

ORIGINAL RESEARCH ARTICLE



Genotypic diversity in queenless honey bee colonies reduces fitness

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Summary

Honey bee queens mate with many males. The resulting genotypic diversity appears to enhance fitness of queenright colonies (those colonies with a reproductive queen present) which is difficult to measure, because measures of long-term fitness include successful matings of produced male sexuals (drones) and number of surviving swarms. The fitness of queenless colonies is, however, limited to worker-produced adult drones until natural colony death. Here we test the impact of genotypic diversity on fitness of queenless honey bee colonies, which were headed by queens inseminated with one, 10 and 20 drones or naturally mated. The data show that genetically diverse queenless colonies (20 subfamilies per colony) produced fewer adult drones, had a delayed onset of worker-derived drone flight activity, and a lower efficacy in drone production / per day colony life span compared to all other groups. Our data suggest that genotypic diversity may reduce fitness of queenless honey bee colonies, probably due to reproductive conflicts among subfamilies after queenloss.

La diversidad genotípica en colonias de la abeja de la miel sin reinas reduce la aptitud biológica

Resumen

Las reinas de la abeja de la miel se aparean con muchos individuos masculinos. La diversidad genotípica resultante parece mejorar la aptitud biológica de las colonias con una reina presente (es decir, aquellas colonias con una reina reproductiva presente), lo cual es difícil de medir, ya que las medidas de la aptitud biológica a largo plazo incluyen los apareamientos exitosos de los machos producidos (zánganos) y el número de enjambres supervivientes. Sin embargo, la aptitud biológica de las colonias sin reina está limitada a los zánganos adultos producidos por obreras hasta la muerte natural de la colonia. Aquí ponemos a prueba el impacto de la diversidad genotípica en la aptitud biológica de colonias de abejas sin reina, que fueron gobernadas por reinas inseminadas con uno, 10 y 20 zánganos o apareadas de forma natural. Los datos muestran que las colonias sin reina genéticamente diversas (con 20 subfamilias por colonia) produjeron menos zánganos adultos, tuvieron un retraso en la aparición de la actividad de vuelo de los zánganos producidos por obreras, y una menor eficacia en la producción de zánganos por día de vida de la colonia en comparación con los demás grupos. Nuestros datos sugieren que la diversidad genotípica puede reducir la aptitud de las colonias de abejas sin reina, probablemente debido a los conflictos reproductivos entre subfamilias después de la pérdida de la reina.

Keywords: *Apis mellifera*, evolution, drone, fitness, laying worker, polyandry

Introduction

One of the most intriguing aspects of the biology of the honey bee genus *Apis* is the extreme level of multiple mating by queens (= polyandry) (Woyke, 1956). The topic has received intensive

research in recent decades (e.g. Page, 1980; Crozier and Page, 1985; Boomsma and Ratnieks, 1996; Palmer and Oldroyd, 2000; Kraus *et al.*, 2004; Tarpy and Seeley, 2006; among many others). Although genetic diversity seems to result in healthier and more productive queenright colonies in various insects (honey bees: Mattila and Seeley, 2007;

Kraus *et al.*, 2011; bumble bees: Baer and Schmid-Hempel, 1999; Baer and Schmid-Hempel, 2003; ants: Hughes and Boomsma, 2004), the evolutionary mechanisms underlying polyandry are not fully understood. If a queen is only single mated the haplo/diploid sex determination system in the Hymenoptera should favour kin selection and the evolution of sociality (Hamilton, 1964). Polyandry, however, decreases the average relatedness of colony members and should therefore weaken selection that maintains eusociality (Wilson, 1971; Crozier and Pamilo, 1996). Thus, polyandry is still one of the unsolved riddles in the evolution of insect societies, particularly in honey bees.

Many hypotheses and several potential mechanisms have been proposed to explain the evolution of polyandry in social insects (Page, 1980; Crozier and Page, 1985; Ratnieks, 1990; Boomsma and Ratnieks, 1996; Crozier and Pamilo, 1996; Sherman *et al.*, 1998). Many theoretical and empirical studies focused on the genetic variance hypotheses (Keller and Hudson, 1994; Palmer and Oldroyd, 2000), which predict fitness gains through decreased intracolony relatedness resulting from multiple mating. Those gains, such as better winter survival (Mattila and Seeley, 2007) must outweigh the costs associated with polyandry, such as those due to multiple nuptial flights (Schlüns *et al.*, 2005). Indeed, the high genetic variance for polyandry in honey bees may result from balanced selection between individual queen and colony level (Kraus *et al.*, 2005); for example, risk during mating flight *vs* increased winter survival of the colony (Mattila and Seeley, 2007).

Mattila and Seeley (2007) reported a higher number of drones produced and a better winter survival of more genetically diverse queenright colonies, suggesting that genetic diversity enhances fitness. An evaluation of honey bee colony long-term fitness is, however, particularly difficult. The long-term fitness of honey bee colonies is the lifetime number of surviving swarms and successful matings by the produced male sexuals (drones). Colony phenotype characteristics enhancing or reducing the likelihood of producing viable swarms and large numbers of adult drones are the cues to colony fitness, but should be carefully interpreted, perhaps just as "tokens" of fitness, that are associated in some way with natural colony survival and reproduction (Page *et al.*, 1995). Moreover, the numbers of surviving swarms and of successfully mated drones as direct measures of colony fitness appear extremely difficult to evaluate over the life time of a honey bee colony.

This picture is, however, very different for hopelessly queenless colonies of arrhenotokous honey bee subspecies, where laying workers produce only drone offspring. Such colonies are doomed, and only have a very limited future, determined by the life span of the remaining work force. Hopelessly queenless colonies cannot therefore produce viable swarms, leaving laying worker produced drone offspring as the only evident measure of fitness. Nevertheless, laying workers colonies can successfully produce a considerable number of adult drones (Page and Erickson, 1988), suggesting that the fitness of

queenless colonies is relevant at the population level (Moritz *et al.*, 1998). Supporting evidence is provided by Hepburn (1994) showing that at least 8% of the colonies observed over a five year period became hopelessly queenless and eventually dwindled. Despite these obvious advantages of hopelessly queenless colonies as a model system to understand the evolution of extreme polyandry in honey bees, to our knowledge, no single study has addressed the potential impact of genotypic diversity on their fitness.

Here we evaluated for the first time the impact of genotypic diversity on the fitness of queenless honey bee colonies. We used instrumentally inseminated queens to generate groups of queenless colonies with distinct differences in genotypic diversity. We then used the number of adult drone offspring produced, the onset of worker produced drone flight activity, and the number of drones produced per day of colony life span as more direct measures of colony fitness until natural colony death. Given that increased genotypical diversity enhances fitness of queenless honey bee colonies, we expect positive effects on our fitness estimates. If, however, genotypic diversity is detrimental, we expect negative effects.

Materials and methods

Two colonies of *A. m. carnica* were established at an apiary in Kortowo (UWM University, Poland). Sister-queens were reared from those colonies in a single breeding colony following routine techniques (Wilde, 1994) to control for any potential impact of colony environment on queen quality. The queens were randomly assigned to groups and were either inseminated with semen of: a single drone (N = 8); of 10 drones (N = 5); or 20 drones (N = 3), using the mixed sperm technique (Skowronek *et al.*, 1995). Before and after insemination, the queens were kept and treated as described by Woyke *et al.* (2008). We used an equal sperm volume (8 ml) for each queen to control for any potential impact of semen volume. Drones used for insemination originated from four equally strong unrelated colonies and were randomly distributed over the treatment groups. These inseminated queens, together with naturally mated ones (N = 4) as controls for the effects of insemination, were introduced into colonies. After overwintering, standardized queenless splits of equal strength from each colony were introduced into standardized nucleus hives (~1500 bees, one honey comb, one pollen comb and one empty comb in each) to further limit potential effects of different starting conditions. We used multi-super nucleus hives with six combs in the box, on frames measuring 215 x 163 mm – mini-plus nucleus (Siuda *et al.*, 2011). There were 14 nucleus colonies in the group inseminated with a single drone, 17 with 10 drones, 9 with 20 drones and 13 with naturally mated queens, a total of 53 hives.

For statistical analysis the results for nucleus originating for the same initial colony were pooled. The number of adult drones

produced was counted on a daily basis using standard drone traps at the flight entrances (Drescher, 1975) for all splits until their natural death. To estimate colony fitness, we used the total number of laying worker produced drones and the onset of drone flight activity. While the former is obvious, the latter may also play a role because later produced drones are likely to miss the peak time window for local queen mating flights. Moreover, the onset of drone flight activity also constitutes an estimate of the time needed to produce worker-derived sexuals. Finally, we divided the total number of drones produced by the life span of each colony in days to provide an estimate of colony efficacy in drone production.

Spearman rank correlations were performed between the number of produced adult drones and the number of inseminations. Kruskal/Wallis ANOVAs and multiple comparisons of mean ranks for all groups were performed to test for differences in the total number of produced drones, the onset of drone flight activity and number of produced drones per day. For statistical analyses, we used the software package STATISTICA.

Results

After queen loss, six colonies died before they produced any drones (one colony each from the one drone and the naturally mated group; two colonies each in the 10 and 20 drones groups respectively). The remaining 53 colonies produced a total of 1462 adult drones until the natural death of the colonies after an average of 77 ± 10 days. The number of adult drones produced and the number of inseminations were significantly negatively correlated ($r = -0.82$, $p < 0.001$; Fig. 1). The number of inseminations had a significant effect on the number of drones produced, with colonies inseminated with 20 drones producing the fewest (Kruskal Wallis ANOVA: $H(3,19) = 12.13472$, $p = 0.0069$, Fig. 2). The onset of drone flight activity was not significantly different between the groups (Kruskal Wallis ANOVA: $H(3, N = 19) = 7.170500$, $p = 0.0667$; Fig. 3). Finally, the number of drones produced per day life span of the queenless units was significantly different between the groups (Kruskal Wallis ANOVA: $H(3, N = 19) = 10.40105$, $p = 0.0154$ Fig. 4)

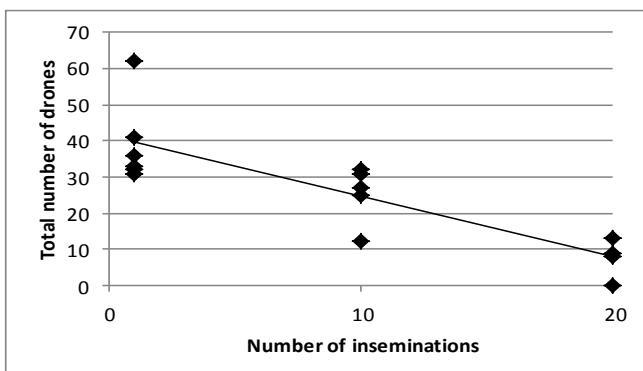


Fig. 1. Correlation of the number of laying worker produced adult drones with the number of inseminations.

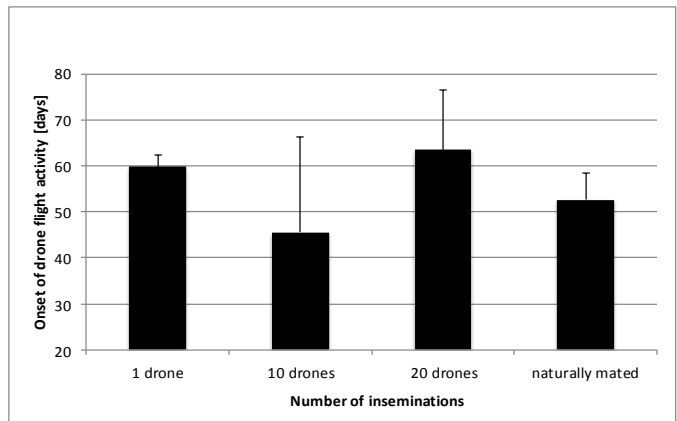


Fig. 2. Total number (mean \pm SD) of laying worker produced adult drones in the nucleus colonies under the different treatments:

1 = inseminated with semen of a single drone; 10 = inseminated with 10 drones; 20 = inseminated with 20 drones; and naturally mated.

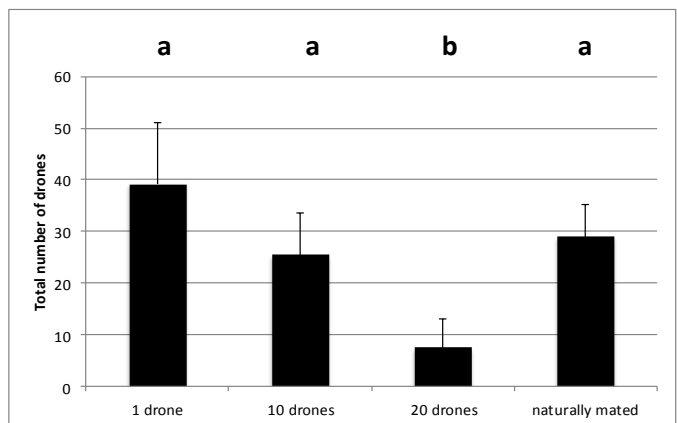


Fig. 3. Onset of laying worker produced drone flight activity in the nucleus colonies after queenloss under the different treatments:

1 = inseminated with semen of a single drone; 10 = inseminated with 10 drones; 20 = inseminated with 20 drones; and naturally mated. Means \pm sd are shown. Different letters (a, b) indicate significant differences for $P < 0.05$.

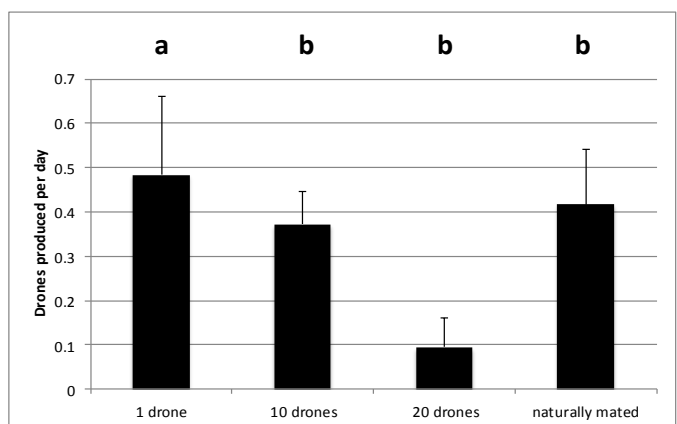


Fig. 4. Number of produced adult drones per day life span of queenless honey bee colonies: 1 = inseminated with semen of a single drone; 10 = inseminated with 10 drones; 20 = inseminated with 20 drones; and naturally mated. Means \pm sd are shown. Different letters (a, b) indicate significant differences for $P < 0.05$.

Discussion

Given that the fitness of queenless colonies is relevant at the population level (Hepburn, 1994; Moritz *et al.*, 1998), our data do not support the genetic variance hypotheses, because the colonies with the highest number of artificially generated subfamilies produced fewer drones than all other groups and had a lower efficacy in drone production per day life span. This suggests that genotypic diversity in queenless honey bee colonies reduces fitness.

It is noticeable that six colonies failed to produce any adult drones and consequently had a fitness of zero, which has already been reported earlier (Page and Erickson, 1988). One possible explanation could be selective egg removal behaviour, so called worker policing, in which workers prevent worker reproduction by other workers (Ratnieks, 1988; Ratnieks and Visscher, 1989). Although such behaviour should collapse when a colony becomes hopelessly queenless (Miller and Ratnieks, 2001), it can nevertheless persist (Chaline *et al.*, 2004), which would result in worker-laid eggs being removed, and may explain the observed lack of adult drone production in some colonies.

Artificial Instrumental insemination may not necessarily reflect natural mating in honey bees, but no significant effect of the insemination sequence was found (Woyke, 1963; Schlüns *et al.*, 2004). Instead, patriline frequencies in colonies headed by artificially instrumentally inseminated queens strongly depend on the semen volume of the respective drones (Schlüns *et al.*, 2004). Since we used the sperm mix technique (Skowronek *et al.*, 1995) and equal sperm volumes per queen, differences in sperm numbers between drones should be equalized, and therefore the situation is likely to reflect natural matings.

In contrast to previous studies using queenright colonies (e.g. Mattila and Seeley, 2007 among others), our data suggest a detrimental effect of genotypic diversity on colony fitness in hopelessly queenless colonies. Depending on the naturally occurring frequency of hopelessly queenless colonies (Hepburn 1994) and on the actual contribution of laying workers to population fitness (Moritz *et al.*, 1998), this supports the view that other more proximate factors such as sperm limitation (Kraus *et al.*, 2004) may be feasible alternative explanations for the evolution of extreme polyandry in honey bees. Alternatively, but not mutually exclusive, queenless honey bee colonies might be special with respect to reproduction. Indeed, first of all egg removal behaviour (policing), which normally prevents successful worker reproduction in queenright honey bee colonies (Ratnieks, 1988; Ratnieks and Visscher, 1989; Pirk *et al.*, 2002; Neumann *et al.*, 2003; Pirk *et al.*, 2003; Pirk *et al.*, 2004) has to collapse before worker reproduction can start (Miller and Ratnieks, 2001). Even more crucial is that upon queen loss workers compete with each other for reproductive dominance using pheromones and/or physical aggression (cf. Neumann and Hepburn, 2002; Neumann and

Moritz, 2002; Wossler, 2002; Diemann *et al.*, 2007; Pirk *et al.*, 2011). Eventually, dominance hierarchies are established and workers from only one or a few subfamilies dominate reproduction (Moritz *et al.*, 1996). Thus, a higher genotypic diversity of the work force may result in a longer time window to resolve reproductive conflicts among subfamilies. The variation, although only just not significant, in the onset of flight activity by the laying worker produced drones could indicate that a prolonged reproductive conflict may have reduced the reproductive output and fitness of the colonies with a higher genotypic diversity. A longer persistence of egg removal behaviour (worker policing) could be the underlying mechanism behind the delay in genotypically more diverse colonies (Fig. 3). One could speculate that in a genetically more diverse colony, the breakdown of worker policing (Miller and Ratnieks, 2001) may be more delayed or removal of worker-laid eggs policing may even persist (Chaline *et al.*, 2004). Moreover, the genotypically more diverse colonies also showed the lowest efficacy in terms of drones produced per day life span. In any case, the lack of respective behavioural observations in our data set obviously prevents more insights into the underlying mechanisms for explaining the observed variation.

In conclusion, our results suggest that although evidence accumulates that an increased genotypic diversity may be beneficial for queenright honey bee colonies (e.g. Tarpy and Seeley, 2006; Mattila and Seeley, 2007), it appears to be detrimental for colony fitness in queenless ones, probably due to increased reproductive conflict among genetically diverse subfamilies. Since the balance between cooperation and conflict appears to be fragile in insect societies, theories on the evolution of polyandry in the social Hymenoptera should take into account that benefits derived from polyandry in queenright colonies must outweigh any resulting costs not only in individual queens (e.g. associated with multiple mating flights), but also in queenless units. We suggest testing this idea in a variety of other social insect species.

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