Polyandrous bee provides extended offspring care biparentally as an alternative to monandry based eusociality

Michael Mikát[a,1], Lukáš Janošík[b], Kateřina Černá[a], Eva Matoušková[a], Jiří Hadrava[a,c], Vít Bureš[a], and Jakub Straka[a]

[a]Department of Zoology, Faculty of Science, Charles University, CZ-12844 Prague, Czech Republic; [b]Department of Botany, Faculty of Science, Charles University, CZ-12801 Prague, Czech Republic; and [c]Institute of Entomology, Biological Centre, Czech Academy of Science, CZ-370 05 České Budějovice, Czech Republic

Edited by Raghavendra Gadagkar, Indian Institute of Science, Bangalore, India, and approved February 12, 2019 (received for review June 12, 2018)

Parental care behavior evolves to increase the survival of offspring. When offspring care becomes complicated for ecological reasons, cooperation of multiple individuals can be beneficial. There are two types of cooperative care: biparental care and worker (helper)-based care (e.g., eusociality). Although biparental care is common in several groups of vertebrates, it is generally rare in arthropods. Conversely, eusociality is widespread in insects, especially the aculeate Hymenoptera. Here, we present a case of biparental care in bees, in *Ceratina nigrolabiata* (Apidae, Xylocopinae). Similar to eusocial behavior, biparental care leads to greater brood protection in this species. Male guarding increases provisioning of nests because females are liberated from the tradeoff between provisioning and nest protection. The main benefit of parental care for males should be increased paternity. Interestingly though, we found that paternity of offspring by guard males is extraordinarily low (10% of offspring). Generally, we found that nests were not guarded by the same male for the whole provisioning season, meaning that males arrive to nests as stepfathers. However, we show that long-term guarding performed by a single male does increase paternity. We suggest that the multiple-mating strategy of these bees increased the amount of time for interactions between the sexes, and this longer period of potential interaction supported the origin of biparental care. Eusociality based on monandry was thought to be the main type of extended brood protection in bees. We show that biparental care based on polyandry provides an interesting evolutionary alternative.

biparental care | social behavior | paternity | mating frequency | bees

In biparental care, females and males cooperate in the care of offspring (1, 2). Several aspects of offspring care can influence the evolution of biparental care: the synergistic effect of male and female care (3), the inability of one parent to care for offspring effectively (4), or care that has similar costs and benefits for both sexes (5, 6). Biparental care is relatively common among vertebrates: it is the dominant type in birds (1) and frequently occurs in mammal, amphibian, and fish lineages (7). Conversely, biparental care is very rare among arthropods, with only a few known examples, including cockroaches from the genus *Cryptocercus,* as well as burying, passalid, and bark beetles (2, 4, 8). Biparental care has also been documented in crabronid wasps of the genus *Trypoxylon* (subgenus *Trypargillum*) belonging to the aculeate Hymenoptera (9, 10). Generally, in this group of insects, females perform nest provisioning, while males perform nest guarding and help with nest construction (10, 11). Among the aculeate Hymenoptera, male participation in offspring care is rare (2, 12). On the other hand, the occurrence of extensive female care is common, and there have been several origins of eusociality (12–14).

Male and female mating strategies are strongly related to the type of parental care (15, 16). Males, in contrast to females, usually benefit more from an increased number of copulation events than from substantial investment in offspring care (17, 18). Male behavior can be further influenced by female availability. Monopolization of multiple females is usually the most beneficial strategy for males; however, when this is not possible, it is best for males to monopolize at least one female (15). This situation typically occurs in species with low population density (as individuals rarely meet) (19) or with male-biased sex ratios (as most of the females are occupied by other males) (20). Mate guarding of a single female leads to social monogamy. Social monogamy is an association between a single male and a single female, which can last for the whole reproductive life or only temporarily (21). Male participation in offspring care can be a byproduct of mate guarding (19, 22). In this situation, no tradeoff between care and mating opportunities occurs. In the case of social monogamy, investment into parental care is beneficial for the male because he is less likely to gain from interactions with other females (15). It is important to remember that social monogamy does not necessarily mean genetic monogamy: extrapair copulation is not uncommon (21, 23).

Here, we describe biparental care in the solitary apid bee, *Ceratina nigrolabiata*, which represents a case of biparental care in bees. Bees and most other aculeate Hymenoptera have specific natural history traits that can strongly modulate the evolution of parental care, including (*i*) nest-making behavior; (*ii*) haplodiploid sex determination, in which males only genetically participate in female offspring; and (*iii*) a spermatheca, which allows the female to store sperm for months or even years (24). Parallel evolution of biparental care in *C. nigrolabiata* and *Trypoxylon* crabronid wasps is likely, as *Trypoxylon* and *Ceratina*

Significance

Biparental care is a rare strategy of offspring protection in insects. We discovered a case of biparental care in bees, in *Ceratina nigrolabiata*. Benefits of biparental care were identified for both males and females; yet, we found that an extraordinarily high proportion of offspring were unrelated to guard males. This is because females of *C. nigrolabiata* are polyandrous and store sperm. Therefore, the most important benefit for males is increased mating opportunities. We suggest that female polyandry and prolonged female receptivity supported the origin and/or maintenance of biparental care concomitant with extended male lifespan. Biparental care is an alternative method of brood protection to eusociality, which provides similar benefits but arises under different female mating conditions.

Author contributions: M.M., K.Č., and J.S. designed research; M.M., L.J., K.Č., E.M., V.B., and J.S. performed research; M.M. and J.H. analyzed data; and M.M., J.H., and J.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Published under the PNAS license.

[1]To whom correspondence should be addressed. Email: michael.mikat@gmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1810092116/-/DCSupplemental.

Published online March 11, 2019.

share natural history traits typical of nest-making aculeate Hymenoptera (9–11). As biparental care should increase the fitness of both sexes (2, 6), we tested the benefits of this strategy for males and females separately. We identified the maternity and paternity of brood individuals using microsatellites, and we compared the mating frequency of *C. nigrolabiata* with that of solitary and facultatively eusocial bee species in which single mating is known (14, 25–27). Importantly, the presence of solitary, biparental, and eusocial nesting within the same bee genus allows for comparisons of the existing cooperative strategies.

Results

Presence of a Male–Female Pair in Active Brood Nests. A male–female pair was found in 88% of the active brood nests of *C. nigrolabiata* (404 out of 460; *SI Appendix*, Table S1). In 1% of the active brood nests (5 out of 460; *SI Appendix*, Table S1), one female and multiple males were present. In the remaining nests, there were only adults of a single sex (only a male in 5%: 26 out of 404 nests; only a female in 4%: 18 out of 404 nests; *SI Appendix*, Table S1) or no adults at all (2%: 8 out of 404 nests; *SI Appendix*, Table S1). We did not observe any nest with more than one adult female.

There was a pronounced division of labor between males and females. In all cases, while females were foraging, males remained in their nests (78 nests, each observed for 1.5 h). The male remained inside the nest, near the nest entrance, and when the female departed or arrived, he let her pass (Fig. 1). Ants are natural enemies of *Ceratina* bees (28); therefore, to test whether guard males of *C. nigrolabiata* effectively protect the nest against invaders, we placed an ant at the nest entrance and observed the behavior of the guard male. Guard males prevented the ant from entering the nest in all cases ($n = 41$), and in 61% of cases, males actively pushed the ant out of the nest.

Duration of Male Guarding. Usually, *C. nigrolabiata* males do not stay in the same nest for the entire provisioning season (the provisioning season lasts ∼42 d; see *SI Appendix*). On average, males remain for 7.756 d in a nest (SD = 8.211); therefore, the male inhabitant of the majority of nests changed one or more times during the provisioning season (*SI Appendix*, Figs. S1 and S2). Only in rare cases (11 out of 93) did a single male remain in the same nest for the whole provisioning season. The frequency of male switches significantly influences nesting productivity, measured as the number of offspring in egg and larval stages in an active brood nest. In each year of the study, nesting productivity decreased with increased frequency of male switches [Fig. 2 *A*–*C*; general linearized model (GLM) Poisson; 2013: deviance = 4.97, $P = 0.026$, $n = 27$; 2014: deviance = 5.13, $P = 0.024$, $n = 120$; 2015: deviance = 8.97, $P = 0.003$, $n = 102$].

Experimental Removal of Males from Their Nests. When a *C. nigrolabiata* male was removed from a nest, he was replaced by another male within the first day postremoval in 61% of cases (47 out of 77 nests; *SI Appendix*, Table S2). By the fourth day after removal, the proportion of nests with a guard male did not significantly differ between manipulated and control nests (Fisher's exact test, $P = 0.153$; $n = 76$; *SI Appendix*, Table S2). Removal of the male was not associated with the presence of brood cell damage (Fisher's exact test, $P = 0.694$; $n = 162$); however, it did result in decreased nesting productivity in nests where the couple was successfully reestablished, as measured by the number of eggs (Fig. 2*D*; GLM Poisson, $df = 1$, deviance = 5.09, $P = 0.024$, $n = 73$) and the number of offspring (at or before the third larval stage; Fig. 2*E*; GLM Poisson, $df = 1$, deviance = 5.59, $P = 0.015$, $n = 73$). Interestingly, male removal had a significant influence on female behavior. Females in male-removed nests performed fewer foraging trips [in 1.5 h of observation time post-male removal; Fig. 3*A*; paired Wilcoxon test, V (test criterium of Wilcoxon paired test) = 221, $P = 0.002$; $n = 24$], and they spent less time outside their nests (Fig. 3*B* and Table 1; paired Wilcoxon test, V = 264, $P < 0.001$; $n = 24$).

Experimental Removal of Females from Their Nests. Removal of *C. nigrolabiata* females had a significant effect on the departure of guard males (Fisher's exact test, $P < 0.001$; $n = 94$). When we removed the female from a nest, we checked for the presence of the male the following day: the guard male was present in only 38% of the female-removed nests (18 out of 48), in comparison with 89% of the control nests (41 out of 46). Importantly, female removal resulted in a significantly increased incidence of brood cell damage in the nest (Fisher's exact test, $P < 0.001$; $n = 194$). The presence of natural enemies, such as another *Ceratina* female that had usurped the nest (Fisher's exact test, $P < 0.001$; $n = 194$) or ants (Fisher's exact test, $P = 0.018$; $n = 194$), was also significantly influenced by female removal.

Comparison of the Duration of Foraging Trips Among Species. We compared the average duration of *C. nigrolabiata* foraging trips with that of two sympatric solitary *Ceratina* species. The average duration of a foraging trip was 16.47 min for *C. nigrolabiata* (SD = 10.74 min, $n = 110$ trips of 55 individuals), 10.30 min for *Ceratina chalybea* (SD = 10.01 min, $n = 42$ trips of 21 individuals), and only 4.77 min for *Ceratina cucurbitina* (SD = 7.50 min, $n = 84$ trips of 42 individuals). *C. nigrolabiata* had significantly longer foraging trips than *C. chalybea* (Fig. 3*C*; post hoc Nemenyi test, $P = 0.008$) and *C. cucurbitina* (Fig. 3*C*; post hoc Nemenyi test, $P < 0.001$). Data are summarized in Table 1.

Male Survival During the Nesting Season. The proportion of *C. nigrolabiata* males in the population fluctuated around 54% for the duration of the nesting season [$n = 408$ individuals from all season (May 1 to August 15); fluctuation between 44% and 65%

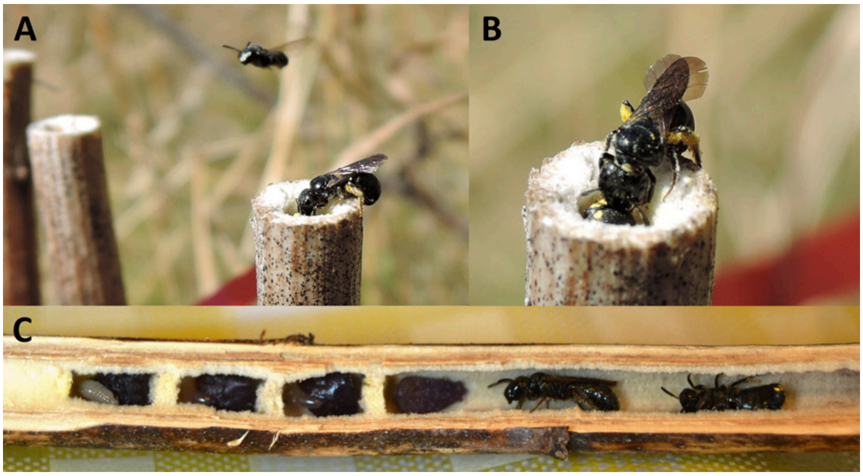

Fig. 1. *C. nigrolabiata* male performs nest guarding; female performs nest provisioning. (*A*) The female arrives at the nest entrance with a pollen load. She uses her fore legs to scratch the metasoma of the male to let her pass. Another male flies around, probably searching for a nest without a guard male. (*B*) Detailed image of the female scratching on the male's metasoma. The male's metasoma is marked yellow. (*C*) Dissected active brood nest with three completed brood cells and one cell currently being provisioned. In *C*, a female (left) and male (right) are present in the burrow.

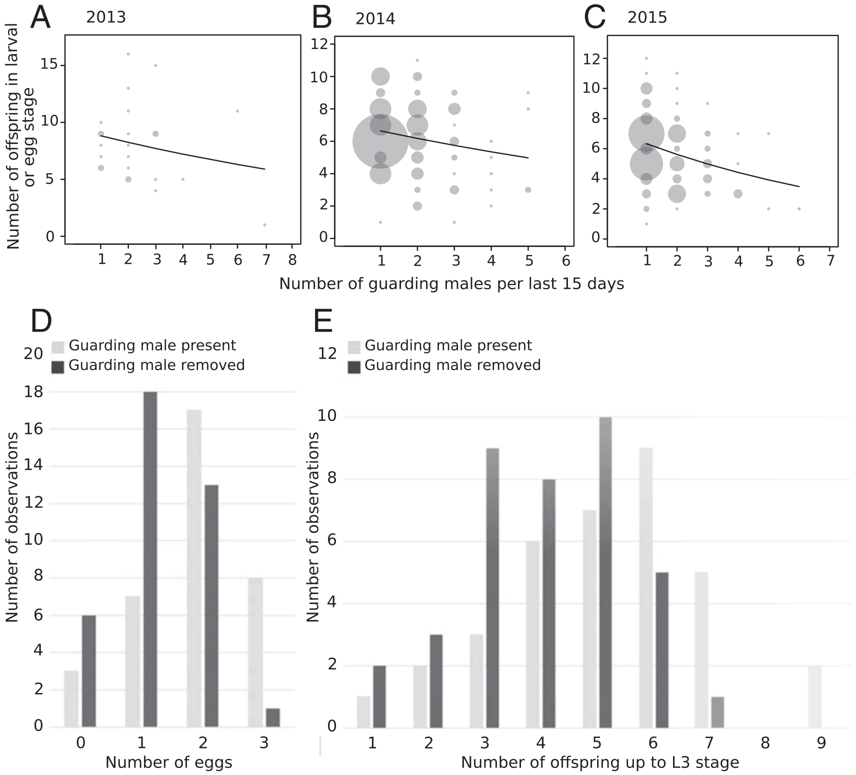

Fig. 2. Effect of male switches on nesting productivity. (*A*–*C*) Relationship between the number of different guard males in the last 15 d and the number of offspring in egg and larval stages from 2013 (27 nests) (*A*), from 2014 (120 nests) (*B*), and from 2015 (102 nests) (*C*). The relationship is significant for all 3 y (GLM Poisson, 2013: deviance = 4.97, $P = 0.026$; 2014: deviance = 5.1264, $P = 0.023$; 2015: deviance = 8.97, $P = 0.003$). The size of the circle corresponds to the number of observations with the same value. The line indicates the result of the Poisson model. (*D* and *E*) Comparison of nesting productivity between male-removed nests and control nests. (*D*) Number of eggs in the nest. (*E*) Number of offspring up to the larval (L)3 stage in the nest (eggs plus young larvae). We used 38 male-removed nests and 35 control nests from 2014 and 2015. Results were statistically significant for both the number of eggs (GLM Poisson, $df = 1$, deviance = 5.09, $P = 0.024$) and the number of offspring up to the L3 stage (GLM Poisson, $df = 1$, deviance = 5.59, $P = 0.015$).

in different months; *SI Appendix*, Fig. S3]. The proportion of *C. chalybea* males in the population decreased over the nesting season from 48% ($n = 23$ individuals) in the first half of May to 8% ($n = 66$ individuals) in the first half of August. Therefore, few *C. chalybea* males survived for the entire season. In contrast, *C. cucurbitina* males died early. In the first half of May, 56% ($n = 52$) of adults were male; however, the last *C. cucurbitina* male of the parental generation was observed in the second half of June, well before the end of the nesting season.

Mating Frequency and Paternity. At nests, we observed instances of mating throughout the nesting season. *C. nigrolabiata* females predominantly mate multiply: on average, offspring of 3.25 males were found in completely provisioned nests (ranging from 1 to 8, SD = 1.70, $n = 31$ nests). Nests with all offspring belonging to a single father were rare (4 out of 31). Moreover, offspring from different fathers were often intermixed in the nest (*SI Appendix*, Fig. S4). Female multiple mating is also present in two related *Ceratina* species, *C. chalybea* (2.57 males, SD = 0.98, $n = 7$ nests) and *Ceratina cyanea* (2.5 males, SD = 0.70, $n = 2$ nests; Table 1).

Kinship of the guard male to nest offspring was low. At least one offspring of the guard male was detected in 29% of active brood nests ($n = 265$; *SI Appendix*, Fig. S5). On average, active brood nests contained 6.604 offspring, with only 0.638 offspring (10%) fathered by the guard male. However, as hymenopteran males do not contribute genetically to male (haploid) offspring,

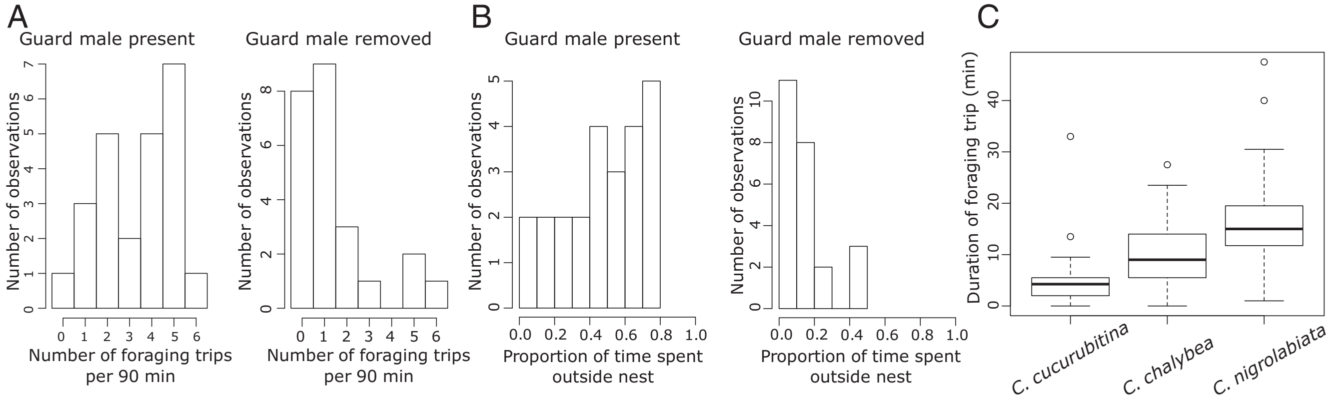

Fig. 3. Effect of male removal on female foraging behavior. (*A*) Histograms of the number of foraging trips comparing male-removed nests and control nests. One treatment and one control nest were observed simultaneously. The difference is statistically significant (paired Wilcoxon test, V = 221, $P = 0.002$; $n = 24$ nest pairs). (*B*) Histograms of the proportion of time spent outside of the nest in foraging activity per one female in male-removed nests and control nests. One treatment and one control nest were observed simultaneously. The difference is statistically significant (paired Wilcoxon test, V = 264, $P < 0.001$; $n = 24$ nest pairs). (*C*) Comparison of the duration of foraging trips between sympatric species of the genus *Ceratina*. The duration of a foraging trip was calculated as the mean of two subsequent trips of one female. We observed 55 nests of *C. nigrolabiata*, 21 nests of *C. chalybea*, and 42 nests of *C. cucurbitina*.

Table 1. Comparison of female mating frequency and duration of foraging trips in four *Ceratina* species

Species	*C. nigrolabiata*	*C. chalybea*	*C. cucurbitina*	*C. cyanea*
Nesting strategy	Biparental	Solitary	Solitary	Solitary
Average mating frequency	3.25 (*n* = 31)	2.57 (*n* = 7)	Not available	2.5 (*n* = 2)
Average duration of foraging trip, min	16.47 (*n* = 55)	10.30 (*n* = 21)	4.77 (*n* = 42)	Not available

n is the number of nests used in the analysis.

the number of female offspring fathered is more important. Guard males, on average, fathered only 17% (169 out of 1005) of female offspring. The maximum number of offspring belonging to the guard male in a nest was seven (*SI Appendix*, Fig. S5).

We also tested how male fitness is affected by the duration of guarding. Both the number of offspring (Fig. 4; linear model, $t = 14.55$, $P < 0.001$; $n = 301$) and the number of offspring per day of guarding (*SI Appendix*, Fig. S6) increase with longer durations of guarding. A male gains an average of 0.638 offspring per 7 d of guarding (which is the average duration of guarding). The provisioning season of *C. nigrolabiata* lasts ∼42 d; therefore, guarding nests for most of the provisioning season (for example, guarding five nests, each for the average duration) would lead to ∼3.19 offspring per male per season.

Discussion

We discovered social monogamy and biparental care in the bee species *C. nigrolabiata*. These strategies were previously unknown in bees. There is a pronounced division of labor between the sexes in this species, similar to another biparental arthropod group, crabronid wasps of the genus *Trypoxylon* (10, 11, 29). While females perform provisioning, males are responsible for nest guarding (Fig. 1). A male–female couple was found in the majority of active brood nests of *C. nigrolabiata* (88%). Nests without an adult couple most commonly contained only male adults, but we also found nests with only female adults and nests without either parent present. Because female-only nests were not the most prevalent, it is likely that active brood nests without a couple were either recently orphaned or deserted by the parents. This suggests that female-only nests are not stable.

The importance of nest and resource defense can select for biparental care (8); in the aculeate Hymenoptera, selective pressures for nest protection are thought to favor eusociality (13). Unguarded nests of *Ceratina* bees are highly vulnerable to natural enemies, especially ants (28); therefore, the benefit of a guard male is most likely similar to the benefit of worker guards in facultatively eusocial nests (30–32). One important benefit of eusocial nesting is nest protection when foraging individuals depart the nest to collect provisions for brood cells (33, 34). Eusocial nesting thus significantly decreases mortality caused by natural enemies (31, 35). We have shown that *C. nigrolabiata* males defend the nest from attacks by enemies such as ants. Interestingly, removal of the guard male did not increase the incidence of brood or cell damage, probably because the removed male was replaced by another male within 1 d in the majority of cases (and maximally within 4 d). By contrast, on a short time scale (hours), we found that absence of the guard male affected female behavior. Male removal resulted in a decreased number of female foraging trips and a decreased amount of time spent outside the nest. In another known biparental hymenopteran, the crabronid wasp, *Trypoxylon vagulum* Richards, females do not perform foraging when the guard male is not present in the nest (29). Similarly, females spend a longer time outside of eusocial nests than solitary nests in the closely related facultatively eusocial bees of the genus *Xylocopa* (30, 36). This difference is also observed in *Ceratina calcarata* Robertson, as females spend more time away from the nests in which they feed adult offspring compared with those in which only vulnerable brood cells are provisioned (37). Likewise, we determined that *C. nigrolabiata* performs significantly longer foraging trips than two sympatric solitary species without biparental care—*C. chalybea* and *C. cucurbitina*. Therefore, biparental care and eusociality, by using two different types of guarding, are strategies that result in similar benefits for foragers, allowing them more time for resource collection thereby increasing nesting productivity.

The presence of a guard male should increase nesting productivity if it allows the female to forage more effectively. Indeed, we have shown that male removal results in altered female foraging behavior as well as decreased nesting productivity. A similar benefit to nesting productivity is known for eusocial nests of *Xylocopa sulcatipes* in comparison with solitary nests (30, 32). Furthermore, effective guarding by males allows females to invest more into female offspring, which contain paternal DNA but are costlier than male offspring (without paternal DNA), as was shown for the biparental crabronid wasp, *Trypoxylon politum* Drury (10). Nesting productivity was also positively influenced by male fidelity in *C. nigrolabiata*; nests with a lower frequency of male switches had a greater number of offspring.

In biparental care, males commonly benefit from caring for their own offspring; therefore, although female infidelity is possible, most of the offspring usually belong to the guard male. In bird and mammal species, more than 90% of the offspring belong to the male who is providing care (21, 23), although some exceptions exist [e.g., the birds *Tachycineta bicolor* (Vieillot) (Hirundinidae) with 31% (38) and *Malurus cyaneus* (Ellis) (Maluridae) with 24% (39) of their own offspring]. In the only other genetically studied biparental hymenopteran, *Trypoxylon albitarse* Fabricius, the guard male fathers 78% of his female partner's offspring (9). Conversely, we found that, for *C. nigrolabiata* males, the fitness benefit from nest protection is generally very low. For *C. nigrolabiata*, only 10% of all offspring (including male offspring, which have no father) and 17% of female offspring were guarded by their own father. Males guarded nests

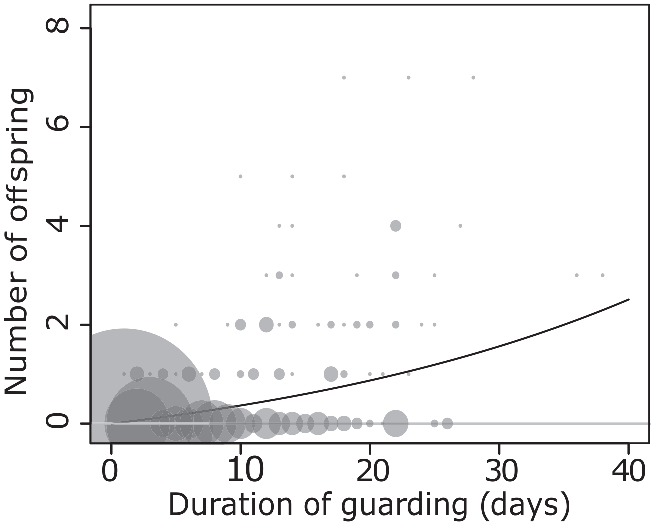

Fig. 4. Influence of the duration of male guarding on the number of his own offspring in the nest. The size of circle corresponds to the number of observations with the same value. The line indicates the result of the log-normal model (*n* = 301 nests).

with at least one of their own descendants in only 29% of cases. Therefore, *C. nigrolabiata* males regularly guard offspring of alien males or offspring with no paternal DNA. Although male fitness gained by guarding any specific nest is low, the total benefit from guarding behavior per entire season is much higher because males guard several nests sequentially within a season. We calculate that a male gains 3.19 offspring by guarding nests for the entire provisioning season.

As there is little parental fitness benefit from protecting one's own offspring for *C. nigrolabiata* males, we posit that nest guarding behavior (which increases offspring survival via nest protection) is primarily a byproduct of mate guarding. This claim is supported by findings from the female-removal experiment: when we removed the female from a nest, the male usually departed within a few days. Additionally, males do not try to protect offspring without the presence of a female.

In *C. nigrolabiata,* biparental care coexists with high female mating frequency. The mixing of offspring from different fathers in the nest (*SI Appendix*, Fig. S4) indicates that females can store and mix sperm from previous matings in their spermatheca. In most bee species, mating with a single male early in the season is the prevailing mating pattern (25, 26); however, we observed females mating throughout the nesting season in *C. nigrolabiata* and a related species, *C. chalybea*. Interestingly, nests of the related solitary *Ceratina* species, *C. chalybea*, and *C. cyanea*, also have offspring from multiple fathers, suggesting that multiple mating arose before biparental care in the genus *Ceratina*. Generally, it is thought that the multiple mating in females selects against male participation in offspring care (2, 4, 40). Contrary to this assumption, we propose that female multiple mating in *C. nigrolabiata* represents a key preadaptation for biparental care in bees. Bee species with single-mated females usually mate before the provisioning season (26) and have short-lived males (24, 41). For example, there is very low male survival for the duration of the nesting season in *C. calcarata* (42), where 81% of females are singly mated (27). Long-term persistence of mating opportunities selects for prolonged life of males (43, 44), and only a long-living male has the opportunity to participate in offspring care. In accordance with this hypothesis, we detected long-term survival of males throughout the nesting season in *C. nigrolabiata* and, partially, also in *C. chalybea* (*SI Appendix*, Fig. S3).

Biparental care has similar maternal benefits as eusociality. Eusociality originated in the context of monandry, which ensures high relatedness between offspring, encouraging cooperation (14). Indeed, the only eusocial *Ceratina* species with known mating frequency, *Ceratina australensis* (Perkins), is monandrous (31). By contrast, biparental care in *C. nigrolabiata* originated in a situation of multiple mating. Therefore, we suggest that primitive eusociality and biparental care represent two alternative ways to reach the same primary benefits of extended parental care. The evolution of parental care in *Ceratina* is notable for its complicated origin, as the ancestor of the genus *Ceratina* was most likely facultatively eusocial, with obligate solitary behavior being a derived state (45). In *C. nigrolabiata*, we did not find any case of multifemale nesting; however, it is possible that some of the advanced behavioral traits observed in *C. nigrolabiata* are inherited from their eusocial ancestors.

Materials and Methods

Study Species. We used *C. nigrolabiata* Friese (Hymenoptera: Apidae) for most of our analyses. We performed field studies on this species, with the nest being the main unit for almost all of our analyses. A nest usually contains two parents (one male and one female) and juvenile offspring. We compared some traits of *C. nigrolabiata* with those of other sympatric *Ceratina* species: *C. cucurbitina* (Rossi), *C. chalybea* (Chevrier), and *C. cyanea* (Kirby).

Study Site. Research was performed at Havranické Vřesoviště, Podyjí National Park, Czech Republic (48°48′32.2″N 15°59′33.7″E). This locality consists of dry steppe grasslands with heather and scattered shrubs. We conducted our research in 2013, 2014, and 2015. Some additional data are from 2012 and 2016.

Field Experiments.

Installation of nesting opportunities. We used sheaves of cut twigs as artificial nesting opportunities. A sheaf was made from 20 twigs, 30–45 cm in length. For further details about the artificial nesting opportunities, see *SI Appendix*.

Observation of nests and dye marking of males. We marked nests and the males that stayed inside them. We checked all studied nests daily in almost all cases for the duration of the provisioning season. Each unmarked male was marked by a colored dot on the metasomal terga near the apex of the metasoma, visible from the nest entrance. We did not observe any case of a male abandoning the nest immediately after marking. We used five different colors for marking. When we found a marked male, we noted its color. This enabled us to assess the duration of nest guarding by males. We used these data to evaluate the average duration of male guarding and for several other analyses where we correlated the duration of male guarding with other variables. For further details about the marking of males and nests, see *SI Appendix*.

Dissection of nests. Nests selected for dissection were collected after the end of foraging activity (after 6:00 PM; Central European Summer Time). The nest entrance was plugged to ensure that adult individuals were retained inside. Collected nests were stored in a fridge until dissection.

Nests were cut by knife and the following data were recorded: number and sex of adult individuals, length of the nest, length of the entrance burrow (distance between the nest entrance and outer septum), number of provisioned cells, number of live offspring, and presence of natural enemies. Additionally, developmental stage was recorded for each offspring. Details about the developmental stage of offspring are described in *SI Appendix*. We used active brood nests for most of the analyses. An active brood nest was still being provisioned at the time of nest collection and therefore contained an egg in the outermost brood cell (42). We used full brood nests for some of the analyses. These nests contained a larva or pupa in the outermost brood cell. We used data from the dissection of nests to assess the length of the provisioning season and changes to the adult sex ratio during the nesting season; this is described in more detail in *SI Appendix*.

Existence of pairs. In this analysis, we estimated the proportion of nests in which a male and a female were both present. We used all dissected active brood nests (excluding nests from those experiments that involved removal of an adult, male or female). We analyzed 133 nests in 2013, 183 in 2014, and 144 in 2015; therefore, 460 nests were analyzed in total.

Impact of male switches on nesting productivity. We examined the influence of guard male switching frequency on nesting productivity, measured as the number of offspring in the egg or larval stage. Specific details for this analysis are presented in *SI Appendix*.

Removal of Males. We randomly selected some of the nests with both a male and a female present for removal experiments. We performed male removal on some of these nests, while others were retained as controls. We used the same nests to determine the influence of male removal on the presence of brood cell damage, to assess the replacement of removed males, and to examine the impact of male removal on nesting productivity. In a subsequent experiment, we evaluated the effect of male removal on female behavior. Specific experimental details are presented in *SI Appendix*.

Removal of Females. In randomly selected nests (not those used for the male removal experiment), the guard female was removed. Other randomly selected nests were appointed as controls. For each nest in this experiment, we confirmed the presence of both a female and a male before the female removal or control treatment (using the same procedures as the male removal experiments). Specific experimental details are presented in *SI Appendix*.

Comparison of the Duration of Foraging Trips Between Species. The duration of foraging trips in *C. nigrolabiata* and two other sympatric species, *C. chalybea* and *C. cucurbitina*, were observed. On each observational day, all nests present on selected sheaves were observed. Each nest was observed until two intervals between departure and arrival were successfully noted. We calculated the average duration of two foraging trips for each individual. For further details, see *SI Appendix*.

Laboratory Analyses. We developed 10 polymorphic microsatellite loci to genotype 3,547 *C. nigrolabiata* individuals. We calculated the probability of two adults having the same genotype using the Cervus program (46). This probability was 0.00000004. It was also possible to use some of the loci developed for *C. nigrolabiata* for other species: *C. chalybea* and *C. cyanea*. The sex of each genotyped offspring was determined by ploidy. Details about microsatellite locus development, the genotyping process, and sex determination are described in *SI Appendix*.

Computing the relatedness of individuals in the nest.

Maternity. Maternity was tested by manual comparison of the guard female's genetic profile with offspring from the same nest. The guard female was considered to be the mother when all loci agreed or when only one locus disagreed. Individuals that were not offspring of the guard female were found only in rare cases. In these cases, they were always at the bottom of the nest and usually evidently older than the other offspring. These individuals disagreed with the guard female in at least minimally three loci. We suppose that in these cases, the offspring belong to the previous owner of the nest and that this situation arises from nest usurpation with incomplete removal of the usurped female's brood cells. Nonexclusion probability computed by Cervus 3 (46) was 0.055 for the first parent.

Paternity. Paternity of the offspring was tested using two procedures: (*i*) manual comparison—the offspring agreed with the guard male (potential father) in all alleles or disagreed in only one; or (*ii*) Colony software (47). Male offspring (haploid) were not analyzed. The settings used for Colony software and details about the paternity calculations are presented in *SI Appendix*.

Female mating frequency (number of fathers in the nest). We examined how many different fathers had offspring in a nest based on microsatellite analysis. This variable also represents the minimum number of sexual partners of the female. We used Colony software for this analysis. We analyzed mating frequency in 31 nests of *C. nigrolabiata*, 7 nests of *C. chalybea*, and 2 nests of *C. cyanea*. For further details about the calculations of mating frequency, see *SI Appendix*.

Influence of the duration of guarding on male fitness. Selected nests were dissected, and the male–female pair and all offspring were genotyped. Paternity of offspring and relatedness to the guard male were analyzed. We modeled and tested the relationship between the duration of time a male spent guarding a nest and the number of his own offspring in that nest. To test whether the relationship between the duration of male guarding and guard male fitness was statistically significant, a linear model on log-transformed data was used. Assumptions of the model were checked using diagnostic plots. We constructed polynomial models (up to the fifth degree of the polynomial), each of them with and without an intercept. Based on the Akaike information criterion, the best model was determined to be a linear model without an intercept. Statistical analyses were done using R software, version 3.2.5 (48). In total, 301 nests were analyzed (54 in 2013, 171 in 2014, 76 in 2015), containing 2,082 offspring, of which 1,189 were female.

Datasets. The datasets used for this study are provided in Dataset S1.

ACKNOWLEDGMENTS. We thank the people who helped us with field experiments: Daniel Benda, Lenka Burešová, Albert Damaška, Jana Habermannová, Celie Korittová, Karel Kodejš, Zuzana Matějková, Lenka Macháčková, Miroslav Mikát, Blanka Mikátová, Šárka Mikátová, Jasna Simonová, Šárka Škorpíková, Jan Pražák, Tomáš Smyčka, Lucie Studená, and Kateřina Votýpková. We thank Rachel Kolisko for comments and stylistic improvement of our manuscript. We thank the Podyjí National Park for research permission and helpfulness. The Grant Agency of Charles University in Prague (Grant GAUK 358315/2015) and the Specific University Research project Integrative Animal Biology (Grant SVV 260434/2018) supported this research.

1. Cockburn A (2006) Prevalence of different modes of parental care in birds. *Proc Biol Sci* 273:1375–1383.
2. Suzuki S (2013) Biparental care in insects: Paternal care, life history, and the function of the nest. *J Insect Sci* 13:131.
3. Fromhage L, Jennions MD (2016) Coevolution of parental investment and sexually selected traits drives sex-role divergence. *Nat Commun* 7:12517.
4. Tallamy DW, Wood TK (1986) Convergence patterns in subsocial insects. *Annu Rev Entomol* 31:369–390.
5. McNamara JM, Wolf M (2015) Sexual conflict over parental care promotes the evolution of sex differences in care and the ability to care. *Proc Biol Sci* 282:20142752.
6. Westneat DF, Craig Sargent R (1996) Sex and parenting: The effects of sexual conflict and parentage on parental strategies. *Trends Ecol Evol* 11:87–91.
7. Reynolds JD, Goodwin NB, Freckleton RP (2002) Evolutionary transitions in parental care and live bearing in vertebrates. *Philos Trans R Soc Lond B Biol Sci* 357:269–281.
8. Gilbert JDJ, Manica A (2015) The evolution of parental care in insects: A test of current hypotheses. *Evolution* 69:1255–1270.
9. Bergamaschi ACB, Almeida JDC, Campos LADO, Del Lama MA (2015) Sociogenetic structure in nests of the mud dauber wasp *Trypoxylon (Trypargilum) albitarse* (Hymenoptera: Crabronidae). *Eur J Entomol* 112:722–727.
10. Brockmann HJ, Grafen A (1989) Mate conflict and male behaviour in a solitary wasp, *Trypoxylon* (*Trypargilum*) *politum* (Hymenoptera: Sphecidae). *Anim Behav* 37:232–255.
11. Brockmann HJ (1992) Male behavior, courtship and nesting in *Trypoxylon* (*Trypargilum*) *monteverdeae* (Hymenoptera: Sphecidae). *J Kans Entomol Soc* 65:66–84.
12. Korb J, Heinze J (2016) Major hurdles for the evolution of sociality. *Annu Rev Entomol* 61:297–316.
13. Wilson EO (1971) *The Insect Societies* (Belknap Press of Harvard Univ Press, Cambridge, MA), pp 75–102.
14. Hughes WO, Oldroyd BP, Beekman M, Ratnieks FL (2008) Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* 320:1213–1216.
15. Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215–223.
16. Liker A, Freckleton RP, Remeš V, Székely T (2015) Sex differences in parental care: Gametic investment, sexual selection, and social environment. *Evolution* 69:2862–2875.
17. Queller DC (1997) Why do females care more than males? *Proc R Soc Lond B Biol Sci* 264:1555–1557.
18. Royle NJ (2016) Parental care: When the sex has to stop. *Curr Biol* 26:R478–R480.
19. Lukas D, Clutton-Brock TH (2013) The evolution of social monogamy in mammals. *Science* 341:526–530.
20. Harts AM, Kokko H (2013) Understanding promiscuity: When is seeking additional mates better than guarding an already found one? *Evolution* 67:2838–2848.
21. Cohas A, Allainé D (2009) Social structure influences extra-pair paternity in socially monogamous mammals. *Biol Lett* 5:313–316.
22. Van Rhijn JG (1991) Mate guarding as a key factor in the evolution of parental care in birds. *Anim Behav* 41:963–970.
23. Petrie M, Kempenaers B (1998) Extra-pair paternity in birds: Explaining variation between species and populations. *Trends Ecol Evol* 13:52–58.
24. Michener CD (2007) *The Bees of the World* (Johns Hopkins Univ Press, Baltimore), 2nd Ed, pp 6–11.
25. Paxton RJ (2005) Male mating behaviour and mating systems of bees: An overview. *Apidologie* 36:145–156.
26. Alcock J (2013) Sexual selection and the mating behavior of solitary bees. *Advances in the Study of Behavior*, eds Brockmann HJ, Naguib TJRM, Mitani JC, Simmons LW, Barrett L (Academic), pp 1–48.
27. Shell WA, Rehan SM (2017) The price of insurance: Costs and benefits of worker production in a facultatively social bee. *Behav Ecol* 29:204–211.
28. Mikát M, Černá K, Straka J (2016) Major benefits of guarding behavior in subsocial bees: Implications for social evolution. *Ecol Evol* 6:6784–6797.
29. Coville RE, Griswold C, Coville PL (2000) Observations on the nesting biology and behavior of *Trypoxylon* (*Trypargilum*) *vagulum* (Hymenoptera: Sphecidae) in Costa Rica. *Pan-Pac Entomol* 76:28–48.
30. Gerling D, Hurd PD, Hefetz A (1983) *Comparative Behavioral Biology of Two Middle East Species of Carpenter Bees (Xylocopa Latreille)(Hymenoptera: Apoidea)* (Smithsonian Institution Scholarly Press, Washington, DC), pp 1–33.
31. Rehan SM, Richards MH, Adams M, Schwarz MP (2014) The costs and benefits of sociality in a facultatively social bee. *Anim Behav* 97:77–85.
32. Stark RE (1992) Cooperative nesting in the multivoltine large carpenter bee *Xylocopa sulcatipes* Maa (Apoidea: Anthophoridae): Do helpers gain or lose to solitary females? *Ethology* 91:301–310.
33. Clouse R (2001) Some effects of group size on the output of beginning nests of *Mischocyttarus mexicanus* (Hymenoptera: Vespidae). *Fla Entomol* 84:418.
34. Richards MH, Packer L (1998) Demography and relatedness in multiple-foundress nests of the social sweat bee, *Halictus ligatus*. *Insectes Soc* 45:97–109.
35. Andersson M (1984) The evolution of eusociality. *Annu Rev Ecol Syst* 15:165–189.
36. Hogendoorn K, Velthuis HHW (1995) The role of young guards in *Xylocopa pubescens*. *Insectes Soc* 42:427–448.
37. Mikát M, Franchino C, Rehan SM (2017) Sociodemographic variation in foraging behavior and the adaptive significance of worker production in the facultatively social small carpenter bee, *Ceratina calcarata*. *Behav Ecol Sociobiol* 71:135.
38. Barber CA, Robertson RJ, Boag PT (1996) The high frequency of extra-pair paternity in tree swallows is not an artifact of nestboxes. *Behav Ecol Sociobiol* 38:425–430.
39. Mulder RA, Dunn PO, Cockburn A, Lazenby-Cohen KA, Howell MJ (1994) Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. *Proc R Soc Lond B Biol Sci* 255:223–229.
40. Kokko H, Jennions MD (2008) Parental investment, sexual selection and sex ratios. *J Evol Biol* 21:919–948.
41. Stubblefield JW, Seger J (1994) Sexual Dimorphism in the Hymenoptera. *The Differences Between the Sexes* (Cambridge Univ Press, Cambridge, UK), pp 71–103.
42. Rehan SM, Richards MH (2010) Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). *Can Entomol* 142:65–74.
43. Gotthard K, Nylin S, Wiklund C (2000) Mating opportunity and the evolution of sex-specific mortality rates in a butterfly. *Oecologia* 122:36–43.
44. Heinze J (2016) The male has done his work - the male may go. *Curr Opin Insect Sci* 16:22–27.
45. Rehan SM, Leys R, Schwarz MP (2012) A mid-cretaceous origin of sociality in xylocopine bees with only two origins of true worker castes indicates severe barriers to eusociality. *PLoS One* 7:e34690.
46. Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16:1099–1106.
47. Jones OR, Wang J (2010) COLONY: A program for parentage and sibship inference from multilocus genotype data. *Mol Ecol Resour* 10:551–555.
48. R. Core Developmental Team (2016) R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna), Version 3.2.5.