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ORIGINAL RESEARCH ARTICLE

Mating frequency in *Apis mellifera iberiensis* queens

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Summary

Honey bees exhibit high levels of polyandry. This mating behaviour provides high levels of genetic diversity within the colonies which increases colony productivity, queen fitness and resistance to diseases. We investigated the mating frequency in two Iberian populations of *Apis mellifera iberiensis*, one from northern Spain inhabiting the Cantabrian Mountains and showing west European (M) mitochondrial haplotypes, the other located in south-eastern Spain close to the Mediterranean Sea and bearing African (A) mitochondrial DNA. Queens of the northern apiaries mated with 8 - 25 drones (average 15.73 ± 4.58), those of the south-eastern with 10 - 29 drones (average 18.92 ± 5.07), a difference that was significant ($p = 0.036$). Genetic relatedness among workers was 0.30 ± 0.02 in both populations. It is discussed whether climatic conditions and genetic background may influence honey bee queen mating behaviour.

Frecuencia de apareamiento en reinas de *Apis mellifera iberiensis*

Resumen

La abeja de la miel exhibe altos niveles de poliandría. Este comportamiento de apareamiento produce altos niveles de diversidad genética en las colonias lo cual aumenta la productividad de las mismas, la adaptabilidad de la reina y la resistencia a las enfermedades. Hemos investigado la frecuencia de apareamiento en dos poblaciones ibéricas de *Apis mellifera iberiensis*, una del norte de España (Cordillera Cantábrica) con haplotipos mitocondriales de Europa occidental (M), y otra localizada en el sureste español cerca del Mar Mediterráneo portando ADN mitocondrial africano (A). Las reinas de los apiarios del norte se aparearon con 8-25 zánganos (media 15,73 ± 4,58), y las del sureste con 10-29 zánganos (media 18,92 ± 5,07), diferencia que es significativa ($p = 0,036$). La relación genética entre las abejas obreras fue de 0,30 ± 0,02 en ambas poblaciones. Se discute si las condiciones climáticas y el origen genético puede influir en el comportamiento de apareamiento de las reinas de abejas.

Key words: honey bee, polyandry, evolutionary lineages, climate, Iberian Peninsula

Introduction

It is well known that honey bee queens mate with more than one drone during their nuptial flights (Hughes *et al.*, 2008). Polyandry has been extensively studied in the different *Apis* species and subspecies (reviewed in Palmer and Oldroyd, 2000; Crozier and Fjerdinstad, 2001) including the observed, estimated and effective number of matings (see Tarpy *et al.*, 2004 and further references therein). Furthermore, polyandry is widespread among species of social Hymenoptera. A number of explanations have been put forward to account for the high mating frequency of the honey bee (Crozier and Page, 1985; Tarpy and Page, 2001; Palmer and Oldroyd, 2000). There is now experimental evidence that multiple mating can enhance colony productivity, increase resistance to disease, and improve division of labour, a phenomenon known as task specialization (Oldroyd and Fewell 2007; Oldroyd *et al.,* 1992; Tarpy and Page, 2002; Tarpy, 2003; Jones *et al.*, 2004; Tarpy and Seeley, 2006; Mattila *et al.*, 2008). Another explanation is the sperm limitation hypothesis (Schlüns *et al.*, 2005) that predicts that honey bee queens mate with as many drones as necessary to ensure the complete filling of her spermatheca.

Mating frequency in the honey bee is likely to be influenced by racial background. Polyandry observed in naturally mated queens of eight subspecies of *Apis mellifera* ranged from 5 to 44 (Franck *et al.*, 2000); the highest values were observed in the South-African *A. m. capensis* (24-44 in 20 colonies analysed by Estoup *et al*., 1995) and the lowest in *A. m. sicula* (5-12 in 98 colonies analysed by Franck *et al*., 2000). It is known that drones of the African *A. m. capensis* have fewer sperm (Buys, 1990) than drones of the European *A. m. carnica* (Schlüns *et al*., 2003), which could explain the higher mating frequencies of *A. m. capensis* queens in comparison to those of *A. m. carnica* (Kraus *et al*., 2004). Likewise, environmental conditions may influence mating frequency as suggested by the results of Kraus *et al*. (2004), who found low mating levels (3-13) in *A. m. carnica* honey bee populations located on islands, where drone density was low and there were bad weather conditions (Alber *et al*., 1955; Neumann *et al*., 1999a,b).

Honey bee populations of the Iberian Peninsula (*Apis mellifera iberiensis* Engel, 1999) show a marked gradient in genetic background (Cánovas *et al*., 2008 and references therein), as northern populations are more similar to the black European *A. m. mellifera*, whereas those from the south are more similar to African *A. m. intermissa*. Likewise, there is a notable shift from a Mediterranean climate in the south to an oceanic climate in the north. We have conducted a preliminary study on the mating frequency of Iberian honey bee by investigating three apiaries from Asturias (north Spain) located in sharp mountain valleys that are *mellifera*-like, and compared them with other three *intermissa*-like apiaries located in Murcia (southeast Spain) in semiarid and open habitats. The aim is to investigate the extent of influence of racial background and environmental conditions in the mating behaviour of these two well-differentiated groups of honey bee populations.

Materials and Methods

Sampling

Three apiaries from Asturias (northern Spain) and three from Murcia (southeastern Spain) were sampled. In the first region apiaries are distantly located from each other (Valle de Lago-Somiedo: seven colonies; Felechosa: eight colonies; Campo de Caso, seven colonies) by 20 km or more, and separated by deep valleys and high peaks of Cantabrian Mountains (1600 m altitude or more). Climate is of oceanic type (abundant rainfall, 1.000-2.000 mm per year and 12-14ºC on average). Apiaries from the south-east are located in litoral (Escombreras valley: nine colonies) or sublitoral (Pto. del Garruchal: ten colonies; Cañada de la Cruz: seven colonies) sites, and are likewise apart from each other by 20 km or more and separated by a hilly terrain. Climate is much drier and warmer (rainfall 350 mm per year and 15-19ºC on average). These two north and southeastern Spanish regions are separated by 860 km. Honey bee workers were taken from the inner frames. All colonies were headed by naturally mated un-reared queens. Samples were stored in absolute ethanol at -20ºC until DNA processing.

Evolutionary lineage determination

Determination of the evolutionary lineage (M or A) was performed following the routine procedure of Garnery *et al*. (1993).

Genotyping

One pair of legs was dissected from each worker honey bee. DNA was extracted using the Chelex® method (Walsh *et al*., 1991). Samples were genotyped with five microsatellite loci (Am059, Am005, Am098, Am109 and Am061, Solignac *et al*., 2003). One primer of each pair was fluorescence labelled (FAM, HEX and NED). The allelic fragment sizes were scored in a DNA sequencer (ABI 3100) with GeneScan 500 as internal size standard at the Genotyping Services of the University of Valencia.

Polyandry analysis

The genotype of the queen of each colony was inferred from the honey bee worker offspring. Haploid paternal genotypes of each worker were deduced by subtraction following Estoup *et al*. (1994). When a worker bee did not carry any of the alleles present in the genotypes of the queen and the drones it was considered to be a drifting bee. The effective number of matings (*m*) was calculated following the formula of Starr (1984), the estimated number of matings (*ke*) was computed as given in Neumann and Moritz (2000) and the relatedness among nestmate workers (*G*) was obtained in each colony using the formula from Pamilo (1993).

Statistical analysis

The differences in the observed, estimated and effective number of matings, genetic relatedness and number of drifters of the two types of colonies (A or M) were statistically analysed with non-parametric Mann-Whitney *U*-tests. The tests were performed by the Statistical Analysis Service at the University of Murcia.

Results

Haplotype and evolutionary lineage determination

All colonies from the northern region corresponded to the North European evolutionary lineage M and bear *A. m. mellifera* haplotypes, and all those from the south-eastern region belonged to the African evolutionary lineage as they showed *A. m. intermissa* haplotypes (Table 1).

Table 1. Mitochondrial haplotypes and genetic data for the 22 northern and 26 southern colonies of *Apis mellifera iberiensis*, sampled from three apiaries in each region. All values show mean ± standard deviation. N = number of workers genotyped per colony; k_o = number of observed matings; k_e = estimated number of matings; m_e = effective number of matings; G = worker relatedness within colonies. The number of bees within the samples that had drifted from other colonies is also shown.

Region	Apiary	Haplotype	N	K_o	K_e	m_e	G	Drifters
North	1	5 M4′, 2 M8	44.70±5.00	17.57±3.74	20.74±6.52	11.92±3.30	0.29±0.01	3.90±4.70
	2	7 M4′, 1 M8	41.90±4.90	15.13±3.87	17.67±5.85	9.08±1.34	0.31±0.01	3.10±4.20
	3	5 M5, 2 M12	38.10±5.90	14.57±5.99	18.59±9.63	10.47±5.38	0.31±0.03	3.30±4.30
Average			41.60±5.70	15.73±4.58	18.94±7.19	10.43±3.66	0.30±0.02	3.40±4.20
Southeast	1	1 A1, 8 A2	46.89±1.97	20.11±4.73	26.34±11.03	13.72±4.24	0.29±0.01	4.44±4.64
	2	7 A2	46.29±1.38	17.57±7.27	28.97±21.94	11.21±5.81	0.31±0.03	2.14±2.85
	3	3 A1, 6 A2, 1 A8	46.60±1.17	18.80±3.65	26.28±9.46	13.30±3.17	0.29±0.01	1.50±1.18
Average			46.60±1.50	18.92±5.07	27.03±13.71	12.88±4.31	0.30±0.02	2.70±3.30

Mating frequency in the northern Spanish colonies

A total of 915 (41.60 ± 5.70 on average) workers bees were genotyped and assigned to different patrilines. The sample sizes of each colony and apiary, numbers of the observed, estimated and effective matings, and the relatedness coefficient among workers and number of drifters found in the M colonies are summarised in Table 1. We detected drifting bees in 14 out of 22 colonies, with an average of 3.4 ± 4.2 (range 0-12) being found per colony sample. The genetic relatedness was calculated among nestmate workers (excluding the drifters) and averaged 0.30 ± 0.02.

Mating frequency in the south-eastern Spanish colonies

A total of 1212 (46.60 ± 1.50 per colony) honey bee workers were genotyped. Results are summarised as in north Spanish colonies (Table 1). Drifting bees were found in 17 colonies, varying in number from 0 to 14 (average 2.70 ± 3.30). The genetic relatedness calculated among nestmate workers (excluding the drifters) also averaged 0.30 ± 0.02.

Comparison between northern and south-eastern Spanish colonies

Three variables analyzed (observed, estimated and effective number of matings) were significantly different between the two groups of Iberian honey bee populations (Mann-Whitney *U*-tests: $U = 185$; $P = 0.036$ for K_o; $U = 187$; $P = 0.040$ for K_e, $U = 180.5$; $P = 0.028$ for m_e). The genetic relatedness and number of drifting bees were not significantly different (Mann-Whitney *U*-tests: $U = 192.5$; $P = 0.053$ for *G*; $U = 274.5$; $P = 0.814$ for drifters).

Discussion

When estimating the level of polyandry, we found a high number of bees whose genotype did not match that of the colony queen, in both the African and the west European colonies, which could lead to an overestimation of queen mating frequency. These bees may correspond to worker daughters of the old queens if queen replacement has taken place (Franck *et al.*, 2000), but microsatellite data allow to distinguish with high confidence the actual patrilines and thus we may assume that they probably represent drifting bees. Drifting may be frequent under particular environmental and apiary layout conditions such as high wind (Betts, 1932; Jay, 1965) and little space between hives. Furthermore, when colonies have abundant food reserves drifting bees are more easily admitted (Downs and Ratnieks, 2000). In our case sampling was conducted in a period of nectar and pollen abundance.

Mating frequencies observed in *A. m. iberiensis* populations are within the range detected in other *A. mellifera* subspecies, although a significant difference exists between the two groups of populations in the average number of matings. The variance in the mating frequency (degree of polyandry) is high among the colonies of both regions. In both northern and south-eastern apiaries the extreme values of polyandry were found within the same apiary. This indicates a considerable variance in queen mating frequency in Iberian populations, which may be due to varying environmental conditions at the time of individual nuptial flights, individual differences, or possibly genetic effects as found in *A. m. carnica* (Kraus *et al.*, 2005, Neumann *et al.*, 1999a,b). These authors concluded that the degree of polyandry is a heritable behavioural trait with remarkable genetic variance in relation to the environmental conditions, such as weather and drone density. Our colonies were located in habitats markedly differing in climate, as south-eastern colonies were situated in stable dry and warm environment but those from north Spain were placed in close valleys with abundant rainfall and humid and windy surroundings. It is known that queens show adaptive responses to unfavourable conditions and stay in the nest during bad weather conditions (Alber *et al.*, 1955). Thus it is likely that queens from northern populations perform fewer nuptial flights in order to avoid risks, leading to a lower mating frequency. Franck *et al.* (2000) postulated that honey bee queens modulate their number of nuptial flights according to climatic conditions. This would explain the large variance in queen mating frequencies among colonies within the same locality. In addition the number of drones making up the drone congregation areas, where they mate with a queen in rapid succession (Koeniger *et al.*, 1979), may vary depending on the stability of weather conditions. These are possibly more stable and less adverse in the south-eastern region, which allows queens to perform more successful nuptial flights during the long Spring period from March to June.

Concerning the genetic background, the results of Kraus *et al.* (2004) concerning the evolution of extreme polyandry in honey bees, predict that drones of African subspecies with smaller size would have fewer sperm than drones of European subspecies with larger body size, which may result in a higher level of polyandry as a consequence of the sperm-limitation hypothesis. This hypothesis states that African queens will evolve towards higher mating frequencies than European queens because of the lower average amount of sperm of African drones. The hypothesis may be valid for *A. m. iberiensis* queens, as these clearly differ in genetic background, those from the south are more African-like queens whereas those from the north are clearly related to west European populations (Cánovas *et al.*, 2008 and references therein).

In brief, these results show a small, but significant, difference in mating frequency between colonies from the North and the South-east of the Iberian Peninsula, which may be due to climatic or genetic effects.

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