Ecology and Evolution

Outbreeding and lack of temporal genetic structure in a drone congregation of the neotropical stingless bee *Scaptotrigona mexicana*

Matthias Y. Mueller, Robin F. A. Moritz & F. Bernhard Kraus

Institute for Biology, Molecular Ecology Work Group, Martin-Luther-University Halle-Wittenberg, Hoher Weg 4, 06122 Halle(Saale), Germany

Abstract

Drone aggregations are a widespread phenomenon in many stingless bee species (Meliponini), but the ultimate and proximate causes for their formation are still not well understood. One adaptive explanation for this phenomenon is the avoidance of inbreeding, which is especially detrimental for stingless bees due to the combined effects of the complementary sex-determining system and the small effective population size caused by eusociality and monandry. We analyzed the temporal genetic dynamics of a drone aggregation of the stingless bee *Scaptotrigona mexicana* with microsatellite markers over a time window of four weeks. We estimated the drones of the aggregation to originate from a total of 55 colonies using sibship reconstruction. There was no detectable temporal genetic differentiation or substructuring in the aggregation. Most important, we could exclude all colonies in close proximity of the aggregation as origin of the drones in the aggregation, implicating that they originate from more distant colonies. We conclude that the diverse genetic composition and the distant origin of the drones of the *S. mexicana* drone congregation provides an effective mechanism to avoid mating among close relatives.

Introduction

Animal aggregations are frequent phenomena throughout the animal kingdom, and evolved in response to various ultimate and proximate causes depending on species and environmental context. Potential benefits of grouping behavior include antipredator effects, cooperative foraging, or energy saving (Krause and Ruxton 2002). One particular form of grouping behavior that particularly has caught the attention of evolutionary biologists are male aggregations or so-called "leks." The term "lek" or "lekking behaviour" was first used in the context of mating behavior of birds (Lloyd 1867), and describes the phenomenon of aggregations of males that gather at a particular place in space and time to wait for females to mate with (Höglund and Alatalo 1995).

Such lek mating systems are known from a wide variety of taxa, including vertebrate as well as invertebrate species. Among the latter, the male aggregations of many eusocial bee species are very spectacular with hundreds or even thousands of males forming a single aggregation. The best studied of all eusocial bees is the honey bee *Apis mellifera*, and its mating system probably represents one of the most panmictic mating systems among terrestrial animals (Baudry et al. 1998). Here, drones from hundreds of colonies gather in midair at so-called drone congregation areas, genetically representing nearly the whole surrounding population (Kraus et al. 2005; Jaffè et al. 2009). Thus, honey bee queens have the chance of mating with any drone from their population, which in combination with the high polyandry results in a nearly panmictic population structure. The most prominent adaptive explanation for the formation of large drone aggregations is inbreeding avoidance (Page 1980; Paxton 2005). Social hymenoptera are expected to be especially sensitive to inbreeding and easily prone to the so-called "extinction vortex" (Zayed and Packer 2005) due to the combined effects of the genetic load of the complementary sex determination system (Beye et al. 2003) and the small effective population size (Chapman et al. 2003). Therefore, any mating system that increases the chances for matings of unrelated individuals in eusocial hymenoptera would be selectively advantageous and an effective mechanism to break any potential extinction vortex.

Picture 1. Worker bees of *Scaptotrigona mexicana* guarding an entrance tube.

Queens of stingless bee species (Meliponini) are typically monandrous and mate with a single male at the beginning of the colony cycle (da Silva et al. 1972; Green and Oldroyd 2002; Palmer et al. 2002; Cameron et al. 2004). Polyandrous mating is the exception and was only reported for four species of Meliponini so far (Falcão and Contel 1991; Imperatriz-Fonseca et al. 1998; Paxton et al. 1999a; Paxton 2000). Mating with a related male, which shares one of the queen's *csd* alleles, generates extreme fitness costs because it results in 50% of the diploid offspring developing into sterile males and natural selection should favor any behavioral adaptation that prevents inbreeding in the first place. In stingless bees, drone aggregations are often substrate based near or directly on conspecific colonies and can last from a few days up to several weeks (Lopez and Kraus 2009). The drones, which never return to their mother colony once they left it, disperse every evening to feed on flowers and return in the morning from their roosts to congregate again. Studies on the composition of drone aggregations in three stingless bee species showed an extremely high genetic diversity of the participating drones. The number of drone-contributing colonies ranged from about 20 to 40 in genus *Scaptotrigona* (Paxton 2000; Kraus et al. 2005, 2008) up to 135 colonies in *Trigona collina* (Cameron et al. 2004). However, all previous studies are based on a single sampling event at a particular drone congregation allowing no insight into the temporal dynamics of the congregation. In this study, we therefore analyzed the genetic composition of a single drone aggregation of the stingless bee *Scaptotrigona mexicana* over a four-week time window and included a genetic characterization of colonies neighboring the drone aggregation. This approach allowed for not only determining temporal genetic turnovers within the drone congregation, but also to test for potential disassortative matings of unrelated males and queens.

Materials and Methods

Study species

Scaptotrigona mexicana is a common and abundant stingless bee of the Neotropics, distributed over Central America from around 24° to 8° north latitude (Ascher et al. 2007). It nests in tree cavities with pyramidal stacked layers of brood cells in the middle, surrounded by storage pockets. A guarded entrance tube is the only way into the colony (Picture 1). Wild Meliponini colonies are often used as honey sources by indigenous families, but *S. mexicana* is also kept in apiaries, the so-called meliponaries, for honey production. As important pollinators of many wildflowers and crops, *S. mexicana* has both ecological and economical importance. Colonies of *S. mexicana* potentially can rear reproductives throughout the year when conditions are favorable. However, the climate conditions at our sampling site are characterized by a distinct change between dry and wet season over the year. The heavy rainfalls during wet season inhibit mating flights, and high levels of drone rearing and mating season take place at the beginning of the dry season, when also food supply for the colonies is highest.

Sampling

Sampling was conducted at a meliponary of *El Colegio de la Frontera Sur* (*Ecosur*) in southern Mexico, Chiapas, 12 km

Picture 2. The meliponary where the sampling was conducted: a typical bee hut.

northwest of the city of Tapachula (14°95′N, 92°18′W), near the Guatemalan border. The meliponary consisted of an open and roofed hut where 26 colonies of *S. mexicana* were kept on wooden planks (Picture 2). Drone samples were taken from a single drone congregation comprising around 500 drones, located directly outside a conspecific colony on the meliponary at the beginning of the dry season 2007 (Picture 3).

A total of 150 drones were collected on four different days within a four-week period. This covered the whole duration of the drone congregation, after which it disappeared, thus being well within the typical time span for drone congregations of *S. mexicana* (Lopez and Kraus 2009). Between the four sampling days (27th of May, $n = 30$; 3rd of June, $n = 40$; 4th of June, $n = 40$; 24th of June, $n = 40$), time intervals were of seven, one, and 20 days, respectively. The different time intervals between the sampling days allowed for the measurement of the speed of potential genetic turnovers within the drone congregation. In order to determine the genotypes of drone producing queens from the colonies at the meliponary, worker brood was collected from each of the 26 colonies present there. All samples (drones and worker larvae) were kept in 95% ethanol at −20°C until genetic analyses.

DNA extraction and genotyping

Total genomic DNA was extracted from one hind leg of each drone and the anterior half of at least 12 larvae of each colony, using a standard Chelex protocol (Walsh et al. 1991). We used eight microsatellite loci that had initially been developed for other Apidae species (Estoup et al. 1993; Paxton et al. 1999b; Green et al. 2001) but proved to cross-amplify in *S. mexicana* (cross-species amplification) (Kraus et al. 2008). Two primer sets were assembled to be combined in two multiplex poly-

Picture 3. The drone congregation under study at the front of a wooden hive.

merase chain reactions. Set 1 consisted of six primers: T3–32, T4–171, T7–5, Tc3–302, Tc4–287, and B 124; Set 2 consisted of two primers: T1–35 and T8–40.

Each reaction (10 µL) contained the fluorescence-labeled primers (Set 1: 2.4 µL/Set 2: 0.8 µL), ready-to-use Promega PCR master mix (5 µL), H_2O (Set 1: 1.6 µL/Set 2: 3.2 µL), and the DNA template (1 µL) (Shaibi et al. 2008). Reactions with no DNA were included in all PCR plates as negative controls. The amplicon fragments were length determined with an automated MegaBACE® capillary sequencer using the Genetic Profiler software (Amersham Bioscience, UK). The microsatellite genotypes for all drones ($n = 150$) and worker larva (12 larvae per colony to deduce queen genotypes, 312 workers in total) were identified with FRAGMENT PROFILER (Amersham Bioscience).

Assigning drones to colonies

The queen genotypes of the 26 colonies of the meliponary were derived by assigning the maternal alleles from the genotypes of the 12 worker larvae of each colony. Because in *S. mexicana* males are haploid and queens are singly mated (Palmer et al. 2002), all workers from the same colony have the identical paternal allele and either one of the two maternal alleles. If the queen is homozygous at a given locus, a differentiation between paternal and maternal allele is not possible. In these cases, both alternative queen genotypes were used in further analysis. To test if drones originated from the 26 meliponary colonies on the sampling site, the drone genotypes were compared with the deduced genotypes of the queens—their potential mothers. In *S. mexicana*, queens dominate the production of males and there is no worker reproduction via laying of unfertilized eggs (Palmer et al. 2002). The allele combination of the eight loci for every single drone was checked to match those of the queens from the meliponary.

Population genetic analysis

Since drones are parthenogenetically produced in hymenoptera (arrhenotoky), queen genotypes can be directly inferred from their son's genotypes. The number of putative mother colonies and the genotypes of the queens were determined using COLONY 1.3, a maximum likelihood algorithm for sibship reconstruction based on the population allele frequencies (Wang 2004). The dataset was analyzed with COLONY with five replicate runs using different seed numbers to test whether stable results are obtained.

The number of colonies remaining undetected (nonsampling error) was calculated with a fitted Poisson distribution (Chapman et al. 2003), using the frequency of the null category as the nonsampling error with the program STATISTICA. The nondetection error (NDE; the probability of similar genotypes in different individuals by chance and

not by descent) was calculated based on the allele frequencies at the used marker loci following Boomsma and Ratnieks (1996).

To test the population substructuring between the sampling days, we used a Fisher's exact test for population differentiation based on the allele frequencies and calculated the pairwise F_{ST} values (Weir and Cockerham 1984) between the sampling days by employing the software package GENEPOP ON THE WEB (Raymond and Rousset 1995). Further, we used the software STRUCTURE 2.3.3 (nonadmixture model; burn-in length 100,000; run length 100,000; $K = 1$ to $K = 6$ subpopulations tested) to analyze the genetic composition and structure of the drone congregation and to infer the number of subpopulations from which the drones might have originated (Pritchard et al. 2000).

Results

Genetic characterization of the drone congregation

The eight microsatellite markers used had a sufficient variability for rigorous population genetic analyses ranging from three alleles for locus Tc4–287 to up to 19 alleles for locus T1–35. The average number of alleles per locus (A_n) over all drones was 11.75 ± 5.6 (mean $\pm$ standard deviation [SD]), the allelic richness (A_r) was 11.70 ± 5.8 (mean $\pm$ SD), and the effective number of alleles (A_e) was 5.21 ± 2.0 (mean $\pm$ SD). For all drones, the overall NDE was negligibly small 5.0×10^{-6} (Table 1), not substantially affecting our estimates. Based on the allele frequencies of the drones, the expected heterozygosity of their source ranged from 0.31 for locus Tc4–287 up to 0.87 for locus T8–40 with an average of $H_e = 0.76 \pm 0.18$ (Table 1). The Fisher's exact test for population substructuring among the four sampling days did not show any significant population differentiation. Similarly, pairwise F_{ST} did not differ significantly from zero (t-test) indicating no significant population substructure (Table 2). The analysis of the genotypic data with Structure 2.3.3 was run for one to six subpopulations and yielded the highest probability for $K = 1$ subpopulations. There is no recognizable substructuring among the four sampling days (tested with $K = 4$) (Figure 1).

Hence all four sampling days were pooled for further analyses and the sibship reconstruction.

Genetic characteristics of the meliponary queens

All genotypes of the queens of the 26 meliponary colonies (Q_M) could unambiguously be deduced from the larval genotypes by Mendelian inference (Table 1). For the queens, the average number of alleles per locus was 8.75 ± 3.5 (mean $\pm$ SD) with an allelic richness of 8.13 ± 3.1 (mean $\pm$ SD). The effective number of alleles was 5.05 ± 2.3 (mean $\pm$ SD). The average expected heterozygosity was 0.74 ± 0.19 and the average observed heterozygosity was 0.73 ± 0.19 ranging from 0.34 (H_e) and 0.33 (H_o) in locus Tc4–287 up to 0.88 (H_e) and 0.92 (H_o) in locus T8–40.

Assigning drones to meliponary queen

When comparing the 150 drone genotypes with the 26 deduced genotypes of the meliponary queens, not a single drone was found originating from the colonies in the apiary. Therefore, no meliponary queen could have been the mother of one of the males in the drone congregation.

Number of drone producing colonies and sibship reconstruction

Since there was no significant genetic population differentiation among the drones of all four sampling days, the genotypic data of all drones were pooled. These pooled data were used for estimating the number of drone-contributing colonies. All five replicate COLONY runs for sibship reconstruction over all 150 drones gave similar results. The average number of reconstructed queens (and thus colonies) was 54.6 ± 0.55 (mean $\pm$ SD) with a nonsampling error of 1.27 ± 0.08 (mean $\pm$ SD) undetected colonies (frequency of the null category of a fitted Poisson distribution). The number of drones per colony in the congregation (number of brothers) ranges from one to five. The highest proportion of drone-contributing colonies (45.5%) was represented by three males in our sample (Figure 2).

For further analyses, we used the estimated queen genotypes from the COLONY run with the best likelihood

Table 1. Population genetic parameters (mean and standard deviation [SD]) of the pooled drones (D_A), the drone-contributing queens (Q_D), and the meliponary queens (Q_M).

	n	$A_n \pm$ SD	$A_r \pm$ SD	$A_e \pm$ SD	$H_o \pm$ SD	$H_e \pm$ SD	NDE
D_A	150	11.75 ± 5.6	11.70 ± 5.7	5.21 ± 2.0	-	0.76 ± 0.18	5.0×10^{-6}
Q_D	55	11.75 ± 5.6	11.48 ± 4.5	5.10 ± 2.0	0.71 ± 0.17	0.73 ± 0.18	1.3×10^{-5}
Q_M	26	8.75 ± 3.5	8.13 ± 3.1	5.05 ± 2.3	0.73 ± 0.19	0.74 ± 0.19	7.8×10^{-6}

A_n, average number of alleles per locus; A_r, allelic richness; A_e, effective number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; NDE, nondetection error.

Table 2. Shown are the details of the pairwise tests for population differentiations (chi square [χ^2], degrees of freedom [df]) between all four sampling days and the pairwise F_{ST} values. After Bonferroni adjustment for multiple tests and the correction to a significance level of $P = 0.0083$, none of the pairwise tests for differentiation remained significant (SL).

Population pair	Fisher's exact test				Pairwise F_{ST} values
	χ^2	df	*P*-value	SL	F_{ST}
D_1 and D_2	10.9	16	0.817	n.s.	0.002 ± 0.003
D_1 and D_3	13.9	16	0.605	n.s.	-0.003 ± 0.003
D_1 and D_4	4.9	16	0.996	n.s.	-0.017 ± 0.002
D_2 and D_3	30.0	16	0.018	n.s.	0.014 ± 0.003
D_2 and D_4	19.6	16	0.240	n.s.	0.008 ± 0.002
D_3 and D_4	20.3	16	0.209	n.s.	0.001 ± 0.003

estimate. In this particular run, the average number of alleles of the reconstructed queens was 11.75 ± 5.6 (mean $\pm$ SD), the allelic richness 11.48 ± 4.45 (mean $\pm$ SD), and the effective number of alleles 5.10 ± 2.0 (mean $\pm$ SD). The expected heterozygosity was 0.73 ± 0.18 and the observed heterozygosity was 0.71 ± 0.19 (Table 1). Thus, there was no significant deviation from Hardy–Weinberg equilibrium ($\chi^2 = 14.11$, df $= 14$, $P = 0.44$). For the queens of the meliponary (Q_M) and the drone producing queens (Q_D), a pairwise F_{ST} value of 0.013 was calculated. A Fisher's exact test of population differentiation ($\chi^2 = 42.34$; df $= 16$; $P = 3.5 \times 10^{-4}$) showed a significant genetic difference between these two groups.

Temporal variations in drone congregation composition

To test if there is genetic variation in the composition of drone congregation over time, we compared the compositions and contribution of colonies to the aggregation on the four sampling days (Figure 3). Two colonies were present at all four

days, whereas drones from 16 colonies were only found at single days.

Discussion

In this study, we could show that the formation of drone congregations in the stingless bee *S. mexicana* is an effective behavioral mechanism to decrease the chances of inbreeding and to lower the probability of sister–brother matings. First and most strikingly, over a four-week time window, not a single genotyped drone originated from one of the 26 colonies in immediate proximity of the drone congregation. This implies that drones from nearby colonies, even though being attracted to aggregations of conspecific drones (Lopez and Kraus 2009), seem to actively avoid joining the close by congregation, thereby reducing the chances of mating with one of their sisters or otherwise close relatives. Indeed such disassortative mating would be highly adaptive for stingless bees, since colony reproduction via colony fission leads to clusters of closely related colonies in stingless bees (Wille 1983; Cameron et al. 2004). Hence, if there was random mate
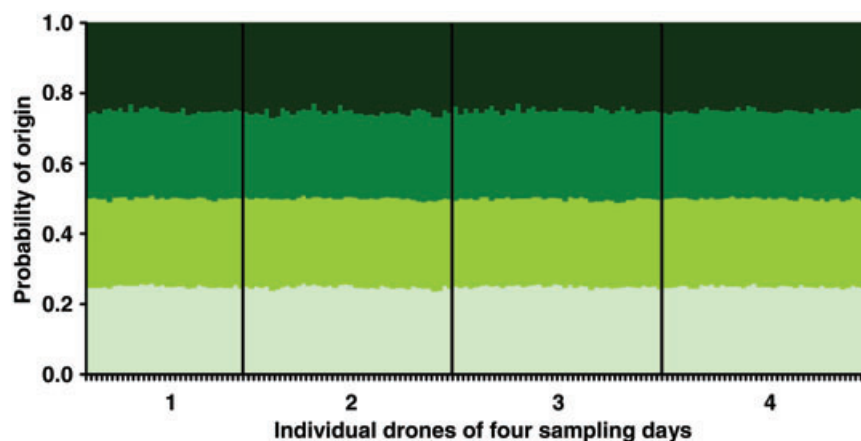

Figure 1. A graphical visualisation for the STRUCTURE 2.3.3 results for four assumed subpopulations (sampling days). All 150 drones have similar proportions of membership to the four pre-defined clusters (K=4) supporting their origin from one population.
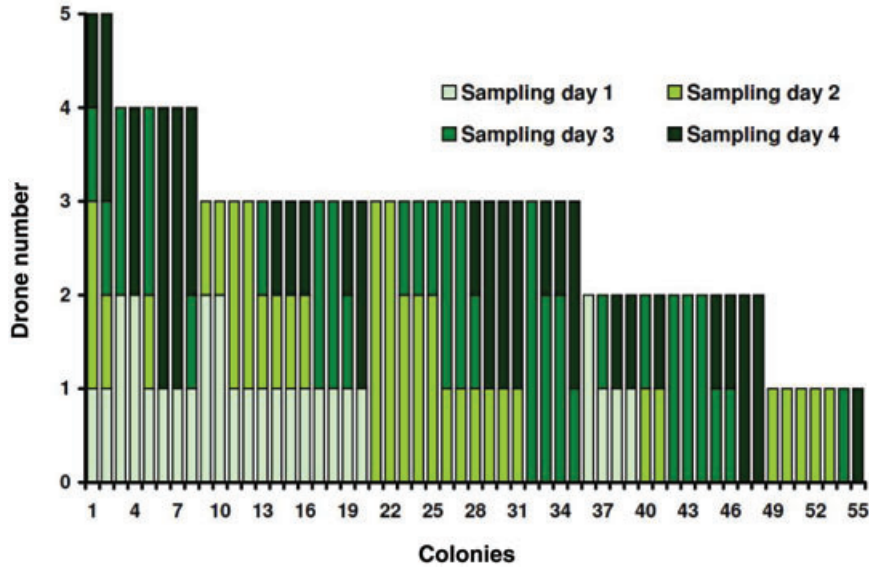
Figure 2. Distribution of the drones over the 55 reconstructed colonies. The number of drones per colony reaches from 1–5. The different colours indicate the sampling day of the particular drone.

choice among locally abundant males, queens would have a high probability to mate with related drones, unless the drones would leave the area and join more distant drone aggregations, as indicated by our results.

Since there were no other meliponaries in the vicinity, the drones of the congregation must have originated from the surrounding wild population. Because nothing is known about the flight ranges of drones in stingless bees so far, it is difficult to give an estimate on the size of the area this population is distributed over. Prospective studies on that topic could potentially use two methods to estimate the

flight radius of drones. One possibility is to mark and re-capture drones to measure the covered distance. The other would be to infer the colony density based on the number of colonies found within a defined area. However, both approaches would be confronted with the problem to detect inconspicuous individuals or colonies in difficult terrain in a tropical region. We estimated the number of surrounding drone producing colonies to be as high as 55 colonies. Even higher colony numbers were estimated for a population of the stingless bee *T. collina* with 132 colonies based on a sample of a single drone aggregation (Cameron et al. 2004).
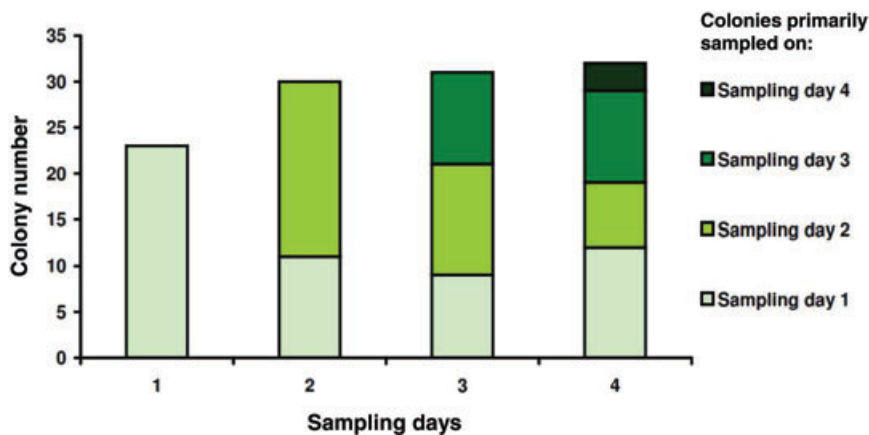

Figure 3. Cumulative number of reconstructed drone contributing colonies over time. The number of newly detected queen genotypes (derived from drone genotypes) decreases over the sampling days.

These numbers are comparable to the honey bee *A. mellifera* where drone congregations can be constituted of drones from 47 colonies, like found in wild African populations (Moritz et al. 2008), up to 240 in dense beekeeper settings (Baudry et al. 1998).

Despite the lack of genetic differentiation among the sampling days, the feral colonies reconstructed from the drone samples and the colonies of the meliponary showed a significant genetic differentiation. This is not surprising, given none of the drones of the aggregation originated from the colonies of the meliponary, which are derived from just a few colonies by artificial colony splits and are therefore close relatives. However, since stingless bee species propagate via colony fission and new colonies establish in close proximity to the mother nest, also under natural conditions clusters of closely related colonies may be common (Wille 1983; Cameron et al. 2004). Thus, the genetic structure of wild populations might be to some extent similar to the situation at a meliponary.

We observed no major temporal turnover of drone-contributing colonies in the aggregation over time. Although two colonies contributed drones on all four sampling days, others were just sampled on a single day. However, the detection of a decreasing number of new drone-contributing colonies over the sampling days can be easily explained by the successive approximation to the total number of colonies. The low nonsampling error of 1.27 colonies supports this assumption.

The common hypotheses for lek formation assume that the males of an aggregation are divided into dominant and subordinated males. This assumption however seems not applicable to highly eusocial Apidae due to the specific characteristics of their drone congregations, which are lacking display territories. Given the selective pressure to avoid inbreeding and the strongly male biased sex ratio, different evolutionary mechanisms might have driven the evolution of lek-like male aggregations in social bees than in other animal species.

In summary, monandrous stingless bee species such as *S. mexicana* have a considerable benefit from every strategy promoting outbreeding because the fitness loss for a colony due to inbreeding can be enormous. The scenario described by our data, where not a single drone from the meliponary could be detected in the congregation, describes a plausible mechanism for avoiding brother–sister matings, which would also help to overcome a potential extinction vortex (Zayed and Packer 2005). Since similar mechanism has been suggested to promote outbreeding in other stingless bees such as *T. collina* (Cameron et al. 2004) and *S. postica* (Paxton 2000), male overdispersal may be a general feature and widespread strategy in stingless bees to avoid inbreeding.

References

Ascher, S., J. G. Rozen, T. Schuh, R. G. Goelet, and J. Pickering. 2007. Discover life Apoidae species guide. Am. Mus. Nat. Hist. Available at www.discoverlife.org 2009-08-26.

Baudry, E., M. Solignac, L. Garnery, M. Gries, J-M Cornuet, and N. Koeniger. 1998. Relatedness among honeybees (*Apis mellifera*) of a drone congregation. Proc. R. Soc. B 265:2009–2014

Beye, M., M. Hasselmann, M. K. Fondrk, R. E. Page, and S. W. Omholt. 2003. The gene csd is the primary signal for sexual development in the honeybee and encodes an SR-type protein. Cell 114:419–429.

Boomsma, J. J., and F. L. W. Ratnieks. 1996. Paternity in eusocial Hymenoptera. Philos. Transac. R. Soc. B 351:947–975.

Cameron, E., P. Franck, and B. Oldroyd. 2004. Genetic structure of nest aggregations and drone congregations of the southeast Asian stingless bee *Trigona collina*. Mol. Ecol. 13:2357–2364.

Chapman, R. E., J. Wang, and A. F. G. Bourke. 2003. Genetic analysis of spatial foraging patterns and resource sharing in bumblebee pollinators. Mol. Ecol. 12:2801–2808.

da Silva, D. L. N., R. Succhi, and W. E. Kerr. 1972. Biological and behavioural aspects of the reproduction in some species of Melipona (Hymenoptera, Apidae, Meliponinae). Anim. Behav. 20:123–132.

Estoup, A., M. Solignac, M. Harry, and J-M Cornuet. 1993. Characterization of (GT)n (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. Nucleic Acids Res. 21:1427–1431.

Falcão, T. M. M. A., and E. P. B. Contel. 1991. Genetic variability in natural population of Brazilian bees: II electrophoretic data for PMG and MDH give evidence for multiple fertilizations in stingless bees. Rev. Bras. Genet. 14:47–59.

Green, C. L., and B. P. Oldroyd. 2002. Queen mating frequency, maternity of males, and diploid male production in the stingless bee *Trigona carbonaria*. Insectes Sociaux 49:196–202.

Green, C. L., P. Franck, and B. P. Oldroyd. 2001. Characterization of microsatellite loci for *Trigona carbonaria*, a stingless bee endemic to Australia. Mol. Ecol. Notes 1:89–92.

Höglund, J., and R. V. Alatalo. 1995. Leks. Princeton Univ. Press, Princeton, NJ.

Imperatriz-Fonseca, V. L., E. T. Matos, F. Ferreira, and H. H. W. Velthuis. 1998. A case of multiple mating in stingless bees (Meliponinae). Insectes Soc. 45:231–233.

Jaffè, R., V. Dietemann, R. Crewe, and R. F. A. Moritz. 2009. Temporal variation in the genetic structure of a drone congregation area: an insight into the population dynamics of wild African honeybees (*Apis mellifera scutellata*). Mol. Ecol. 18:1511–1522.

Kraus, F. B., N. Koeniger, S. Tingek, and R. F. A. Moritz. 2005. Temporal genetic structure of a drone congregation area of the giant Asian honeybee (*Apis dorsata*). Naturwissenschaften 92:578–581.

Kraus, F. B., S. Weinhold, and R. F. A. Moritz. 2008. Genetic structure of drone congregations of the stingless bee *Scaptotrigona mexicana*. Insectes Soc. 55:22–27.

Krause, J., and G. D. Ruxton. 2002. Living in groups. Oxford Univ. Press, Oxford, U.K.

Lloyd, L. 1867. Game birds and fowl of Sweden and Norway. Warne, London.

Lopez, J. C. G., and F. B. Kraus. 2009. Cherchez la femme? Site choice of drone congregations in the stingless bee *Scaptotrigona mexicana*. Anim. Behav. 77:1247–1252.

Moritz, R. F. A., V. Dietemann, and R. M. Crewe. 2008. Determining colony densities in wild honeybee populations (*Apis mellifera*) with linked microsatellite DNA markers. J. Insect Conserv. 12:455–459.

Page, R. E. 1980. The evolution of multiple mating behavior by honey bee queens (*Apis mellifera* L.). Genetics 96:263–273.

Palmer, K. A., B. P. Oldroyd, J. J. G. Quesada-Euán, R. J. Paxton, and W de J May-Itz. 2002 Paternity frequency and maternity of males in some stingless bee species. Mol. Ecol. 11:2107–2113.

Paxton, R. J. 2000. Genetic structure of colonies and a male aggregation in the stingless bee *Scaptotrigona postica*, as revealed by microsatellite analysis. Insectes Soc. 47: 63–69.

Paxton, R. J. 2005. Male mating behaviour and mating systems of bees: an overview. Apidologie 36:145–156.

Paxton, R. J., N. Weissschuh, W. Engels, K. Hartfelder, and J. J. G. Quezada-Euan. 1999a. Not only single mating in stingless bees. Naturwissenschaften 86:143–146.

Paxton, R. J., N. Wießschuh, and J. J. G. Quezada-Euán. 1999b. Characterization of dinucleotide microsatellite loci for stingless bees. Mol. Ecol. 8:685–702.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

Shaibi, T., H. M. G. Lattorf, and R. F. A. Moritz. 2008. A microsatellite toolkit for studying population structure in *Apis mellifera*. Mol. Ecol. Res. 8:1034–1036.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as amedium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. Genetics 16:1963–1979.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wille, A. 1983. Biology of the stingless bees. Ann. Rev. Entomol. 28:41–46.

Zayed, A., and L. Packer. 2005. Complementary sex determination substantially increases extinction proneness of haplodiploid populations. Proc. Natl. Acad. Sci. U.S.A. 102:10742–10746.

Supporting Information

Additional Supporting Information may be found online on Wiley Online Library:

Table S1. Given are the allele lengths in base pairs and frequencies of all microsatellite markers (T1–35, T3–32, T4–171, T7–5, T8–40, Tc3–302, Tc4–287, B124) for the following sample groups: drones of the four sampling days (D_1, D_2, D_3, D_4); pooled drones (D_A), the drone-contributing queens (Q_D), and the meliponary queens (Q_M).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.