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Mating frequency and genetic colony structure of the neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae)

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Abstract – So far, nearly all studies concerning the mating frequency of bumblebees have been conducted with temperate species, showing that single mating seems to be the predominant pattern in bumblebees. Studies involving tropical species, however, are still scarce. Here, we determined the mating frequency of queens of the tropical bumblebee species, *Bombus wilmattae* by using microsatellite genotyping based on a sample of nine colonies from Chiapas, Southern Mexico. A total of 204 workers were genotyped with microsatellite markers to infer the queen genotype and the number of males with which each queen had mated. Two of the nine queens were doubly mated and seven singly mated. In the colonies with the double-mated queens, the distribution of the patriline was not even, resulting in effective mating frequencies of 1.34 and 1.70, respectively, and an average relatedness of $g=0.58\pm0.06$.

Bombus wilmattae / bumblebees / eusocial Hymenoptera / mating frequency / microsatellites

1. INTRODUCTION

The mating frequency of queens in monogynous social hymenoptera determines the genetic colony structure and relatedness among the workers in the colony. Single mating results in a high intracolony relatedness of $g=0.75$ (Crozier and Pamilo 1996), but each additional mating reduces the relatedness and increases intracolony genetic variability. Because a high intracolony relatedness is one of the base arguments for the evolution of sociality (Hamilton 1964a, b), polyandry has gained strong attention from researchers during the last decades (Moritz et al.

1995; Boomsma and Ratnieks 1996; Schmid-Hempel and Schmid-Hempel 2000; Foster and Ratnieks 2001; Payne et al. 2003). Multiple mating is, however, a rare trait and seems to be only common in more derived, highly eusocial Hymenoptera like honeybees, wasps, leafcutter ants or army ants (Strassmann 2001).

Bumblebees are characterised by an annual life cycle, and their colonies are headed by a single queen. Their colony size is rather small, ranging from about 50 to 2,000 individuals per colony (Sladen 1912; Plath 1934; Michener and LaBerge 1954). Studies on the mating frequencies of bumblebee queens in European, North American and Japanese *Bombus* species using microsatellite data revealed that the majority of species are singly mated ($N_{\text{species}}=72\%$). However, there are also occasional reports on

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polyandry of bumblebee queens. For example, queens of the temperate climate species *Bombus hypnorum* and *Bombus mixtus* have been shown to mate with up to six males (Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000; Paxton et al. 2001; Sauter et al. 2001; Payne et al. 2003; Takahashi et al. 2008a, b; Kokuvo et al. 2009). For tropical *Bombus* species, we are only aware of a study by Garofalo et al. (1986), who described the polyandrous mating behaviour of *Bombus atratus* with up to three matings per queen. However, since these observations were conducted under highly artificial experimental conditions in small containers it remains unclear whether this also occurs under natural conditions. Also for *Bombus terrestris*, behavioural observation of matings turned out to be less reliable than those based on genetic analyses (Röseler 1973; Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000).

One of the main hypotheses for the evolution of polyandry suggest that the increased within-colony genetic variability, caused by polyandry, results in an enhanced resistance towards parasites (Sherman et al. 1988; Keller and Reeve 1994; Mattila and Seeley 2007). Indeed, increased genetic variability, based on artificial insemination with sperm from multiple males, leads to lower parasite loads in *B. terrestris*, even though this is a single-mated species under natural conditions (Baer and Schmid-Hempel 1999). Since parasite pressure and parasite load exhibit a longitudinal cline and are markedly higher in tropical regions (Moller 1998; Nunn and Altizer 2005; Poulin and Rohde 1997; Cumming 2000; Poulin and Morand 2004), one might expect that tropical bumblebees are under greater parasitic pressure and queens should be more likely to exhibit polyandry compared to *Bombus* spp. from temperate climates.

In this study, we analysed the mating frequency and genetic colony structure of the neotropical bumblebee, *B. wilmattae*, using microsatellite analysis to obtain insight into the genetic colony structure and mating behaviour of tropical bumblebees on a reliable basis.

2. MATERIALS AND METHODS

2.1. Species in focus

The bumblebee species used in this study was *B. (Pyrobombus) wilmattae*, a species whose distribution is restricted to Chiapas, the southernmost state of Mexico and Guatemala. Although *B. wilmattae* was considered conspecific with the more widely distributed species *Bombus ephippiatus* by some authors (Labougle 1990; Williams 1998) a more recent study placed it closer to *Bombus impatiens* (Hines et al. 2006).

2.2. Sampling

B. wilmattae workers ($n=216$) were sampled from nine colonies (C1-C9) from 2005 to 2008 in the vicinity of Unión Juárez, Chiapas, Mexico, close to the Guatemalan border (15°3'52.31" N, 92°4'52.32" W) (Figure 1). Samples were collected as whole individuals or as single hind legs of workers (non-lethal sampling, Holehouse et al. 2003) and stored in 95% ethanol at -20°C until DNA extraction.

2.3. Molecular analysis

DNA was extracted by using one leg of each individual following a *Chelex*-extraction protocol by Walsh et al. (1991). The sampled individuals were genotyped with five microsatellite markers (B100, B124, B126, B131, B132; Estoup et al. 1993, 1995). Additionally, four newly developed microsatellite markers (BTMS0065, BTMS0125, BTMS0126, BTMS0141; Stolle et al. 2009) were used for colony C9 (data not shown) to obtain sufficient resolution as the queen and the siring male shared identical alleles at two of the five initially used markers.

All individuals were genotyped following standard polymerase chain reaction (PCR) protocols and the obtained microsatellite fragments were scored in an automated DNA capillary sequencer (MegaBACE 1000) according to manufacturer's instructions.

2.4. Data analysis

The number of alleles (A_n) and the observed and the expected heterozygosities (H_o , H_e) were assessed

Figure 1. Sampling sites of the *Bombus wilmattae* colonies in the mountain region of the municipality Unión Juárez, Southern Mexico, near the Guatemalan border (Talquian C1, C2, C6, C7; Desenlace C3; Monte Perla C4; Chiquihutes C5, C9; América C8).

with the Excel Microsatellite Toolkit (Park 2001). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium were tested using GENEPOP (version 1.2; Raymond and Rousset 1995).

For each colony, the queen genotype, the father genotypes and the observed number of matings were directly inferred from the genotypic data of the workers applying Mendelian rules. Additionally, MATESOFT 1.0 (Moilanen et al. 2004) was used for inferring the mating frequencies in each of the nine colonies to independently confirm the genotype annotations. In all nine colonies, the maternally and paternally transmitted alleles could be unambiguously distinguished. The probability of not detecting identical genotypes of two different fathers (d_p) was calculated with $d_p = \prod (\sum q_i^2)$, where q_i is the frequency of the i th allele, with summation over all alleles at a locus and multiplication over all loci (Boomsma and Ratnieks 1996). In case of more than one mating of a given queen, the effective queen-mating frequency (k_E) was calculated according to Starr (1979, 1984), taking into account the proportion of workers fathered by each male. The intracolony relatedness (g_{ww}) between the workers was calculated according to Crozier and Pamilo (1996) assuming that males were unrelated to queens.

3. RESULTS

3.1. Population genetic parameters

The analysed loci were highly variable resulting in observed heterozygosities (H_o) ranging from 0.89 to 1 (Table I). There was no

significant deviation from HWE (global test, $P=0.97$) and no linkage disequilibrium among the tested loci ($P>0.09$). The non-detection error was $d_p=3.6\times 10^{-4}$ based on the parental generation's allele frequencies.

3.2. Observed and effective queen-mating frequency

From 216 genotyped workers, 204 (n_w , Table II) were used to determine the number of observed matings, the effective queen-mating frequency (k_E) and the intracolony relatedness for each colony (Table II). Genotyping completely failed for nine workers and three were identified as alien workers from a different colony, having no alleles in common with the mother queen. Colonies C2 and C4 were headed by doubly mated queens, whereas all others were headed by singly mated queens, resulting in an average mating frequency of 1.22 ± 0.44 . The deduced number of matings in all nine colonies was confirmed by using the software MATESOFT 1.0 (Moilanen et al. 2004). In colonies with doubly mated queens, the patriline lines were not evenly distributed with the dominant male siring 71% or 82%, respectively. This paternity skew was significant for colony C2 ($\chi^2=4.96$; $P=0.03$). In both cases, the effective mating frequency (k_E) was less than two (Table II) with an average effective mating frequency of 1.12 ± 0.25 . The intracolony relatedness (g_{ww}) among the workers inferred from the parental generation ranged from 0.54 to 0.75 with a mean of 0.71 ± 0.08 (Table II).

Table I. Given are the number of alleles (An), range and size of the microsatellite fragments in bp, and observed heterozygosity (H_o) as well as expected heterozygosity (H_e) per locus and population for all nine *B. wilmattae* colonies.

	B100	B124	B126	B131	B132	Population
An	11	6	8	8	4	37
Range	156–192	254–274	150–166	118–146	158–174	118–274
H_o	1.00	0.89	1.00	1.00	1.00	0.98 ± 0.02
H_e	0.92	0.72	0.83	0.86	0.71	0.81 ± 0.04

Table II. Given is the number of observed matings (m_{obs}), effective mating frequency (k_{E}), intracolony relatedness (g_{ww}) and number of analysed workers (n_{W}) of the nine colonies of *B. wilmatiae*.

Colonies	m_{obs}	k_{E}	g_{ww}	n_{W}
C1	1	1.00	0.75	23
C2	2	1.34	0.62	22
C3	1	1.00	0.75	24
C4	2	1.70	0.54	24
C5	1	1.00	0.75	23
C6	1	1.00	0.75	24
C7	1	1.00	0.75	23
C8	1	1.00	0.75	21
C9	1	1.00	0.75	20

4. DISCUSSION

Our results suggest that the neotropical bumblebee, *B. wilmatiae*, is predominantly a monandrous species, with seven out of nine analysed colonies being headed by a singly mated queen. However, in two of the analysed colonies, we found offspring of two males showing that also double mating occurs, albeit at a lower frequency. The mating frequency estimates can be considered highly reliable, since the non-detection error was very low due to the highly variable markers. Also the sample size of at least 20 workers per colony is sufficiently large to detect potential additional patriline unless these are very rare and genetically marginally represented in the colony. The results are in accordance with the mating frequencies determined for the majority of bumblebee species from temperate regions (Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000; Paxton et al. 2001; Sauter et al. 2001; Payne et al. 2003; Takahashi et al. 2008a, b; Kokuvo et al. 2009). Although the analysis of our *B. wilmatiae* samples reveals two double-mated colonies, the effective queen-mating frequency in these colonies was lower than two, as one father sired the majority of worker offspring. The pattern is very similar to that found in Swiss populations of *B. hypnorum*

where the majority of queens are also single mated and only few double matings occur (Schmid-Hempel and Schmid-Hempel 2000). Interestingly, in this particular species, there seems to be considerable geographical variation in mating frequency, since in Swedish *B. hypnorum* populations, queens mated up to six times (Paxton et al. 2001). As in our study, in both Swiss and Swedish populations of *B. hypnorum*, the effective mating frequency in the multiple-mated queens was always lower than the observed mating frequency due to the unequal contribution of the males.

Studies on the mating frequency and behaviour of tropical *Bombus* species are scarce so far and based on behavioural observations solely. In *B. atratus*, for example, a species which like *B. wilmatiae* is also found in tropical highland rainforests, Garofalo et al. (1986) described up to three matings per queen. However, because they were carried out under laboratory conditions, it is uncertain if these observations are also valid in the wild. Towards the equator, parasite pressure and parasite load show a distinctive cline, with tropical regions having the highest parasite pressure, a correlation which was found for numerous species from several taxa (Poulin and Rohde 1997; Moller 1998; Cumming 2000; Poulin and Morand 2004; Nunn and Altizer 2005). Given also that tropical bumblebees are exposed to more parasites, they might have evolved some form of adaptation towards a higher parasite pressure. Baer and Schmid-Hempel (1999, 2001) artificially inseminated *B. terrestris* queens with semen from a varying number of males and could show that colonies with a higher intracolony genetic variability had also a lower parasite load. In spite of the potentially enhanced parasite pressure on bumblebees in the tropics, *B. wilmatiae* did not evolve towards polyandry and increased intracolony genetic variability. Monandry seems to be the common mating pattern in this species and *B. wilmatiae* may have evolved other ways to cope with the higher parasite load typical for the tropics. In any case, our results show that high degrees of polyandry as in *B. hypnorum* remain the exception rather than the rule in bumblebee mating biology.

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Fréquence d'accouplement et structure génétique des colonies chez le bourdon néotropical *Bombus wilmattae* (Hymenoptera: Apidae)

Bombus wilmattae / insectes sociaux / fréquence d'accouplement / microsatellites / Mexique

Paarungsfrequenz und genetische Koloniestruktur der neotropischen Hummel *Bombus wilmattae* (Hymenoptera: Apidae)

Bombus wilmattae / Hummeln / eusoziale Hymenopteren / Paarungsfrequenz / Mikrosatelliten.

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