varroa mite transmission serves as an activator of viral replication, leading to acute onset with a dramatic increase in mortality and colony loss (Ball and Allen, 1988).

Typical symptoms of infection by these Dicistroviridae include impaired flight, trembling progressing to paralysis and loss of hair, which gives honey bees a dark appearance. These characteristics, however, can be difficult to notice at the colony level due to the rapid death of diseased individuals. ABPV has been suggested as a cause of high honey bee mortality since the host shift of varroa mites to *Apis mellifera* (Ball and Allen, 1988; Bakonyi et al., 2002). Moreover, IAPV was one of the main suspects of so-called Colony Collapse Disorder (the mass die-off of colonies) that took North American beekeepers and scientists by surprise with a sudden wave of colony depopulation in winter 2006/2007

(vanEngelsdorp et al., 2009). However, these three viruses are so virulent when transmitted by varroa mites that they kill pupae in the pupal stage, where they die un-opened. In the short term, then, these viruses can lead to colony loss. But, in the long term, they are so virulent when vectored by varroa mites that they are not transmitted by infected pupae because pupae die in their sealed brood cells. In effect, these viruses eliminate themselves from a honey bee population (Martin, 2001; Sumpter and Martin, 2004; McMahon et al., 2018) and their prevalence is typically very low (Genersch et al., 2010; Traynor et al., 2016).

2.2.3 Slow bee paralysis virus

Slow bee paralysis virus (SBPV) is a (+)ssRNA virus of the family Iflavirus infecting a variety of bees, managed and wild (de Miranda et al., 2010). Transmission by varroa mites results in high viral titres and subsequent high colony mortality (Carreck et al., 2010). Other routes of transmission result in only covert infections. Affected honey bees exhibit paralysis in two anterior leg pairs 10 days after infection. After a spike in mortality closely following varroa introduction (Carreck, 2005; Carreck et al., 2010), SBPV is now considered a rare virus (McMahon et al., 2015) and has not been detected in a multi-year survey of honey bees in the United States (Traynor et al., 2016).

2.2.4 Chronic bee paralysis virus

Chronic bee paralysis virus (CBPV) is an unclassified (+)ssRNA virus with wide distribution and apparently increasing prevalence (Traynor et al., 2016). It has a distinct set of symptoms comprised of two syndromes that may occur simultaneously in the colony; type 1 syndrome is characterised by trembling, paralysis, distended abdomen and loss of flight ability (crawling). Type 2 syndrome results in loss of hair on the abdomen, which gives honey bees a black, shiny appearance (Dittes et al., 2020a). Moreover, affected honey bees are refused entry to the hive and can even be attacked by healthy hive-mates, which may create an illusion to beekeepers of robbing. The symptomatic similarity to ABPV lessens with the length of host survival; CBPV-affected honey bees can live for several days, whereas honey bees infected with ABPV die already after 1–2 days. Despite its noticeable symptoms, CBPV is rarely thought to result in the collapse of a colony but can impair its performance due to a lack of healthy, capable worker honey bees.

2.2.5 Sacbrood virus

Sacbrood virus (SBV) is another varroa-transmitted (+)ssRNA virus in the family Iflaviridae (Shen et al., 2005). Its name derives from the typical appearance of

infected honey bee larvae which fail to pupate, accumulating fluid within the unshed skin, which resembles a sac. Subsequently, the dead larvae dry out and darken, acquiring a gondola-like shape and forming a scale at the base of the brood cell. Loss of brood at the point of pupation places the colony in a disadvantageous position; not enough new worker honey bees emerge to provide care and supplies (pollen and nectar) for the colony. Covert infections in adult honey bees facilitate the spread of the virus to other larvae through feeding (Beaurepaire et al., 2020). Similar to DWV, SBV is prone to mutation and recombination, which results in the emergence of different variants with altered virulence (Huang et al., 2021b). Despite its global distribution (Beaurepaire et al., 2020) and the damage it inflicts on brood, SBV is not considered a major factor in the loss of *Apis mellifera* colonies.

2.2.6 Black queen cell virus

Black queen cell virus (BQCV) is a (+)ssRNA virus in the family Dicistrovirus that is widely distributed (Beaurepaire et al., 2020) and at high prevalence in honey bees (e.g. McMahon et al., 2015; Traynor et al., 2016). It has a primarily faecal–oral transmission route and is apparently not transmitted by varroa mites. As suggested by the name, BQCV poses a threat to the queen-rearing industry, with high titres responsible for elevated mortality of queen larvae. Following their death, remnants of queen larvae acquire a black colour that stains the queen brood cell.

Although BQCV rarely results in symptomatic infections in managed hives, experimental infection of worker larvae results in high host mortality (Doublet et al., 2015), suggesting that the virus might exert a significant impact on colony health. BQCV, like the other Dicistroviridae ABPV, IAPV and KBV, is highly virulent when experimentally injected into host pupae and adults (Remnant et al., 2019; Al Naggar and Paxton, 2020). Research suggests a connection between infection of BQCV and the Microsporidian *Nosema ceranae* in adult honey bees; mixed infections result in higher mortality of adult workers (Doublet et al., 2015).

2.2.7 Novel viruses

Due to advances in molecular biological methods, and especially next-generation sequencing technologies, myriad novel viruses have been detected in honey bees (Beaurepaire et al., 2020). Although distinguishing between viruses causing active infections and viruses passively present on or within honey bees is problematic, recent discoveries shed light on how abundant and diverse is the world of bee viruses.

2.3 Fungi: Nosema *spp. and chalkbrood*

Nosema spp. are Microsporidia, highly derived spore-forming fungi that are intracellular parasites of animal hosts. The genus contains two highly honey bee-pathogenic species: *Nosema apis*, whose primary host is *Apis mellifera*, and *Nosema ceranae*, whose primary host is *Apis cerana* of south and east Asia but which has jumped host to *Apis mellifera* and is nowadays found across the world (Klee et al., 2007). Note that *N. apis* and *N. ceranae* have recently been reclassified to the genus *Vairimorpha* (Tokarev et al., 2020). Of many possible colony collapse culprits, *Nosema* spp. seconds the varroa mite and its associated viruses in the number of reported cases (e.g. Higes et al., 2008).

Nosema ceranae in *Apis mellifera* originally appeared better adapted to higher temperatures and to dominate in warm climates whilst *N. apis* retained its reign in cool temperate climates (Natsopoulou et al., 2015). More recent evidence suggests that *N. ceranae* may even dominate *N. apis* as a parasite of *Apis mellifera* in Canada's cooler, northern climate (Emsen et al., 2016). Both species are characterised by seasonality, with higher incidence and spore loads reported in the spring. Both parasitise the adult host's ventriculus (part of the gut), resulting in dysentery and within-hive defaecation, though experimental evidence suggests that *N. ceranae* may also infect larvae (Urbieta-Magro et al., 2019). The disease may also progress furtively, with a sudden collapse of the colony. Symptoms such as a distended abdomen and loss of flight ability, which results in crawling behaviour, are often found in colonies heavily infected with *N. ceranae*. The comparative virulence of these two *Nosema* species in *Apis mellifera* varies across studies from slight to moderately higher virulence of *N. ceranae* (Milbrath et al., 2015; Natsopoulou et al., 2016). Nevertheless, both species may have deleterious effects on colony fitness and ultimately lead to its collapse (Higes et al., 2008).

A more recognisable fungal pathogen of honey bees is the spore-forming *Ascosphaera apis*, the causative agent of chalkbrood (Aronstein and Murray, 2010). If spores are ingested by a honey bee larva, it then succumbs to the developing fungus, drying out to form a 'mummy' that seems to be covered in a white or mottled black-and-white 'chalk'. *Ascosphaera apis* is likely omnipresent in *Apis mellifera* colonies, at least in temperate regions, and is thought to break out only under conditions stressful for larvae, particularly if they are chilled below 35°C during development.

2.4 Bacteria: American and European Foulbrood

The two most economically important bacterial diseases of honey bees, American Foulbrood (AFB) and European Foulbrood (EFB), are exclusively diseases of brood (larvae).

AFB, caused by the Gram-positive, rod-shaped bacterium *Paenibacillus larvae*, is a worldwide disease of *Apis mellifera* known for its detrimental effects on colonies during, usually sudden, outbreaks (Hansen and Brødsgaard, 1999; Genersch, 2010). It consists of four genotypes (ERIC I-IV), of which the first two are the most prevalent. Spores, the infectious form of *P. larvae*, are extremely resilient and capable of surviving for 60 or more years under harsh environmental conditions. AFB is highly contagious; less than a dozen spores are required to elicit an infection in a honey bee larva. Upon ingestion, the pathogen germinates and massively proliferates in the midgut. Typical symptoms are observed in capped brood, in which the cap sealing the cell is dark and concave. Excreted proteases decompose the larval body within, which transforms into a brown, colloid-like mass known as the ropey stage (with a fishy or foul smell, hence the name 'foulbrood'). The remnants of the larva then dry, forming a scale that adheres to the floor of the brood cell, called the foulbrood scale. The classic symptoms are often obscured during infection with highly virulent strains (ERIC II-IV) by the rapid death of infected larvae before capping. Dead larvae are removed by worker honey bees and do not form foulbrood scales, producing an irregular brood pattern (so-called 'shotgun' pattern), though this pattern can have a number of other causes, too (e.g. queen infertility). All four genotypes of AFB lead to almost 100% mortality in honey bee larvae, with the time of death varying from 7 to 12 days post-infection. Infection can profoundly affect colony strength and lead to complete colony collapse.

EFB is caused by the Gram-positive bacterium *Melissococcus plutonius*, frequently accompanied by infection with other bacteria: *Enterococcus faecalis*, *P. alvei* and *Achromobacter Euridice*, the latter also commonly occurring in healthy honey bees (White, 1912; Forsgren, 2010). Affected honey bee larvae may die before capping and be removed from the hive or die post-capping, where the contagious material is preserved (and, when fresh, has a foul smell). The disease does not inflict 100% mortality; honey bee pupation, although delayed, may occur and result in the emergence of small, asymptomatic adults which serve as a bacterial reservoir. Sudden outbreaks of EFB disease are often followed by equally sudden recovery. The onset of symptoms may mirror a period of unfavourable weather conditions or lack of food resources. Similar to ABF, if EFB-infected larvae die, they first form a dark colloid-like mass which then dries into a scale. Co-infection with other bacteria may alter the characteristics of the disease, most notably changing the scent of decomposing larvae.

2.5 Trypansomes: Crithidia mellificae *and* Lotmaria passim

Despite growing interest in the Trypanosomatidae of honey bees, the pathogenicity of the two most prevalent members, *Crithidia mellificae* and *Lotmaria passim,*

remains elusive. The former seems well adapted to the warm and acidic gut of adult honey bees, whereas the latter may spill over from other hosts into honey bees (Palmer-Young et al., 2021). These gut-infesting kinetoplasts occur in two forms: flagellated, motile choanomastigote and non-flagellated amastigote. Studies report contradictory results regarding their impact on infected honey bees, although the outcome of disease might be highly dependent on the dose and the phase of the pathogen's growth, which influences its infectivity (Gómez-Moracho et al., 2020; Liu et al., 2020). Few studies have linked trypanosomatids to colony collapse, although this needs further investigation (Cornman et al., 2012; Ravoet et al., 2013). *Crithidia mellificae* and *L. passim* are often found in colonies infected with *N. ceranae* (Runckel et al., 2011), yet the nature of the interaction between these pathogens remains unclear.

2.6 Common pests: the small hive beetle and wax moths

The small hive beetle (*Aethina tumida*), a minor pest of African *Apis mellifera* (for review: Neumann and Ellis, 2008), came into the spotlight after its discovery in US honey bee hives in 1996. Although African honey bees seem to be resistant to infestation, European *Apis mellifera* in temperate climates seem to lack vital behavioural hygienic traits that can either prevent the beetle from entering the hive or facilitate its eradication (Ellis et al., 2004).

The life cycle of the small hive beetle is divided into the within-hive phase, when reproducing adults and wandering larvae feed on honey bee resources (honey and pollen stores), and a phase outside the hive in the soil, where larvae undergo pupation. The beetle can also survive and reproduce outside the hive by feeding on fruit. Even the strongest European *Apis mellifera* colonies seem to be powerless against the voracious beetle, with reports of colony collapse only 2 weeks after infestation.

Two species of wax moth (Lepidoptera and Pyralidae; see Table 1) infest old wax combs, which their larvae consume. They cause considerable damage to combs stored by beekeepers but do not usually represent a problem inside a hive because honey bee workers can effectively defend their nest against moths.

2.7 Measuring disease impact on honey bee colonies (and pollination)

A healthy colony can grow in size, and a large colony with a numerous worker force is likely to have a considerable number of forager honey bees that can act as pollinators. The size of a colony can be measured directly by visually quantifying the number of honey bees, brood, pollen and honey reserves, e.g. using the so-called Liebefeld method (Dainat et al., 2020), which can be

semi-automated through digital photography linked to AI-informed recognition software. Numerous fully automated devices that can operate remotely are nowadays also available on the market that record the weight of a colony and send the information in real time over the internet to a beekeeper's digital device to report on colony health (Rafael Braga et al., 2020). Poor colony growth might suggest disease impact, which can be investigated through veterinary inspection (Dittes et al., 2020b), incorporating the use of molecular methods for pathogen detection so that the cause of poor colony health can be correctly diagnosed and an appropriate remedy deployed.

The regular inspection of honey bee colonies is an obligatory part of beekeeping, all the more so for commercial colonies managed for the pollination of crops. During the swarm season, which usually coincides with the spring/early summer flowering of many crop plants in temperate regions, hive inspections every 9 days are recommended so as to ensure that a colony does not swarm before the next inspection (Hooper, 1976). A hive inspection should include not only a check (i) on whether the colony is about to swarm (e.g. building of queen cells) but also (ii) for the presence of the queen (iii) that there are sufficient reserves of honey and (iv) sufficient space to store additional honey as well as (v) for diseases, i.e. through visual inspection of adult honey bees (for symptoms of viral infection – discoloration, trembling and deformed wings – and for varroa mites), of the brood (for foulbrood, chalkbrood, other fungal and viral diseases), of the stored honey and pollen (for the small hive beetle) and of hive debris on the hive's bottom board. If disease is suspected, a full veterinary inspection can be undertaken (Dittes et al., 2020b).

Across temperate parts of its native range, most colonies of *Apis mellifera* are managed and can be inspected as described above. Tropical parts of *A. mellifera*'s native range maintain large populations of wild colonies (Jaffé et al., 2010). This is also likely true of feral Africanized *Apis mellifera* in Latin America, where the feral population may exceed that of managed colonies even in localities such as the Yucatan Peninsula of Mexico, with one of the world's highest densities of managed *Apis mellifera* colonies (Moritz et al., 2013). It is very challenging to monitor the health of wild or feral colonies because the colony's brood is often protected within a tree trunk or rock face. In such cases, disease presence can be determined by the molecular quantification of pathogens in honey bees collected from the hive entrance or its drones where they assemble for mating, high in the sky (Yañez et al., 2012; Forfert et al., 2016). But measuring disease impact on colony size or performance *per se* (as in the number of exiting forager honey bees) can only be evaluated indirectly by quantifying the flow of honey bees into and out of a colony.

3 The poorly known wild bee diseases

Concern over the decline of pollinators (and pollination) was initially focussed on wild species of insect and vertebrates such as wild bee species, humming birds and bats, and the wild plants they pollinate (Buchmann and Nabhan, 1996). In-depth analysis of museum records subsequently revealed that wild bees have declined in diversity in the Netherlands and the United Kingdom (Biesmeijer et al., 2006). Garibaldi et al.'s (2013) landmark paper, highlighting the important role of wild bee (and other insects) species in the pollination of crops, has only served to draw more attention to the fate of wild bees and their conservation. A recent analysis of records held by the Global Biodiversity Information Facility (GBIF) has revealed that the decline in wild bee species diversity is a worldwide phenomenon (Zattara and Aizen, 2021).

Bumble bees (*Bombus* spp.) are a social subset of ca. 260 of the world's total ca. 20 000 wild bee species that are rather charismatic because of their large size and characteristic, sometimes species-specific colour patterns. Of all wild bee taxa, they have therefore been relatively well studied. Monitoring data reveal that many *Bombus* spp. have declined in range over the past decades (Williams and Osborne, 2009; Cameron and Sadd, 2020), both in North America (Cameron et al., 2011) and in Europe (Nieto et al., 2014).

Another large group of wild bees, the eusocial (colony-forming) and pantropical stingless bees, are also considered important pollinators of numerous crop plants (Heard, 1999). There is a long history of management of some of these species back into antiquity; e.g. archaeological evidence suggests the Maya peoples have managed the Neotropical stingless bee *Melipona beecheii* for well over 2 millennia, and this and many other stingless bee species continue to be managed nowadays (Osterman et al., 2021). Stingless bee populations are thought to be in decline largely through habitat deterioration and destruction (Freitas et al., 2009); their pests and pathogens are less well known and deserve greater scrutiny. Because of the paucity of data on their diseases, we do not address them further in this chapter.

To what extent are pests and pathogens to blame for the decline of wild bees? In contrast to the relatively good knowledge of honey bee pests and pathogens, the disease organisms of wild bees are poorly researched, and there is little knowledge of the impact they may have on wild bee populations (Brown and Paxton, 2009). Exceptions are the few wild bee species that are managed for use in crop pollination: currently eight solitary bee species and nine *Bombus* taxa (Osterman et al., 2021). The scrutiny to which *Bombus* spp. have been subject to basic ecological and evolutionary research has also provided deeper insight into their pests and pathogens, comprehensively reviewed in Schmid-Hempel (1998). Evidence points to the important role of pathogens in wild bee decline (Dicks et al., 2021), particularly in the decline

of bumble bees (Cameron et al., 2011; Arbetman et al., 2017; Cameron and Sadd, 2020).

We now overview the major known pests and pathogens of (i) bumble bees (Table 2) and (ii) other managed and wild bee species (Table 3), which we collectively term solitary bees.

3.1 Bumble bees

Bombus comprises a genus of large-bodied social (or socially parasitic) bee species that are frequent visitors to flowers in temperate and cooler regions of the world (e.g. the Andes of South America), where they are considered important pollinators (Goulson, 2009). In addition, their large size and often

Table 2 Major parasites and pests of bumble bees (*Bombus* spp.)

Disease/common name	Causative agent
Viruses	
Deformed wing virus	Deformed wing virus
Acute bee paralysis virus	Acute bee paralysis virus
Israeli acute paralysis virus	Israeli acute paralysis virus
Slow bee paralysis virus	Slow bee paralysis virus
Black queen cell virus	Black queen cell virus
Fungi	
Nosemosis	*Nosema bombi*
Nosemosis type C	*Nosema ceranae*
Trypanosomatids	
Crithidia	*Crithidia bombi*
Gregarines	
Apicystis	*Apicystis bombi*
Mites	
Locustacaris tracheal mite	*Locustacaris buchneri*
Nematodes	
Sphaerularia nematode	*Sphaerularia bombi*
Hymenoptera	
Parasitoid wasp	*Syntretus* sp.
Cuckoo bumble bees	*Psithyrus* spp.
Lepidoptera: Pyralidae	
Wax moth	*Aphomia sociella*
Diptera	
Conopid flies	Conopidae spp.

Table 3 Some examples of widespread parasites and pests of wild bees

Disease/common name	Causative agent
Fungi	
Nosemosis	*Nosema thomsoni*
Nosemosis type C	*Nosema ceranae*
Chalkbrood	*Ascophaera* spp.
Trypanosomatids	
Crithidia	*Crithidia* spp.
Gregarines	
Apicystis	*Apicystis bombi*
Diptera	
Conopid flies	Conopidae
Bee flies	Bombuyliidae
Satellite flies	Anthomyiidae
Strepsiptera	
Twisted-wing insects	*Stylops* spp.
Hymenoptera	
Nomad bees	*Nomada* spp.
Pests	
Oil and blister beetles	Meloidae

colourful adult patterns have attracted the attention of lay and scientists alike, contributing to the knowledge of their pests and parasites. Major diseases of bumble bees and their respective causative agents are given in Table 2.

3.1.1 The Microsporidia: Nosema bombi *and* Nosema ceranae

Bumble bees are parasitised by a species-specific Microsporidian, *Nosema bombi*, which can be responsible for a drastic reduction in fertility (Otti and Schmid-Hempel, 2007). Its route of infection is faecal–oral, and ingested *N. bombi* spores can colonise the adult host's intestine, Malpighian tubes and reproductive organs, causing diarrhoea, limiting sperm production in males and elevating mortality (Otti and Schmid-Hempel, 2007). Heavily diseased gynes (queen-destined females) and males have scarcely a chance of successful mating.

The honey bee Microsporidian *Nosema ceranae* is known to infect bumble bees, e.g. the common and widespread *Bombus terrestris* (Fürst et al., 2014), although consensus has not been reached yet on whether the detection of *N. ceranae* is mere contamination (the host may act as a mechanical vector) or reflects an actual infection (Gisder et al., 2020).

3.1.2 Widespread Protozoa: Crithidia bombi *and* Apicystis bombi

The trypanosomatid *Crithidia bombi* is a gut parasite of adult *Bombus* spp., transmitted by the faecal–oral route and often rising to high prevalence (Ruiz-González et al., 2012). Although it does not inflict direct negative effects on the reproductive system of its hosts, infection leads to higher mortality of gynes, and their success rates are lower (Rutrecht and Brown, 2008). Furthermore, *C. bombi* can impair the cognitive function of worker bees and reduce foraging effectiveness. *C. bombi* often occurs in combination with other parasites, which further aggravates the effects of infection.

Apicystis bombi is a gregarine parasite of various bee species, with bumble bees considered to be its primary host. Oral ingestion of spores results in colonisation of the gut and fat body. Parasites reproduce in fat cells, causing the organ to lose its cream colour and become hypertrophic (swollen). It is considered a serious disease organism of adult bumble bees; affected gynes have a shortened lifespan, which hinders successful mating and thus colony establishment (Schoonvaere et al., 2020).

3.1.3 Viruses: Deformed wing virus, black queen cell virus and others

In recent years, increasing attention has been given to emerging infectious diseases in wild pollinators, including bumble bees (Fürst et al., 2014; Nanetti et al., 2021). Advanced molecular techniques have led to the discovery of typical honey bee viruses in bumble bees and other wild bees (Table 2), as well as the presence of a range of novel viruses that are seemingly associated with *Bombus* spp. (Pascall et al., 2019). Of the various honey bee-associated viral pathogens, two appear to be the most prevalent in *Bombus* spp., namely DWV and BQCV (Fürst et al., 2014; McMahon et al., 2015). Although not apparently as pathogenic as in their reservoir host *Apis mellifera*, DWV inflicts negative effects on *Bombus* spp. and has been reported to cause overt infection with characteristic 'deformed wing' symptoms (Genersch et al., 2006).

3.1.4 Other Eukaryote pests and parasites

The nematode *Spherularia bombi* is a serious parasite of *Bombus* spp. queens; it is not known to infect worker bumble bees. Queens are exposed to *S. bombi* during winter hibernation in the soil, when juvenile nematodes enter overwintering females and colonise the host's haemocoel, draining host resources during and after hibernation. Infection results in impaired development of the host's corpora allata and ovaries, usually terminating a

queen's reproduction. Affected queens do not find nests after winter diapause but seek new places to overwinter, where they shed large numbers of juvenile-stage *S. bombi* into the soil (Poinar and van der Laan, 1972). Infection with *S. bombi* is common in many *Bombus* spp. queens.

Bumble bees are associated with many mite species that vary in their supposed impact from parasitic (i.e. detrimental to host health) to beneficial (Alford, 1975). One relatively well-characterised mite is *Locustacaris buchneri* (Table 2), a widely prevalent parasite of multiple bumble bee species which is found in the tracheal tubes of both queens and worker bees, where it feeds on the haemolymph of the host (Rutrecht and Brown, 2008). Yet the scarcity of research about *L. buchneri* means that little is known about its true impact on colony fitness and reproduction. This is true for many of the numerous mite species associated with bumble bees.

The parasitic wasp *Syntretus* sp. is considered a rare parasite of bumble bees. Females lay eggs within *Bombus* individuals, thereby developing offspring feed on the tissues of the host, quickly leading to its death. *Syntretus* spp. may infest queens as well as workers, shortening their lifespan, reducing colony performance and potentially resulting in colony failure (Rutrecht and Brown, 2008).

The wax moth *Aphomia sociella* is considered a pest of bumble bee nests, especially those managed in artificial boxes. The ravenous larvae of *A. sociella* feed on various resources of the colony, from pollen and wax to bee eggs and larvae. Heavy infestation can cause the death of the colony due to a lack of stored resources (pollen and honey) and workers.

Conopidae are a group of true flies (Diptera) that lay their eggs inside their hosts, usually other insects; the conopid egg hatches and consumes the host from inside, eventually killing it. Some conopid species specialise in parasitising *Bombus* spp., manipulating their host so that it digs underground to die, where the conopid can more successfully hibernate (Müller, 1994). They clearly exert a cost on host populations which can be quite profound in temperate regions as summer progresses, with up to 47% of host workers infected in one study (Schmid-Hempel et al., 1990).

3.1.5 Social parasites (cuckoo bumble bees of the subgenus Psithyrus*)*

Social parasitic bumble bee species (genus *Bombus*, subgenus *Psithyrus*; also known as cuckoo or inquiline bumble bees) usurp nests of other *Bombus* species (termed 'true' bumble bees), often killing the host queen; host workers are thereby enslaved and provision the sexual offspring of the social parasite. Though socially parasitic *Bombus (Psithyrus)* spp. could theoretically regulate host populations, evidence from multiannual records in Great Britain suggests

little temporal fluctuation in social parasitism (Antonovics and Edwards, 2011). In New Zealand, where only true bumble bee species (but not their social parasites) have been introduced, spring foundress queens are encouraged to nest around crop fields requiring pollination by offering them underground nesting chambers (Donovan, 2007). The efficacy of this method of boosting *Bombus* colonies for crop pollination may be substantively reduced elsewhere in the world where cuckoo bumble bees are found, e.g. northern temperate regions, because of frequent nest usurpation by cuckoo queens.

3.2 Solitary bees

Under this heading, we include the managed solitary bees, of which 22 species are or have been trialled for pollination management (eight species are currently in use; Osterman et al., 2021), and all other wild bee species are not subsumed within honey bees and bumble bees. This includes ca. 20 000 species of bee, 10% of which are not solitary but possess some degree of sociality and between 10% and 30% of which are cleptoparasitic (brood parasitic) on other wild bee species (Wcislo, 1987; Danforth et al., 2019).

Though the recent review of solitary bees by Danforth et al. (2019) is very informative on solitary bee pests and parasites, we still largely lack detailed information on their impact on host populations, with the most detailed knowledge derived from the foremost commercial solitary bee pollinator, *Megachile rotundata* (Pitts-Singer and Cane, 2011). Other knowledge on solitary bee parasites is largely derived from autecological studies of a single host species at a single field site (e.g. Paxton et al., 1996). Major diseases of solitary bees and their respective causative agents are given in Table 3.

3.2.1 Fungi: Microsporidia and chalkbrood

Microsporidia have been detected in various solitary bees (Grupe and Alisha Quant, 2020; Martínez-López et al., 2021), although their effects on fitness and mortality are poorly researched. Artificial infection of *Osmia bicornis* larvae with *N. ceranae* resulted in poor rates of infection, but treated larvae suffered apparent survival costs (Bramke et al., 2019). The Lepidopteran parasite *Nosema thomsoni* has been found in solitary bees (Ravoet et al., 2014; Schoonvaere et al., 2018), but we lack studies providing an in-depth analysis of its course of infection. Genetic examination of museum specimens of the hoary squash bee (*Eucera pruinosa*) revealed the presence of *Nosema* sp. in over a decade-old pinned specimens (Vaudo et al., 2018). The North European *Andrena scotica* (synonym *carantonica*) harbours the Microsporidian *Antonospora scoticae* (Fries et al., 1999); there is likely a diversity of yet-to-be-discovered Microsporidia within other solitary bee species.

A prominent fungal disease of wild bees is chalkbrood, caused by *Ascosphaera* spp. and closely related to *A. apis* that infects honey bees. Wild bees, especially those of the family Megachilid, are known to be susceptible to *Ascosphaera* spp. infection. Chalkbrood spores are ingested by a larva with stored brood food (the 'bee bread' deposited by its mother bee in an offspring's natal cell). Spore germination, penetration and subsequent growth of the fungus inside the larval host's body cavity cause lethal mechanical and enzymatic damage to the larva that becomes swollen and which, after drying out, resembles a 'mummy' of chalk-like consistency. The high pathogenicity of *Ascosphaera* spp. can lead to the death of 60% of brood. It is therefore a serious pathogen of commercial rearing facilities of *Megachile rotundata* (Richards, 1984).

3.2.2 Other microparasites (those not visible to the naked eye) of wild bees

Two *Crithidia* spp. have been found in solitary bees: *Crithidia mellificae* (reservoir host: honey bees) in *Osmia cornuta* and *Crithidia bombi* (reservoir host: bumble bees) in both *Osmia lignaria* and *M. rotundata*. Similar to their impact on their reservoir hosts, trypanosomatid infections tend to be mild and only subtly affect the mortality of solitary bee hosts (Figueroa et al., 2021).

Infection by *Apicystis bombi* has been documented in *Osmia bicornis*, considered a model solitary bee species; infected individuals, both females and males, show significantly reduced rates of survival, and sublethal effects might include compromised mating and reproductive success (Tian et al., 2018).

We know less about other solitary bee microparasites, such as bacteria, and therefore of their role as drivers of host decline. In some instances, for example, the microorganisms associated with the bee bread provisions of larvae, these microorganisms may play a positive and important role in protecting the provisions from decay and in supporting larval nutrition (Dharampal et al., 2019; Steffan et al., 2019). Increasing awareness of the viruses of honey bees has, though, prompted investigation of the role of these same viruses in the decline of solitary bees, revealing the presence of many of them across the community of flower visitors (Nanetti et al., 2021). We take up this topic more fully below (section: disease transmission, spillover and spillback), though note here that definitive evidence that these 'honey bee' viruses are parasitic (i.e. reduce host fitness) is in many instances lacking.

3.2.3 Macroparasites (those visible to the naked eye)

Close investigation of a solitary bee species often reveals a rich diversity of symbiotic organisms, some parasitic, some potentially beneficial. A case

study of one solitary bee, *Andrena scotica*, revealed adults to be parasitised by conopid flies (Diptera, Conopidae), Strepsiptera, generalist parasitic mermithid nematodes and diplogasterid potentially beneficial nematodes in intersegmental glands whilst brood cells were parasitised by bee flies (Diptera, Bombyliidae), satellite flies (Diptera, Anthomyiidae), oil or blister beetles (Coleoptera, Meloidae) and cleptoparasitic nomad bees (*Nomada marshamella*; Hymenoptera, Anthophila) (Paxton et al., 1996). This rich assemblage of pests and parasites associated with this one solitary bee species, many with highly specialised life histories tied to just one or a few host bee species, is by no means unusual (Danforth et al., 2019). Though up to 40% of adults were parasitised by deadly conopid flies and a further 10% of brood cells (offspring) had been replaced by nomad cleptoparasitic bees (Paxton et al., 1996), it is unclear whether these parasites in any way regulated host *A. scotica* population dynamics (and therefore provision of pollination). A recent review of the literature (Danforth et al., 2019) comes to the same conclusion; i.e. we lack the data to know whether pests and parasites cause the decline of solitary bees.

3.3 Assessing impacts of disease on bumble bees and other wild bee species

Honey bees are eusocial, living in perennial colonies that are usually maintained in a beehive and managed by a beekeeper, features facilitating their inspection and assessment of the impact of pests and pathogens on them. The same may be said for managed (commercial) bumble bee colonies. For wild bees, most of which are solitary, with a single generation per year (Michener, 1974), and nesting in hidden cavities above or below ground, assessment of the impact on their populations of pests and diseases is all the more complicated.

One option to assess the impact on them of disease agents is to undertake long-term monitoring of their populations coupled with an independent assessment of their pests and parasites, including the use of molecular techniques to assay microparasites (as for honey bees: Dittes et al., 2020b). Multi-generation (multiannual) monitoring of wild bees through destructive sampling, e.g. using pan traps/bowls, though costly, is a standard technique for monitoring wild bee species (Westphal et al., 2008) that, from a cost-benefit perspective, pays its way (Breeze et al., 2021), though is not without its critics (Tepedino and Portman, 2021). A downside is that this approach is merely correlational; it can inform about changes in the population size of wild bees and even about the association of a change in population size with a pathogen, but it cannot demonstrate the cause of a decline.

A case study of *Andrena scotica* and its novel Microsporidian *Antonospora scoticae* reveals some of the problems (for details of the study, see Paxton et al., 1997, supplemented by personal observation, RJP). At one nesting aggregation

at Törnbottens Stugby on Öland in South Sweden, over 85% of adults emerging in 1995 were infected with *A. scoticae*, some with over 10^9 spores concentrated in the bees' fat bodies. Nesting females were generally uninfected or with a low spore count, suggesting that *A. scoticae* exerts a heavy fitness cost on hosts. Though the population of this wild bee species at Törnbottens Stugby was studied by RJP over 5 intensive years of fieldwork (1993–1997), formal estimate of its size by mark-recapture was only undertaken in 1994 and 1995, when the population comprised ca. 5000 females; however, by 1997 the field site was devoid of *A. scotica* (RJP, unpublished data). The Microsporidian could have caused the death of the Törnbottens Stugby population of *Andrena scotica*, but it is not possible to exclude other factors such as inclement weather during a critical phase of the life cycle, either alone or in combination with the Microsporidian; correct diagnosis of a decline is necessary to devise effective remedies to restore a population. As the Microsporidian was at the time an undescribed species infecting a little researched host that nests underground, it was difficult to conceive of control measures that could have rescued the population from extirpation. Moreover, the Microsporidian was seemingly host-specific; monitoring of all wild bees by pan-trapping might not have alerted to a decline of *A. scotica*, or its decline might have been ignored as an idiosyncrasy of the species (which is most likely the case).

Cutting the Gordian knot of demonstrating causation requires experimental studies, but these are difficult with non-model species such as the vast majority of wild bees. In lieu of experimentation, next-generation sequencing (NGS) approaches might provide a high-resolution means to infer causation (Grozinger and Zayed, 2020); for example, Tsvetkov et al. (2021) have recently demonstrated that the declining North American *Bombus terricola* often harbours the parasites *Nosema ceranae, Crithidia bombi, Lotmaria passim* as well as the viruses BQCV and SBV, whereupon bees were also associated with an up-regulation of genes employed in defence against these parasites.

4 Disease transmission, spillover and spillback

Many routes of transmission exist for pathogens and pests of the honey bee that are possible because of the host's eusocial colony organisation and perennial lifecycle (Schmid-Hempel, 1998). Intraspecific transmission can occur horizontally through within-hive interactions such as trophallaxis, grooming, cannibalism and direct contact augmented by the constricted space within a hive. Transmission between colonies occurs via hive robbing (honey bees from one colony removing resources from another, usually adjacent, colony), by bee drifting (a returning forager entering the wrong colony), at shared flower resources as well as by human-induced contamination of beekeeping tools, clothing, hive frames, honey or beeswax. Introduction of an infected queen to a

colony may result in the horizontal spread of a disease agent among other colony inhabitants and its vertical spread to offspring, with the route of transmission of considerable significance for disease epidemiology (Schmid-Hempel, 1998; Fries and Camazine, 2001). Though differences in apiary size (number of honey bee colonies per apiary) have been shown theoretically to have little impact on disease prevalence and epidemiology (Bartlett et al., 2019), empirical evidence suggests that disease prevalence increases with colony density, presumably because of greater opportunities for between-colony (horizontal) transmission (Forfert et al., 2016). Maintaining colonies in hives may itself lead to persistent infection within the colony (Bartlett et al., 2021).

Less is known about the transmission and epidemiology of the pests and parasites of wild bees. An exception is *Crithidia bombi*, which parasitises multiple bumble bee species (Ruiz-González et al., 2012) and has been demonstrated to be transmitted through shared use of the same flower (Durrer and Schmid-Hempel, 1994). Even hover flies may pick up *C. bombi* from infected flowers and transport it onwards, acting as mechanical vectors (Davis et al., 2021). This example highlights the important role that flowers play as transmission hubs for a range of parasites across a diversity of pollinator (flower-visiting) insect species (McArt et al., 2014; Graystock et al., 2015), in which one insect deposits (by defecation, by regurgitation, by other forms of excretion or by mere physical proximity) a pathogen propagule onto a flower and from which a subsequent insect visitor to the same flower acquires the pathogen (Fig. 2). It also brings to

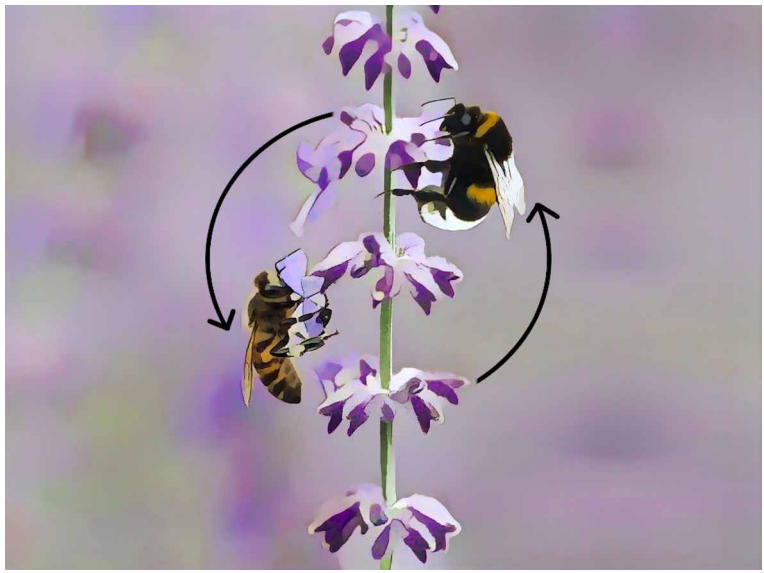

Figure 2 Two or more bee species may sequentially visit the same flower, offering ample opportunity for pathogen transmission (spillover).

the fore the question of whether a transmitted pathogen leads to an infection in a recipient host, with the host potentially acting as a biological vector (in which case the host is defined as being competent), or whether the transmitted pathogen merely resides in or on a host without replicating, with the host acting either as a form of horizontal vector or as a dead-end host. These differences are important because they impact the parasite's epidemiology and evolutionary trajectory as well as host population fitness (McMahon et al., 2018).

The global distribution of managed honey bees, even in otherwise non-native regions (Hung et al., 2018), their increasing abundance (Osterman et al., 2021) and the ubiquity of many of their parasites (see Section 3.1.3) have consequences for wild pollinator species. Pathogen spillover (interspecific disease transmission) from honey bees as reservoir hosts to wild bee species as recipient hosts has been increasingly researched because the potential negative effects of honey bee pathogens could undermine the already fragile health status of wild bee species (Fig. 3).

Spillover has been documented for several honey bee pathogens (e.g. Ravoet et al., 2014; Graystock et al., 2020), with increasing attention now being given to viral spillover. Experimental evidence using flight cages first demonstrated that IAPV can be transmitted from honey bees to bumble bees (Singh et al., 2010). Correlational evidence strongly supports the ongoing viral transmission of DWV and BQCV from honey bees to bumble bees in

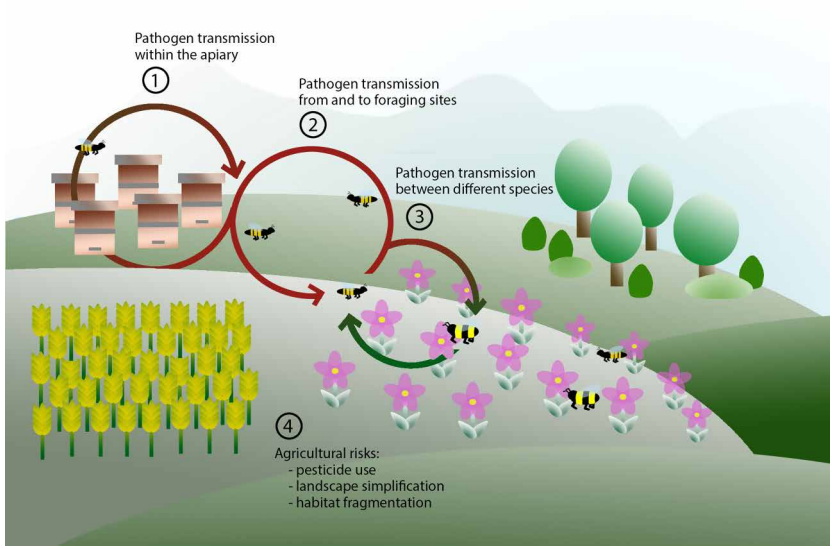

Figure 3 Schematic landscape describing pathogen spillover and spillback among honey bees and wild bee species at shared flowers, a phenomenon that may be promoted by agricultural practices and other global change pressures.

Europe (Fürst et al., 2014; McMahon et al., 2015; Manley et al., 2019), where the honey bee is native, as well as in North America (Alger et al., 2019; Pritchard et al., 2021), where the honey bee is not native. Honey bee viruses have been detected in a large number of wild bee species (Nanetti et al., 2021), suggesting that viral spillover may be a very common phenomenon. Though evidence for the impact of DWV on bumble bees is equivocal (cf. Fürst et al., 2014; Tehel et al., 2020), this and other RNA viruses have high adaptive potential (Holmes, 2009), thereby posing a serious threat to wild pollinator health. Viruses that have spilled over from a reservoir to a novel host may subsequently spill back to the original host with altered virulence due to adaptation to the novel host environment, though we currently lack evidence for this phenomenon.

Spillover is not solely attributable to the honey bee as a reservoir host. *Apicystis bombi*, a pathogen of bumble bees, has been increasingly found in managed honey bee colonies (Plischuk et al., 2011; Schulz et al., 2019), though the consequences of the spillover of *A. bombi* for pathogen epidemiology and recipient honey bee host fitness remain open conjectures. Pathogens may also spill over from managed bumble bees used for commercial pollination to wild bumble bees, both in North America (reservoir host *Bombus impatiens*: Colla et al., 2006) and Europe (reservoir host *Bombus terrestris*: Murray et al., 2013). In Chile and Argentina, the deployment of commercial Eurasian *B. terrestris* in the past 3 decades is associated with the introduction of Eurasian variants of bumble bee parasites (*A. bombi* and *C. bombi*), which have likely caused population collapse of the native giant bumble bee of South America, *Bombus dahlbombi* (Arbetman et al., 2013; Schmid-Hempel et al., 2014).

Hopefully, it is clear from this section that pathogen spillover is a major determinant of the health of pollinator populations and therefore the provision of the ecosystem service of pollination. It needs to be seriously considered in the management of pollinators. Before transporting a pollinator species, whether native or not, to a crop requiring pollination, one should evaluate the risk of pathogen spillover. Encouragement of local pollinators that already exist in or around a crop ought to be favoured in the first instance as a more sustainable approach to pollination service provision.

5 Defence mechanisms of bees

Though challenged by a wide diversity of pests and pathogens, large and small, bees have evolved an impressive array of response mechanisms to fight their foes. We divide these into 'individual immunity', mechanisms possessed by each individual and which function equally in social and solitary insects, and 'social immunity', describing the behavioural and physiological traits employed

by a group of two or more social bees to fight pests and pathogens (Cremer et al., 2007).

5.1 Individual immunity

Despite their vulnerability to infectious diseases, bees have developed well-functioning pathogen defence responses, comprising mechanical, behavioural and physiological (immune) mechanisms (Evans and Spivak, 2010). A hard exocuticle with a waxy epithelial covering provides insects with a barrier against mechanical damage and limits entry points for pathogens. Bees employ a range of behaviours to defend themselves against their macroparasites. A well-studied case in point is the interaction between bumble bees and conopid fly parasitoids (Müller and Schmid-Hempel, 1993). A worker bumble bee normally spends the night in its warm hive. If a concopid fly has laid an egg within its abdomen, it is more likely to spend the night outdoors at a lower temperature, which arrests the development of the conopid egg/larva and may even kill it, thus extending the bumble bee's lifespan. In a wicked twist of coevolution, if the conopid larva does succeed in developing inside its bumble bee host by eating it from the inside out, it manipulates its host to dig itself underground just before host death, where the conopid can pupate with a higher probability of survival than if the bumble bee had died in the open.

Although bees (like other invertebrates) do not possess an adaptive immune response as found in all vertebrates including ourselves, their innate immune mechanisms provide a wide range of physiological and other molecular defences that protect them from bacteria, fungi and viruses. For example, melanisation, encapsulation, nodulation and phagocytosis are performed by haemocytes in response to septic or aseptic trauma. These cellular responses are rapid, in contrast to the humoral mechanisms which deploy the production of antimicrobial peptides (AMPs). In detail, canonical innate immune defence employs pathogen recognition and signalling cascades (e.g. Toll, Imd, JAT/STAT and JNK pathways), leading to the production of AMPs to fight against bacteria and fungi by altering the structure of a pathogen's cell membrane and leading to its destruction. Haemocytes and AMPs are produced by the bee's fat body, a factory for various immune-related compounds (Evans et al., 2006).

For fighting viruses, the best-described dimension of the honey bee's innate immune system is the RNAi (RNA interference) pathway (McMenamin et al., 2018), which is also found in bumble bees (Barribeau et al., 2015) and likely all bee species. In essence, the host cell recognises and then degrades dsRNA (double-stranded RNA), thereafter degrading (knocking down or silencing) other intracellular RNA with the very same sequence, including RNA viruses.

The honey bee's gastrointestinal tract harbours a rich diversity of commensal microorganisms dominated by five major bacterial phylotypes that are very consistent within and between hives (Kwong and Moran, 2016). Their social life enables all individuals in a hive to become colonised by the same gut flora, which are thought to have a major beneficial impact on the health and fitness of the individual by maintaining an optimal gut environment, enabling proper nutrition, detoxification and stimulation of immune pathways (Emery et al., 2017) as well as defending the bee against pathogens (Forsgren et al., 2010). Solitary bees also possess a rich gut microbiota, though it is much more variable in composition compared to that of social bees (Martinson et al., 2011).

5.2 Social immunity

Many behaviours of social bees are considered to be acts of collective defence against pathogens. Through grooming, honey bees can remove external parasites from either their own body or other colony members' bodies (Pritchard, 2016). Collective 'balling' of predatory wasps allows the Eastern honey bee (*A. cerana*) to kill (through heat exhaustion) yellowjackets and hornets much larger than an individual honey bee (Ono et al., 1995). Antimicrobial excretions can be passed to other honey bees together with food via trophallaxis to protect all colony members. This practice is a double-edged sword as pathogens that have escaped the effects of AMPs may also spread rapidly within the colony (Naug, 2008). Hygienic behaviour deploying removal of parasitised brood from the hive can help with parasite management, especially in reducing *Varroa destructor* invasion (Wilson-Rich et al., 2009). When faced with severe conditions (shortage of food and extreme temperatures), bees can cannibalise their brood to provide nutrition for the colony and limit the spread of pathogens (Schmickl and Crailsheim, 2001). Reducing pathogen dispersal among brood can also be achieved through social fever, when adult bees raise the temperature of the brood above the tolerance limit of pathogens, e.g. *Ascosphaera apis* (Starks et al., 2000). Finally, infected individuals may abandon the colony in the act of self-sacrifice to prevent the transmission of pathogens to other nestmates (Schmid-Hempel, 1998).

6 Synergies with other risk factors

The interaction between a host bee and its pests and pathogens is, in many instances, context-dependent, being modulated by, for example, host resource supply, human activities such as agricultural intensification and the associated use of pesticides and climate. Global change pressures may therefore drive altered pest and pathogen epidemiology and virulence (Proesmans et al., 2021).

Nutrition represents an important factor that can alter the host-parasite relationship. Honey bee hosts with adequate resources or an intact microbiome are better able to tolerate viral pathogens than hosts with a depleted microbiome (Dosch et al., 2021). Under a scenario of agricultural intensification, floral scarcity in farmland threatens the provision of an adequate nutritional supply (Jha and Kremen, 2013), which is needed for the maintenance of a well-functioning immune system (Smith, 2007). A mass-flowering crop that increases floral abundance may alleviate nutritional shortage within the agricultural landscape, but it may also lure in pollinators, creating a perfect opportunity for horizontal pathogen transmission (spillover), increasing the prevalence of pathogens among the community of bees (Piot et al., 2019). Alternatively, a superabundance of flowers may dilute pathogens, reducing transmission and prevalence (Graystock et al., 2020).

Pesticides impair a honey bee's detoxification capacities and immune response, increasing its susceptibility to pathogen infection (di Prisco et al., 2013). In the laboratory, synergy is seen between *Nosema* spp. and neonicotinoid insecticides used in farming, aggravating the negative effects of both agents on honey bees (Doublet et al., 2015). A recent, comprehensive review of the impacts of pesticides on bees (Siviter et al., 2021) highlights synergistic interactions between two or more pesticides on host health; the data on pest/pathogen-pesticide interactions on bee health, whilst generally suggesting an additive or synergistic impacts, currently do not permit a definitive statement. This is an area that deserves great research attention across a range of managed and wild bee species.

Climate change, similar to agricultural intensification, may lead to the loss of natural habitats for wild and managed pollinators, depriving them of food resources and niches (Walter, 2020), with knock-on consequences for the impact of pests and pathogens on bees. Climate warming may allow for the expansion of exotic pathogens into new regions, e.g. *Nosema ceranae* (Natsopoulou et al., 2015), which may be especially dangerous due to the lack of adaptation of naïve species to the new threat. Heat *per se* has an ambivalent role in pathogen susceptibility, on the one hand, potentially lowering host immune response (Laughton et al., 2017), on the other, lowering the survivorship of some pathogens (Zaragoza-Trello et al., 2021).

7 Prevention of diseases

Disease prevention in managed pollinators poses many challenges. One is their unrestricted movement from one crop to another for pollination. For example, the transport of ca. 2 million honey bee colonies from across the United States to California every February for almond pollination risks rapid transmission of pests and pathogens within the stock of US honey bees (Cavigli et al., 2016).

For honey bees and potentially also other bee species, drifting of workers from one colony to the next or the robbing by one colony's members of another, diseased colony's resources represent very efficient routes of transmission. Another challenge is the shared use of flowers such that pathogens can easily be transmitted within and between bee species. A honey bee forager visits hundreds to thousands of flowers every day, which creates ample opportunities for pathogen transmission. Since it is not possible or sensible to restrict foraging, disease prevention must be limited to the management or treatment of the hive itself. For social species such as honey bees, a third challenge is their size and dynamism; it is difficult to treat effectively all individuals of a colony that may comprise over 40 000 individual worker honey bees, each replaced on a 3- to 6-week schedule.

Typical disease prevention for honey bees therefore aims at certain hygienic beekeeper standards (for one beekeeping hygiene manual, see: Tyl et al., 2014). In addition, low honey bee density (Forfert et al., 2016), small hive size and periodic brood breaks may further aid in disease control and diminish disease spread, though we note that maintaining a small hive size (and a small number of workers) likely diminishes the pollination potential of a honey bee colony and its likelihood of surviving the winter in northern climates like experienced in Canada.

Given the risk of disease spillover from managed to wild bumble bees (Colla et al., 2006; Murray et al., 2013), some countries or regions have imposed restrictions on the importation of non-native species or subspecies of *Bombus*, e.g. the United Kingdom and the Canary Isles. It is unclear whether these retrospective measures have prevented disease spread, though evidence from South America clearly points to the introduction of the non-native *Bombus terrestris* as the cause of disease spread among native bumble bees (Arbetman et al., 2013; Schmid-Hempel et al., 2014).

Every year, US alfalfa farmers import cocoons of *M. rotundata* from further north (Canada), where *Ascosphaera* spp. are absent. The widespread proliferation of chalkbrood in US facilities does not permit a sustainable US *M. rotundata* industry. This is not so much 'prevention' as 'mitigation' by the annual replacement of diseased bees.

Next, we address additional topics in disease prevention, though note that they largely relate to the honey bee and its diseases.

7.1 Varroa control

Given the importance of the varroa-virus nexus for honey bee health, it is understandable that the most commonly applied measures to control honey bee diseases are associated with the reduction in varroa mite infestation. Since *V. destructor* is a vector and 'virulence amplifier' of multiple viruses, managing

varroa infection in the hive contributes greatly to maintaining the vitality of the colony. Purchasing varroa-free colonies is not a long-term solution as honey bees will eventually acquire the mites from other hives through robbing and drifting.

Chemicals (miticides) are frequently used to control varroa and are often unavoidable as the infestation progresses and may lead to a collapse of the colony if untreated after 3–4 years (e.g. McMahon et al., 2016). The choice of chemical substances used to control mite infestation is vast, ranging from essential oils, propolis extracts and algal preparations to synthetic miticides (Box 1). Beekeeping management may also help reduce the number of varroa mites in a hive; examples include artificial swarming and the removal of capped drone brood. Varroa control is a vast topic comprehensively described by Jack and Ellis (2021).

Box 1. Differences between 'hard' and 'soft' chemicals used to control varroa mites in honey bee colonies

Beekeepers often resort to chemical control of *Varroa destructor* mites in their honey bee (*Apis mellifera*) colonies because it is often difficult to otherwise reduce mite numbers. If not controlled, varroa mites (and the viruses they transmit, especially the deformed wing virus) lead to the collapse and death of the colony. Two classes of chemicals ('hard' and 'soft') are often employed in varroa control, each with their merits and demerits, which we bullet point here.

'Hard' chemicals:

- Synthetic compounds
- Examples include amitraz, coumaphos, flumethrin, tau-fluvalinate
- Advantages
  - Well-established efficacy
  - Convenience to use
  - Low in cost
  - Climate-independent efficiency
- Disadvantages
  - Lead to mite resistance of a chemical
  - Negative effects on bee reproduction and memory
  - Accumulation in bee products, potential to enter the human food chain

'Soft' chemicals:

- Compounds found naturally in the environment, even in the hive
- Thymol, formic acid, oxalic acid, lactic acid

- Advantages
  - No resistance yet reported
  - Impact varroa on adult bees but also in capped brood cells (formic acid)
  - Minimal accumulation in bee products
- Disadvantages
  - Limited use in temperate regions (e.g. formic acid at lower temperatures)
  - Use limited to broodless conditions (e.g. lactic and oxalic acids)
  - Lower and poorly quantified efficiency compared to 'hard' chemicals

See Jack and Ellis (2021) for a complete review of methods of varroa control in the beehive.

7.2 Viruses

The prevention of viruses in honey bees, at least those transmitted by varroa mites, is best achieved by controlling varroa mites (see above). For viral diseases of honey bee brood, namely those caused by SBV and CBPV, a break in brood rearing, e.g. by generating an artificial swarm or by re-queening the colony, is an effective means of reducing viral titres and relieving or eliminating overt disease symptoms (Dittes et al., 2020b). For viruses in wild bees (bumble bees and solitary bees) that spill over from honey bees, two plausible options are to decrease honey bee density (by relocating honey bee hives) and increase flower density, which is likely to diminish the force of infection (the probability that a honey bee and a wild bee visit the same flower); the latter effect has been inferred for protozoan parasites (Graystock et al., 2020). However, we lack direct tests of their effectiveness.

Given that bees, like other invertebrates, lack an adaptive (acquired) immune system, they cannot be 'vaccinated' in the way that humans can so as to fight viral disease. As described in Section 5, they do, though, possess an innate immune system, which includes the RNAi (RNA interference) pathway. By administering dsRNA with a specific sequence, one can efficiently knock down a gene's expression or, in the case of RNA viruses, induce host cells to destroy viral RNA. This approach has been used with apparent success to control a range of pathogens in honey bees, including varroa mites and DWV (Desai et al., 2012; Leonard et al., 2020), though successful dsRNA administration requires refinement and its efficacy in the field has not yet been demonstrated (Paxton, 2020). RNAi does, though, offer a breakthrough in the control of pernicious viral diseases such as DWV.

Chemical control of bee viruses such as DWV is an additional approach (e.g. Tang et al., 2021), though contamination of honey (for honey bees) and efficacy in the field remain open questions.

7.3 Breeding

Some honey bee colonies express higher tolerance to varroa mites or viruses, withstanding invasion without treatment (Locke, 2016). Resistance or tolerance characteristics of honey bees can be maintained or enhanced by targeted breeding (Rinderer et al., 2010). Promoting colonies that display resistance against or tolerance to parasites and express social immunity traits, such as hygienic behaviour and grooming that are known to be genetically determined, is highly desirable in order to improve the intrinsic defence mechanisms of honey bees (Rothenbuhler, 1964; Arechavaleta-Velasco et al., 2012).

7.4 Novel methods of diagnosis and treatment

With rapid progress in molecular biology, research on bee diseases has advanced quickly, giving rise to novel methods of disease prevention and treatment. One possible option for varroa control is the use of gene drives (Faber et al., 2021). Promotion of genes reducing mite reproduction, survival rates or resistance to miticides would help immensely with effective varroa management, although successfully establishing gene drive in varroa population poses multiple obstacles, such as mite inbreeding and haplodiploidy, to be first overcome.

Extensive research into the honey bee microbiome has uncovered the positive effects of lactic acid bacteria (LAB) on honey bee health (Royan, 2019). Additionally, LAB has been reported to inhibit the growth of *P. larvae*, the causative agent of AFB (Forsgren et al., 2010); their inclusion in honey bee nutritional supplements may be a powerful means of enhancing honey bee immune defence.

Bee disease diagnosis poses another challenge; two major problems are the great number of asymptomatic individuals in an affected honey bee colony and the very circumstantial switch to overt infection that precludes proper visual detection of a disease. Moreover, due to the small size of individuals, bees often need to be sacrificed in order to identify efficiently an internal pathogen. To circumvent this latter problem, several means of non-lethal collection and detection of infectious agents from individual honey bees have been proposed, with notable successes in haemolymph collection (in which disease agents can be detected by molecular approaches) from antennae (Borsuk et al., 2017) and via a needle (Huang et al., 2021a).

Insight into disease pathogenesis is crucial in order to establish methods of treatment and prevention. A big step for honey bee viral research was the recent

construction of DWV tagged with a green fluorescent protein (GFP) (Evans et al., 2021). Bees infected with GFP-DWV can be non-lethally screened in real time via camera recording. Fluorescence-marked viruses open new opportunities for research on this pathogen, such as DWV propagation, tissue specificity, host species specificity, interference with other pathogens and many others.

8 Future trends

The charm of bees and their various, often peculiar, methods of immune defence does not negate the fact that they suffer considerable annual losses from pathogens. The most important disease agents of honey bees are varroa mites and the viruses they transmit. Both are at high prevalence and act synergistically (Nazzi et al., 2012), often resulting in the death of affected colonies. Despite the lower number of known wild bee parasites, wild bees also face grave consequences from well-established pathogens and others spilling over from heterospecifics.

Notable progress in the detection of bee pathogens has brought to light the desperate need for new and effective methods for disease control, e.g. to treat honey bee viruses, especially DWV. Well-known disease-causing agents and newly emerging pests and pathogens, in interaction with climate change and agricultural practices, reduce pollinator survival. On a more upbeat note, it is important to emphasise that the initial dramatic effect of an emerging exotic pathogen may lessen with time as the host adapts and evolves greater resistance or tolerance towards the disease agent.

Human interference in honey bee colony life (disease treatment, food supplementation, swarm prevention and breeding focussed on production traits) may impede the process of natural selection and leaves the managed hive vulnerable to infestation and disease outbreak. Moreover, pathogens do not remain evolutionarily stagnant, undergoing change through mutation and selection, not only adapting to new hosts but also to new vectors. This scenario may be tempered, however, by human practices aimed at appropriate conservation efforts and bee management that aim at reducing pathogen virulence.

Together with changes in hosts and parasites, future research and monitoring techniques may be better able to capture data on honey bee diseases in real time (Rafael Braga et al., 2020). The use of NGS (genomics and metagenomics) marks a new standard in bee molecular research and may prove invaluable in elucidating the genetic diversity of bees, their parasites and their interactions as drivers of their diversity (Tsvetkov et al., 2021). The obscure pathogenesis of many honey bee diseases is now being challenged by means of reverse genetics (Gusachenko et al., 2020). Novel methods of disease

treatment arising from recent research, such as the use of LAB, RNAi and gene drive, promise hope for limiting pathogen spread and mitigating bee decline.

9 Where to look for further information

9.1 Honey bees

COLOSS (https://coloss.org) is a worldwide network of researchers interested in the health and well-being of honey bees, with numerous working groups ('task forces') addressing pressing issues in honey bee health. Membership is open to all. As well as organising two conferences per year, they have co-published with the International Bee Research Association (https://ibra.org.uk) the *BeeBook*, a compendium of (open access) chapters addressing all matters related to honey bees and bee research; volume II, titled *Standard Methods for* Apis mellifera *Pest and Pathogen Research*, contains chapters on each major taxon of a honey bee disease-causing organism (https://coloss.org/beebook/volume-2/).

9.2 Wild bees

Schmid-Hempel's (1998) *Parasites in Social Insects* is a tour de force of the major pests and pathogens of social insects, with much emphasis on bumble bees, whilst Richards' (1984) pamphlet on managing the alfalfa leafcutter bee (*Megachile rotundata*) reviews the diseases and control measures for this species, the most important managed solitary bee. The literature on the pests and pathogens of other wild bees is scattered, though Danforth et al. (2019) provide an excellent overview in their book *The Solitary Bees*.

The International Union for the Conservation of Nature (IUCN) has a wild bee specialist group (WBSG) tasked with the development of best practice guidelines for the implementation of conservation measures for wild bees (https://www.iucn.org/ssc-groups/invertebrates/wild-bee/aims-wbsg). Initiated in 2021 and set to run till 2025, at the time of writing this chapter, WBSG's website promises to provide details on how best to conserve wild bees.

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