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RESEARCH ARTICLE

Caught in an evolutionary trap: worker honey bees that have drifted into foreign colonies do not invest in ovary activation

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Abstract Drifting, the phenomenon whereby workers from one colony find their way into a foreign colony, is widespread in social insects. In apiaries of the honey bee *Apis mellifera*, orientation errors lead to high rates of worker drift. Given that *A. mellifera* workers in apiaries enter foreign colonies accidentally, do they continue to refrain from laying eggs in the foreign colony, or do they behave in their evolutionary interests and attempt to lay eggs? We propose two hypotheses: the "lost losers" hypothesis, where lost workers do not invest in personal reproduction, and the "lost social parasites" hypothesis, where lost workers detect that they are in a foreign colony and do invest in personal reproduction. Previous work has used complete ovary activation as an assay for testing whether workers invest in personal reproduction, but this may not detect subtle reproductive investments in queenright colonies. We instead look at the full range of ovary activation in natal and non-natal workers, because partial activation may signal preparation for future reproduction. We show that in queenright colonies, non-natal workers have the same low degree of ovary activation as their natal counterparts, which supports the hypothesis that drifted bees are "lost losers" caught in an evolutionary trap.

Keywords Evolutionary trap · Honey bee · Ovary activation · Reproductive parasitism · Drifting

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Introduction

Drifting, the phenomenon whereby insect workers from one colony find their way into a foreign colony, is widespread among bees and wasps (*Apis mellifera*—Free 1958; Neumann et al. 2001; Chapman et al. 2010; *Apis dorsata*—Moritz et al. 1995; Paar et al. 2002; *Lasioglossum malachurum*—Paxton et al. 2002; *Bombus terrestris*—Lopez-Vaamonde et al. 2004; *Bombus impatiens* and *Bombus occidentalis*—Birmingham et al. 2004; *Apis florea*—Nanork et al. 2005; Chapman et al. 2009a; *Apis cerana*—Nanork et al. 2007; *Polistes canadensis*—Sumner et al. 2007; *Halictus scabiosae*—Ulrich et al. 2009). In some cases, this drifting may be an adaptive behavior of social parasites seeking to reproduce in foreign colonies (e.g., Neumann et al. 2001; Lopez-Vaamonde et al. 2004; reviewed in Beekman and Oldroyd 2008). In other cases, this drifting is likely the result of an orientation error (Free 1958). In apiaries of the Western honey bee, *A. mellifera*, where hives are commonly arranged in rows, facing the same direction, and painted the same color, it is unsurprising that workers make frequent orientation errors (Jay 1965, 1966a, b). Indeed, as many as 40 % of the workers within a colony located in an apiary can be non-natal (Free 1958; Pfeiffer and Crailsheim 1998). Once assimilated into a foreign colony, do these lost workers live as evolutionary losers, or do they detect that they are in a foreign colony and act to increase their genetic fitness?

We propose two hypotheses: the "lost losers" hypothesis, by which drifted workers do not invest in personal reproduction, and the "lost social parasites" hypothesis, by which drifted workers do invest in personal reproduction. In both hypotheses, the bees are lost—the difference is what the lost bees do once in the foreign colony. In the "lost losers" hypothesis, the drifted workers behave in a foreign

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colony as if they were their own colony. They presumably follow the same colony cues that indicate what work should be done, even though this behavior is now maladaptive because they are not related to the foreign colony's queen. This is an example of an evolutionary trap: when a formerly reliable cue no longer leads to an adaptive response (Schlaepfer et al. 2002). We know that drifted workers continue to work when in a foreign colony (Pfeiffer and Crailsheim 1999), but perhaps they are not as ignorant of their surroundings as it might seem.

In the "lost social parasites" hypothesis, drifted workers invest in personal reproduction while in a foreign colony. They may have joined the foreign colony due to an orientation error, but they then detect that they are in a foreign colony and adaptively respond by investing in personal reproduction, even as they perform work in the hive. In support of this "lost social parasites" hypothesis, drifted *A. mellifera* workers have been shown to work less than non-drifted workers when in a foreign colony (Pfeiffer and Crailsheim 1999). Non-natal workers also reproduce more than natal workers when worker reproduction begins in a queenless colony (*A. florea*—Nanork et al. 2005; *A. cerana*—Nanork et al. 2007; *A. mellifera*—Chapman et al. 2010). Workers may have evolved an adaptive response to being in a foreign colony because orientation errors may have been common in their evolutionary history. Indeed, some species of *Apis* do aggregate their nests naturally (*A. dorsata*—Paar et al. 2002; *A. florea*—Wattanachaiyingcharoen et al. 2008), so it is plausible that drifting between colonies would have occurred.

In both the "lost losers" hypothesis and the "lost social parasites" hypothesis, the non-natal workers have arrived in the foreign colony due to an orientation error. The critical difference between the two hypotheses is whether workers invest more in personal reproduction when in a foreign colony than when in their natal colony. In queenright colonies, we know that non-natal workers do *not* reproduce more than natal workers (*A. florea*—Nanork et al. 2005; *A. cerana*—Nanork et al. 2007; A. *mellifera*—Holmes et al. 2013), but this might be because worker policing prevents worker reproduction unless the colony becomes queenless (Ratnieks and Visscher 1989). In a queenright colony, workers with completely activated ovaries are harassed by nestmates (Visscher and Dukas 1995; Smith et al. 2009; but see Dampney et al. 2002), and the eggs laid by workers are eaten by other workers (Ratnieks and Visscher 1989). Therefore, non-natal workers may do better to not completely activate their ovaries in a queenright colony, but it is possible that they could invest more subtly in personal reproduction.

Worker ovary activation progresses through stages (Velthuis 1970; Pernal and Currie 2000), so non-natal workers could partially activate their ovaries. The studies described above only classify an ovary as active if an egg >50 % of mature size is present, but partial ovary activation is widespread among eusocial bees and wasps (Smith et al. 2013). Non-natal workers could partially activate their ovaries to a point that is below the threshold for receiving harassment from nestmates. In the event of queen loss, they could then complete ovary activation and reproduce more quickly than natal workers without partially activated ovaries. These non-natal workers with partially activated ovaries would be preparing their reproductive physiology to be the first "horse out of the gate" in the race for worker reproduction, if it becomes possible. This would explain how non-natal workers have higher reproductive success than natal workers after a colony's queen is removed experimentally (Chapman et al. 2010). Unlike natal workers, these non-natal workers experience no indirect fitness costs from partial ovary activation (i.e., the effects of their not being good workers; Mattila et al. 2012; Roth et al. 2014), and hence they may be expected to have higher rates of partial ovary activation than natal workers. This would support the "lost social parasites" hypothesis.

This study aims to determine whether *A. mellifera* workers that drift into a foreign queenright colony partially activate their ovaries. If they do, this suggests that these non-natal workers are lost bees that prime themselves to be social parasites, effectively "making the best of a bad job." If they do not partially activate their ovaries, then these non-natal workers are lost bees that do not adaptively respond to their environment-losers in an evolutionary trap.

Materials and methods

The basic experimental design was to dissect the ovaries of honey bees and see whether non-natal workers in a foreign colony have higher levels of ovary activation than natal workers in their native colony. Two pairs of *A. mellifera* colonies were used in the experiment. Each pair consisted of one colony producing only black workers and another colony producing only yellow workers. All of the colonies were queenright throughout the experiment, which was conducted after the Ithaca, NY swarming season (May/June). Each colony was kept >200 m away from any other colony for 30 days before the start of the experiment, to ensure that each colony's worker force consisted exclusively of either yellow or black bees at the start of the experiment. Therefore, when the experiment began, any yellow bees in the colony producing black bees were easily detected as non-natal workers, and vice versa.

On June 13, 2013, the colonies were moved to the Liddell Field Station of Cornell University, in Ithaca, NY (42° 27.6′ N, 76° 26.7′ W). For each pair, the two colonies (one with only yellow workers and one with only black workers) were placed 1 m apart. The two pairs of colonies were spaced >50 m away from other colonies. Given published rates of drift at different inter-colony distances (Seeley and Smith 2015), we assume that the foreign bees collected in each colony came from the adjacent hive (1 m away) and that drift from other colonies (50 m away) was negligibly low. To encourage drift, we placed the colonies in identical white hives on June 24, 2013, and an additional empty white hive was placed on both sides of the pair on July 2, 2013, so the experimental colonies were now at the center of a row of identical hive boxes, where most drifting occurs (Jay 1965).

Our goal was to collect 20 natal and 20 non-natal workers, identified by color, from each colony's brood nest on five dates (June 14, July 15, July 29, August 12, and August 26, 2013). On June 14, however, only natal workers were collected, because there were not yet any non-natal workers in the colonies. Also, on August 26, only 10 non-natal workers were sampled from one of the four colonies, because there were not enough non-natal workers to collect. The sampled bees were stored in Prefer fixative (Anatech Ltd.) until they were dissected in May 2014 to expose their ovaries. Each bee's ovaries were scored for ovary activation on a conventional 0–4 scale, where 0 = resting and 4 = completely activated (Pernal and Currie 2000). KJL performed the dissections and the scorings, and was kept blind to whether the workers in each group were natal or non-natal.

Statistical analyses

All statistical analyses were performed using R software version 3.0.3 and the packages lme4 and fitdistrplus (R Core Team 2014; Bates et al. 2014; Delignette-Muller et al. 2014). To see whether ovary activation was more likely in non-natal workers, we compared the number of natal and non-natal workers with activated ovaries versus inactive ovaries, using a Fisher's exact test. Workers with both ovaries scoring a 0 or 1 were marked as inactive. If either ovary scored a 2–4, the worker was marked as activated. Whereas other studies classify worker's ovaries as activated if their ovary score was 4, our classification of activated workers includes those with at least partially active ovaries (2 and 3). For this data analysis, all sampling dates were pooled.

We further analyzed the data in a generalized linear mixed effects model to test for differences in any level of ovary activation between natal and non-natal workers. This analysis used the summed ovary score for each individual to include the full range of ovary activation. Colony number was set as a categorical random effect. Whether an individual was natal or non-natal was analyzed as a categorical fixed effect. Birth colony (i.e., the colony from which an individual eclosed) was analyzed as a categorical fixed effect. The Julian date of sample collection was analyzed as a continuous fixed effect. The response variable was the summed ovary score for each individual, using a Poisson distribution, after confirming that the data fit a Poisson distribution. The best-fit model was determined by using likelihood ratio tests (Lewis et al. 2011) implemented in R using the anova() function. These tests compare nested models to see whether an additional factor improves the model over the simpler version without the factor added.

Results

Of the 400 natal bees sampled (100 from each colony), 40 had partially activated ovaries (at least one of the two ovaries scoring 2–3), and 360 had inactive ovaries (both ovaries scoring 0–1). Of the 310 non-natal bees sampled from the neighboring foreign colony, 24 had partially activated ovaries and 286 had inactive ovaries. None of the natal or non-natal bees had fully activated ovaries (ovary scoring 4). We found no significant difference in ovary activation between natal and non-natal workers (Fisher's exact test, $p = 0.36$). In Table 1, we report the mean level of ovary activation for bees from each of the four colonies when sampled in their natal colony and when sampled in the neighboring non-natal colony.

In the model assessing the full range of ovary activation, adding whether an individual was natal or non-natal did not improve the model versus a null model (natal/non-natal worker: $\chi^2 = 0.012$, $df = 1$, $p > 0.05$). Adding either birth colony or sample date did improve the model versus the null model (birth colony: $\chi^2 = 66.36$, $df = 3$, $p < 0.001$; sample date: $\chi^2 = 19.98$, $df = 1$, $p < 0.001$), consistent with other studies of worker ovary development that show variation based on colony and season (Jay 1968; Kropacova and Haslbachova 1969; Holmes et al. 2013). But, again, including whether an individual was natal or non-natal did

Table 1 Mean level of ovary activation for bees from each of the four colonies when sampled in their natal colony and when sampled in the neighboring non-natal colony (colonies were paired, with colonies 1 and 2 together, and colonies 3 and 4 together)

Colony	Bees sampled from	Mean ± SD level of ovary activation
1	Natal colony	0.26 ± 0.44
1	Non-natal colony	0.46 ± 0.62
2	Natal colony	0.68 ± 0.80
2	Non-natal colony	0.34 ± 0.49
3	Natal colony	0.40 ± 0.68
3	Non-natal colony	0.23 ± 0.48
4	Natal colony	0.07 ± 0.19
4	Non-natal colony	0.33 ± 0.52

not further improve either model (natal/non-natal worker + birth colony: $\chi^2 = 0.359, df = 1, p > 0.05$; natal/non-natal worker + sample date: $\chi^2 = 0.511$, $df = 1$, $p > 0.05$).

Discussion

The main finding of this investigation is that non-natal workers do not have greater ovary activation than natal workers in queenright colonies. Even when the full range of ovary activation was analyzed, whether a worker was natal or non-natal did not significantly predict the level of ovary activation. This indicates that the non-natal *A. mellifera* workers in this study did not respond to finding themselves in a foreign colony by partially activating their ovaries. This implies that workers that have joined a foreign colony are "lost losers" caught in an evolutionary trap, not "lost social parasites." Their lack of personal reproduction will not lead to direct or indirect fitness benefits as long as the foreign colony remains queenright, and they do not appear to be preparing for queen loss—they even refrain from partial ovary activation.

Why do these non-natal workers fail to detect and respond adaptively to being in a foreign colony? It seems likely that accidental joining of foreign colonies is uncommon in this species' evolutionary history. Colonies in apiaries often live 1 m apart in nearly identical hives, so orientation errors by workers are common. Colonies in the wild, however, live in tree cavities spaced an average of 850 m apart (Seeley 2007), so orientation errors by their workers are likely rare. Under the conditions in which the behavior of honey bees evolved, it seems likely that there has been little selective pressure for workers to behave selfishly when in a foreign colony. Even in an environment that supports a high density of wild *A. mellifera* colonies, we would still expect the number of accidentally drifted non-natal workers to be low (for example, increasing colony spacing from 1 m to 34 m reduces drone drift from 44–48 to 0–6 %, see Seeley and Smith 2015).

What about *Apis* species for which accidental drifting may be more common? *A. dorsata* and *A. florea* do naturally aggregate, with neighboring nests as close as 0.2 and 1.7 m apart, respectively (Paar et al. 2002; Wattanachaiyingcharoen et al. 2008). Drifted non-natal workers may be common in these species, making it more likely that they exhibit reproductive parasitism following accidental joining of a foreign colony. The same may well be true for colonies of other social insects, such as *Bombus* bees, *Halictid* bees, and *Polistes* wasps. All of these typically have much higher colony densities than *A. mellifera* (e.g., Knight et al. 2005; Sumner et al. 2007; Ulrich et al. 2009), and the workers have been shown to drift (e.g., Lopez-Vaamonde et al. 2004; Sumner et al. 2007; Ulrich et al. 2009). Although partial ovary activation by drifted workers was not observed in *A. mellifera*, it may be found in other species whose colonies live close together naturally, and hence their workers may have evolved an adaptive response to being in a foreign colony.

We have shown that non-natal *A. mellifera* workers do not have a higher level of ovary activation in queenright foreign colonies, contrary to the hypothesis that drifted workers are lost bees that become social parasites. If a colony does become permanently queenless, then non-natal workers will lay more eggs than natal workers, though these non-natal workers are probably not joining the colony after queen loss (Chapman et al. 2009b, 2010). How can we explain that non-natal workers obtain higher reproduction than natal workers if the colony becomes queenless? One possibility is that the bees in Chapman's (2010) study were not lost and instead had actively entered the queenright colony and primed themselves for reproduction, unlike our lost bees. Alternatively, it could be that non-natal workers are more sensitive to queen loss than natal workers and thus activate their ovaries quickly when a colony loses a queen, or swarms. How exactly non-natal workers gain the upper hand once a colony becomes queenless remains an area for further study.

In conclusion, our results show that lost non-natal workers do not partially activate their ovaries to give themselves a head start on reproduction. This suggests that drifted workers living in foreign colonies lack an adaptive response, making them "lost losers" stuck in an evolutionary trap.

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References

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4

Beekman M, Oldroyd BP (2008) When workers disunite: intraspecific parasitism by eusocial bees. Annu Rev Entomol 53:19–37. doi:10.1146/annurev.ento.53.103106.093515

Birmingham AL, Hoover SE, Winston ML, Ydenberg RC (2004) Drifting bumble bee (Hymenoptera: Apidae) workers in commercial greenhouses may be social parasites. Can J Zool 82:1843–1853. doi:10.1139/z04-181

Chapman NC, Makinson J, Beekman M, Oldroyd BP (2009a) Honeybee, *Apis mellifera*, guards use adaptive acceptance thresholds to limit worker reproductive parasitism. Anim Behav 78:1205–1211. doi:10.1016/j.anbehav.2009.08.007

Chapman NC, Nanork P, Gloag RS et al (2009b) Queenless colonies of the Asian red dwarf honey bee (*Apis florea*) are infiltrated by workers from other queenless colonies. Behav Ecol 20:817–820. doi:10.1093/beheco/arp065

Chapman NC, Beekman M, Oldroyd BP (2010) Worker reproductive parasitism and drift in the western honeybee *Apis mellifera*. Behav Ecol Sociobiol 64:419–427. doi:10.1007/s00265-009-0858-7

Dampney JR, Barron AB, Oldroyd BP (2002) Policing of adult honey bees with activated ovaries is error prone. Insectes Soc 49:270–274. doi:10.1007/s00040-002-8313-z

Delignette-Muller ML, Dutang C, Pouillot R, Denis JB (2014) fitdistrplus: help to fit of a parametric distribution to non-censored or censored data

Free JB (1958) The drifting of honey-bees. J Agric Sci 51:294–306

Holmes MJ, Oldroyd BP, Duncan M et al (2013) Cheaters sometimes prosper: targeted worker reproduction in honeybee (*Apis mellifera*) colonies during swarming. Mol Ecol 22:4298–4306. doi:10.1111/mec.12387

Jay SC (1965) Drifting of honeybees in commercial apiaries I: effect of various environmental factors. J Apic Res 4:167–175

Jay SC (1966a) Drifting of honeybees in commercial apiaries II: effect of various factors when hives are arranged in rows. J Apic Res 5:103–112

Jay SC (1966b) Drifting of honeybees in commercial apiaries III: effect of apiary layout. J Apic Res 5:137–148

Jay SC (1968) Factors influencing ovary development of worker honeybees under natural conditions. Can J Zool 46:345–347. doi:10.1139/z68-052

Knight ME, Martin AP, Bishop S et al (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Mol Ecol 14:1811–1820. doi:10.1111/j.1365-294X.2005.02540.x

Kropacova S, Haslbachova H (1969) The development of ovaries in worker honeybees in a queenright colony. J Apic Res 8:57–64

Lewis F, Butler A, Gilbert L (2011) A unified approach to model selection using the likelihood ratio test. Methods Ecol Evol 2:155–162. doi:10.1111/j.2041-210X.2010.00063.x

Lopez-Vaamonde C, Koning JW, Brown RM et al (2004) Social parasitism by male-producing reproductive workers in a eusocial insect. Nature 430:557–560. doi:10.1038/nature02705.1

Mattila HR, Reeve HK, Smith ML (2012) Promiscuous honey bee queens increase colony productivity by suppressing worker selfishness. Curr Biol 22:2027–2031. doi:10.1016/j.cub.2012.08.021

Moritz RFA, Kryger P, Koeniger G et al (1995) High degree of polyandry in *Apis dorsata* queens detected by DNA microsatellite variability. Behav Ecol Sociobiol 37:357–363. doi:10.1007/BF00174141

Nanork P, Paar J, Chapman NC et al (2005) Entomology: Asian honeybees parasitize the future dead. Nature 437:829. doi:10.1038/437829a

Nanork P, Chapman NC, Wongsiri S et al (2007) Social parasitism by workers in queenless and queenright *Apis cerana* colonies. Mol Ecol 16:1107–1114. doi:10.1111/j.1365-294X.2006.03207.x

Neumann P, Radloff SE, Moritz RFA et al (2001) Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): host finding and resistance of hybrid host colonies. Behav Ecol 12:419–428. doi:10.1093/beheco/12.4.419

Paar J, Oldroyd BP, Huettinger E, Kastberger G (2002) Drifting of workers in nest aggregations of the giant honeybee *Apis dorsata*. Apidologie 33:553–561. doi:10.1051/apido:2002040

Paxton RJ, Ayasse M, Field J, Soro A (2002) Complex sociogenetic organization and reproductive skew in a primitively eusocial sweat bee, *Lasioglossum malachurum*, as revealed by microsatellites. Mol Ecol 11:2405–2416. doi:10.1046/j.1365-294X.2002.01620.x

Pernal SF, Currie RW (2000) Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (Apis mellifera L.). Apidologie 31:387–409

Pfeiffer KJ, Crailsheim K (1998) Drifting of honeybees. Insectes Soc 45:151–167. doi:10.1007/s000400050076

Pfeiffer KJ, Crailsheim K (1999) The behaviour of drifted nurse honey bees. Insectes Soc 46:34–40. doi:10.1007/s000400050109

R Core Team (2014) R: a language and environment for statistical computing

Ratnieks FLW, Visscher PK (1989) Worker policing in the honeybee. Nature 342:796–797. doi:10.1038/342796a0

Roth KM, Beekman M, Allsopp MH et al (2014) Cheating workers with large activated ovaries avoid risky foraging. Behav Ecol 25:668–674. doi:10.1093/beheco/aru043

Schlaepfer MA, Runge MC, Sherman PW (2002) Ecological and evolutionary traps. Trends Ecol Evol 17:474–480. doi:10.1016/S0169-5347(02)02580-6

Seeley TD (2007) Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States. Apidologie 38:19–29. doi:10.1051/apido:2006055

Seeley TD, Smith ML (2015) Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. Apidologie. doi:10.1007/s13592-015-0361-2

Smith AA, Hölldobler B, Liebig J (2009) Cuticular hydrocarbons reliably identify cheaters and allow enforcement of altruism in a social insect. Curr Biol 19:78–81. doi:10.1016/j.cub.2008.11.059

Smith ML, Mattila HR, Reeve HK (2013) Partial ovary development is widespread in honey bees and comparable to other eusocial bees and wasps. Commun Integr Biol 6(e25004):1–4. doi:10.4161/cib.25004

Sumner S, Lucas E, Barker J, Isaac N (2007) Radio-tagging technology reveals extreme nest-drifting behavior in a eusocial insect. Curr Biol 17:140–145. doi:10.1016/j.cub.2006.11.064

Ulrich Y, Perrin N, Chapuisat M (2009) Flexible social organization and high incidence of drifting in the sweat bee, *Halictus scabiosae*. Mol Ecol 18:1791–1800. doi:10.1111/j.1365-294X.2009.04154.x

Velthuis HHW (1970) Ovarian development in *Apis mellifera* worker bees. Entomol Exp Appl 13:377–394

Visscher PK, Dukas R (1995) Honey bees recognize development of nestmates' ovaries. Anim Behav 49:542–544. doi:10.1006/anbe.1995.0074

Wattanachaiyingcharoen W, Wongsiri S, Oldroyd BP (2008) Aggregations of unrelated *Apis florea* colonies. Apidologie 39:531–536. doi:10.1051/apido:2008045