

Male flight distance and population substructure in the bumblebee *Bombus terrestris*

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Summary

1. Bumblebees are important pollinators in natural as well as agricultural ecosystems. Estimates of foraging range, population size and genetic population structure so far have been based on worker samples alone. Here we include both males and workers in a population genetic analysis to infer the contribution of males to these important ecological parameters.
2. The population genetic (microsatellite) analyses of *Bombus terrestris* L. populations on the island of Cabrera (Spain) and Halle (Germany) revealed high heterozygosities (0.60 ± 0.08 to 0.77 ± 0.13) and neither a deviation from Hardy–Weinberg equilibrium nor linkage disequilibrium.
3. We detected five colonies (census population size) for the island population and 27 to 68 for the German mainland population. The genetic effective population sizes were $N_e = 7.5$ for the island and 40.5 to 102 for the mainland population respectively.
4. There was a significant genetic subdifferentiation between the male and the worker population samples, suggesting that males originated from different and/or more distant colonies than workers.
5. Based on the colony numbers, we estimated the flight range of males, which ranged from 2.6 km to 9.9 km, much further than worker flight ranges. Bumblebee-mediated pollen flow will therefore be much further than expected based on the foraging range of workers alone if males also contribute to pollination.

Key-words: *Bombus terrestris*, flight distance, microsatellite, population structure, social insects

Introduction

Pollinators are essential for maintaining the function of agricultural and natural ecosystems, because they facilitate the gene flow among populations of flowering plants (Kevan 1999; Kevan & Phillips 2001; Knight *et al.* 2005). Particularly, those pollinators transporting pollen over long distances are important in fragmented ecosystems because they can mediate gene flow between distant plant populations (Kearns & Thomson 2001; Cresswell & Osborne 2004). One of the most abundant and efficient groups of pollinators in temperate climates are the bumblebees (Fussell & Corbet 1992; Bingham & Orthner 1998; Kearns & Thomson 2001; Goulson 2003), which even can exceed the honeybee

Apis mellifera in pollination efficiency (Westerkamp 1991). In particular, bumblebees are renowned for more robust handling of flowers and buzz pollination (Kevan *et al.* 1993; Morandin, Laverty & Kevan 2001a,b; Goulson 2003). In addition, there are fundamental differences in foraging strategies between *Apis* and *Bombus*. Whereas honeybee foragers take advantage of sophisticated communication systems, with mass recruitment to highly rewarding food sources, bumblebees have a less-advanced communication system (Dornhaus & Chittka 2001, 2004). The bumblebee worker forages largely based on individual experience and as a result, bumblebee colonies have more scattered foraging grounds (Westerkamp 1991; Kearns & Thomson 2001; Goulson 2003). Finally, bumblebee nests have large foraging ranges covering areas of up to 47.7 km² (Chapman, Wang & Bourke 2003).

The life cycle of bumblebee colonies includes the production of sexuals, drones and queens. In contrast to workers, drones do not return to the nest once they have started mating

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flights in search for a queen (Free 1982). In several species, males establish distinct patrol routes (Darwin 1886; Haas 1949; Alford 1975; Kearns & Thomson 2001) where they leave pheromone marks to attract females (Bergström *et al.* 1967, 1981; Mauss 1994). In between these patrol flights, they also visit and pollinate flowers. Given the importance of long-distance gene flow for insect-pollinated plants in fragmented ecosystems, and given the potential for facilitating long-distance pollen flow by drones, it seems highly desirable to obtain more information on the actual flight distances of males to assess their pollination potential.

So far, all estimates of flight distance have been carried out for workers only, either indirectly by using molecular markers or directly by traditional field-based approaches (Walther-Hellwig & Frankl 2000a,b; Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005; Osborne *et al.* 2008; Wolf & Moritz 2008). All these studies, however, neglected the potential effects and impact that males might have on both pollination and population ecology. For pollination ecology, males might significantly contribute to the pollination service provided by colonies if male flight ranges exceed those of workers. For population ecology and genetics, male flight ranges are an essential parameter since they might expand mating ranges beyond the flight range of females and, therefore, also increase the genetic effective population size. The latter in turn is a crucial parameter for the conservation of bumblebee populations (Ellis *et al.* 2006).

Using microsatellite DNA analyses, we infer male flight ranges, estimate the number of colonies they originated from and determine effective population sizes in two *Bombus terrestris* L. populations, using both worker and male samples. This allowed comparison of the differences in flight ranges between workers and males and their potential impact on bee-mediated pollen flow.

Materials and methods

SAMPLING

We sampled a population of *B. terrestris* in the Botanical Garden of Halle/Saale (Germany) in two consecutive years (2004 and 2005). Individuals were caught with an insect net between 12 and 22 July 2004 (21 workers and 75 males) and from 30 May to 12 July 2005 (86 workers and 80 males) from flowers or in flight.

Additionally, an island population was sampled in the nature reserve on the Island of Cabrera (Spain) between 2 and 4 April 2003 (25 workers, 3 males) also using an insect net. Cabrera is a small island (13 km²) 10 km south-west of the Island of Mallorca (Spain). We screened the entire island, but only found bumblebees in the agriculturally managed central valley of the island, which was the only region with flowers in bloom during that time of the year.

After species identification in the field, all samples were immediately stored in ethanol (99%) until DNA processing. In the laboratory, each individual was again microscopically checked for species identity and sex using the identification key of Mauss (1994) and genetically confirmed by the restriction fragment length polymorphism (RFLP) methodology described by Murray *et al.* (2008).

DNA ANALYSIS

One leg of each individual was used for DNA extraction following a Chelex extraction procedure as described by Walsh, Metzger & Higuchi (1991). Individuals were then genotyped with up to eight microsatellite loci (B96, B100, B118, B119, B121, B124, B126, and B132; Estoup *et al.* 1993, 1995) following standard polymerase chain reaction (PCR) protocols (Kraus *et al.* 2005) in automated DNA capillary sequencers (ABI PRISM 310 and MegaBACE 1000) according to manufacturers' instructions.

POPULATION CHARACTERIZATION

Before embarking on classical population genetic analyses to test for population subdivision between workers and males, it is important to first assign each individual (both workers and males) to its putative mother colony. We performed four runs with different seed numbers using COLONY 1.3 (Wang 2004) for each population to determine the inferred mother queen genotypes, and used those with the highest *log* probability for further analyses. We used these queen genotypes rather than those of workers and drones for the population genetic analyses to obtain unbiased population comparisons that compensated for the different contributions of the colonies to the sample set. For calculating the number of colonies which remained undetected in our sample, we used a fitted Poisson distribution, using the frequency of the null category as our nondetection error. The genetic effective population size N_e was calculated using the formula for haplodiploid organisms by Wright (1933).

Allele frequencies, observed (H_o) and expected (H_e) heterozygosities and tests for deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were calculated with the software package GENEPOP (online version; Raymond & Rousset 1995). We performed a Fisher's exact test for population differentiation between worker and male-derived queens in the Halle 2004 and 2005 populations and calculated the pairwise F_{ST} values between these groups, again using GENEPOP. To obtain a comparable parameter for allelic diversity, we calculated allelic richness using the software HP RARE by Kalinowski (2005).

ESTIMATION OF FLIGHT RADIUS OF MALES

We estimated the flight radius of males indirectly from the density of worker-producing and/or drone-producing colonies. We first inferred the colony density based on the number of colonies detected (C_{workers}) within the mean maximum foraging range of *B. terrestris* workers of 2.39 km (range 0.87–3.9 km) as determined by Chapman *et al.* (2003) and the resulting mean maximum foraging area of 17.9 km². Assuming that all colonies produce males and assuming a constant colony density, we can derive the flight radius of males (r_{males}) from the number of colonies detected in the male sample. Colonies from which we only sampled males (C_{males}) were considered to be located outside the foraging range of the worker-producing colonies. Hence, we estimated the male foraging area (a_{males}) as:

$$a_{\text{males}} = \frac{C_{\text{males}}}{C_{\text{workers}}} \times 17.9 \text{ km}^2 \quad \text{eqn 1}$$

The male flight radius r_{male} was then calculated from the foraging area assuming a circular shape:

$$r_{\text{males}} = \sqrt{\frac{a_{\text{males}} + 17.9 \text{ km}^2}{\pi}} \quad \text{eqn 2}$$

Table 1. Shown are the genetic characteristics of the Cabrera and Halle 2004/05 populations

| | n | C | N_e | C_{φ} | C_{σ} | $F_{ST \varphi-\sigma}$ | C_j | N_{ej} | $A_n \pm SE$ | $A_r \pm SE$ | $H_O \pm SE$ | $H_E \pm SE$ |
|------------|-----|-----|-------|---------------|--------------|-------------------------|-------|----------|--------------|--------------|--------------|--------------|
| Cabrera | 53 | 5 | 7.5 | 4 | 1 | / | / | / | 4.4 ± 0.5 | 4.2 ± 1.6 | 0.60 ± 0.08 | 0.52 ± 0.12 |
| Halle 2004 | 112 | 27 | 40.5 | 13 | 14 | 0.08* | 19 | 28.5 | 11.0 ± 2.0 | 6.9 ± 2.6 | 0.69 ± 0.07 | 0.79 ± 0.04 |
| Halle 2005 | 252 | 68 | 102 | 48 | 20 | 0.19* | 48 | 72 | 16.0 ± 2.0 | 7.4 ± 1.8 | 0.77 ± 0.13 | 0.81 ± 0.07 |

n gives the sample size (in chromosomal sets), C the total number of colonies in the separate analysis of workers and males, N_e gives the genetic effective populations size based on C . C_{φ} and C_{σ} give the number of worker and male producing colonies from the separate analysis. C_j is the number of colonies based on the joined analysis of workers and males and N_{ej} is the resulting genetic effective population size. $F_{ST \varphi-\sigma}$ gives the F_{ST} value between the male and the worker derived queens and (*) indicates a significant population subdivision between them. A_n is the average number of alleles per locus (mean ± SD), while A_r gives the allelic richness per locus (mean ± SD). H_O and H_E are the observed and expected heterozygosities.

Results

GENETIC CHARACTERISTICS OF THE CABRERA POPULATION

We identified only five colonies from our sample on the island of Cabrera using the COLONY algorithm. One colony was only represented by males, the four others by workers only. The genetic characteristics of the Cabrera population are given in Table 1. The mean number of alleles over all loci was 4.4 ± 0.54 (allelic richness = 4.2 ± 1.6) with an overall observed heterozygosity of $H_O = 0.60 \pm 0.08$ and an expected heterozygosity of $H_E = 0.52 \pm 0.12$. There was neither a significant deviation from HWE nor significant linkage disequilibrium in the population.

GENETIC CHARACTERISTICS OF THE HALLE POPULATION

Table 1 shows the genetic characteristics of the Halle population in both sampling years. The used loci proved to be sufficiently variable with a range of 11.0 ± 2.0 to 16.0 ± 2.0 alleles per locus (allelic richness ranging from 6.9 ± 2.6 to 7.4 ± 1.8), yielding high overall H_E between 0.79 ± 0.04 and 0.81 ± 0.07 . In both years, the population structure obtained from the inferred queen genotypes did not significantly differ from HWE.

POPULATION SUBDIVISION AND NUMBER OF COLONIES IN THE HALLE POPULATION

Using COLONY (Wang 2004), we estimated a total of 27 colonies (14 male- and 13 worker-producing colonies) for the 2004 *B. terrestris* population in the Botanical Garden of Halle. In the following year, we obtained an estimate of 68 colonies (20 male- and 48 worker-contributing colonies) at the same sampling location. Using a fitted Poisson distribution, we calculated a single nondetected colony for the year 2004 and six nondetected colonies for the year 2005, indicating that in both years our sampling sufficiently covered the entire population. Based on the number of colonies detected, we calculated the genetic effective population size using the formula for haplodiploid organisms by Wright (1933). Given

that *B. terrestris* is a single-mated species (Schmid-Hempel & Schmid-Hempel 2000), we obtained a genetic effective population size of $N_e = 40.5$ for the year 2004 and $N_e = 102$ for the year 2005.

We compared the queen mother genotypes inferred from the male and worker samples in a more detailed analysis. In both sampling years, we did not obtain identical mother queen genotypes between the male- and the worker-derived queens (Fig. 1a). There was also a significant population differentiation between worker- and male-derived queens ($P < 0.001$ in both years, Fisher's exact test for population differentiation), with $F_{ST} = 0.08$ for 2004 and $F_{ST} = 0.19$ for 2005 between these two groups.

In a more conservative approach, ignoring the information from the population substructure, we pooled both males and workers in one single analysis within COLONY. Here we obtained a colony number estimate of 19 for 2004 and an estimate of 48 for 2005 (Table 1). As expected, there was a less strong separation between male- and worker-derived colonies (in 2004, 54% and 2005, 37% of the colonies were presented with both workers and males), because the algorithm of COLONY assigns genotypes to minimize the number of possible mother colonies regardless of the substructure at the population level (Fig. 1b). However, also in this approach the distribution of males and workers over the colonies differed significantly from a random distribution, again indicating different male- and worker-producing colonies ($P < 0.01$, Monte Carlo RC contingency table test).

FLIGHT RADIUS OF MALES

Using the proportions of colonies represented only by males, workers or both, we estimated the flight radius of males. With our first estimating approach, where we had a complete separation of the male-contributing colonies from worker-producing colonies (see above), we calculated an average male flight radius of $r = 9.9 \pm 1.4$ km. This result may be an overestimation because it relies heavily on the sorting algorithm of COLONY. We thus consider it as an upper limit for our estimate of the flight radius. In the second, more conservative estimate of the number of male-contributing colonies, the male flight radius is estimated at $r = 2.60 \pm 0.14$ km. Thus, even with this most conservative approach, which ignores the additional

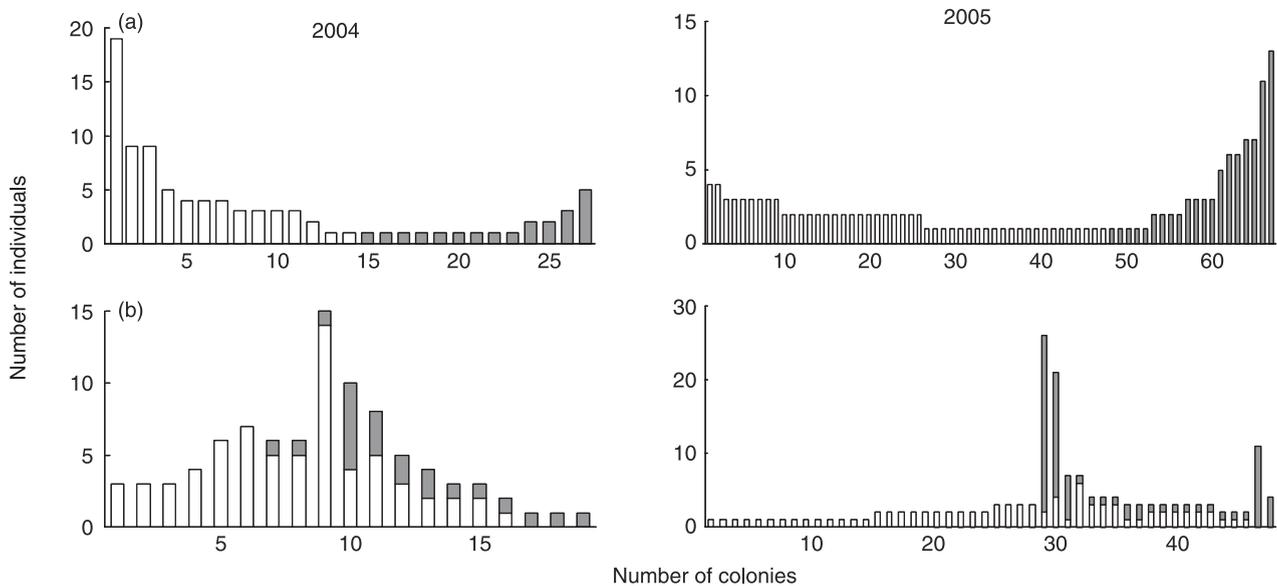


Fig. 1. Estimated number of colonies and the distribution of workers and males in the two sampling years for the Halle population (2004 left; 2005 right). The x -axis in the diagrams gives the total number of colonies detected in a given year, the y -axis the number of individuals in a given colony. White-coloured bars indicate workers and grey bars indicate males assigned to a given colony. The two upper diagrams (a) show the colony assignment when workers and males are treated as independent populations. In the lower diagrams (b) male and worker genotypes are merged and treated as one population.

information we have from the population genetic analyses, the flight radius of males was greater than that of the foraging workers.

Discussion

COLONY NUMBER ESTIMATES

With an estimated number of 27 and 68 colonies (2004 and 2005, respectively) in the urban park environment of the Halle Botanical Garden, our data compare well with the estimates (30–69 colonies) of Chapman *et al.* (2003) for British *B. terrestris* populations in an urban park in London (UK). Based on the formula by Wright (1933), the genetically effective population sizes in our study ranged from 28.5 up to 102, depending on population and estimation procedure. Thus, obviously highly diverse park habitats in urban areas can support large bumblebee populations. However, whether these population estimates from urban areas also apply to agricultural or semi-natural habitats remains doubtful, since these surely provide different nesting and foraging conditions.

The island population on Cabrera had a much lower number of colonies. Here we only found a total of five colonies and thus such a small, isolated island seems not be able to support larger populations of bumblebees. Insect pollinators were generally very rare on this island during our collection trip in early spring and previous studies indicate that alternative animals (e.g. lizards, *Podarcis lilfordi*) may serve as pollinators on the island (Traveset & Saez 1997). Due to the small population size, the allele diversity was low and consequently one would expect the island population to be prone to inbreeding. In bumblebees inbreeding is particularly detrimental due to

the genetic load associated with the sex locus (*csd*) (Crozier 1971; Gerloff & Schmid-Hempel 2005), which causes diploid males that either are inviable, sterile or unable to produce fertile offspring (Mackensen 1951; Duchateau & Mariën 1995; Gadau *et al.* 2001; Darvill *et al.* 2006). Surprisingly, and in contrast to findings in small and isolated *Bombus muscorum* populations (Darvill *et al.* 2006) and in fragmented *Bombus sylvarum* populations (Ellis *et al.* 2006), no inbreeding could be detected in the Cabrera population. Recent introgression might explain this observation, even though the nearest population on the main island Mallorca is about 10 km away from Cabrera. Given that bumblebee workers can fly more than 9 km after nest displacement (Goulson & Stout 2001) and long-distance migration of queens occurs (Mikkola 1984), it seems possible that sexuals can cover a 10-km distance in flight.

SUBSTRUCTURING OF POPULATIONS

The most striking observation in this study is the significant substructuring of the worker and male population in the Botanical Garden in Halle. We found that males and workers collected at the same site originate from quite different colonies. Our estimate might be biased if some of the sampled males had been worker produced (Trivers & Hare 1976; van Doorn & Heringa 1986; Lopez-Vaamonde *et al.* 2003), but we sampled early in the season when worker production of males is rare and the majority of the males are queen produced. Thus, although we did not sample virgin queens in our study, it seems clear that the mating range of bumblebees exceeds their foraging range by far just because of the flight pattern of the males alone.

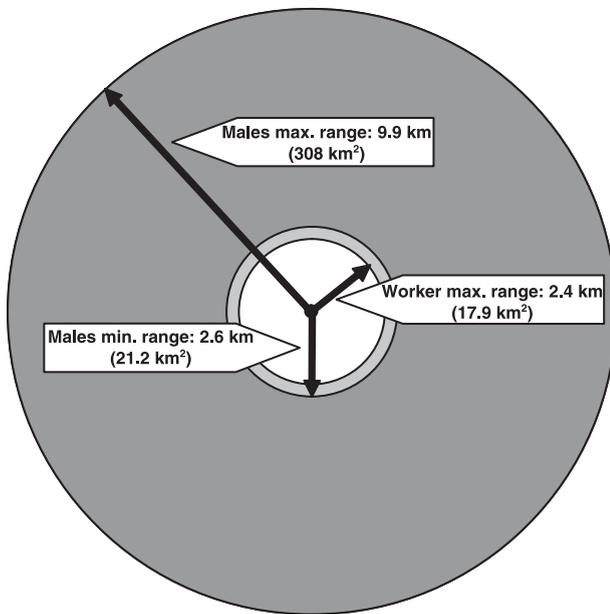


Fig. 2. Foraging range of workers and estimated mating ranges of males for the Halle population (given as a mean over both years). The minimal mating range is calculated from inferred colonies with sub-structure ignored (conservative approach Fig. 1b) and maximal mating range is calculated from colonies inferred under consideration of substructure (male and worker sample separate Fig. 1a). Even the most conservative approach results in drone mating ranges that exceed the foraging range of workers by far.

FLIGHT RADIUS OF MALES

Our estimates of the flight radius of males varied between $r = 2.60 \pm 0.14$ km and $r = 9.9 \pm 1.4$ km for the Botanical Garden population, depending on the estimation procedure (Fig. 2). Even if we look at the minimum values, mating ranges are surprisingly high, showing that bumblebee populations can cover vast areas, in particular because flight ranges of queens add to the maximum mating range. Also, the high heterozygosity observed on the island population on Cabrera convincingly illustrates the high potential of *B. terrestris* for long-distance gene flow.

Interestingly, there is a high variability of estimates for the flight ranges of bumblebee workers in the studies published so far (Walther-Hellwig & Frankl 2000a,b; Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005; Osborne *et al.* 2008; Wolf & Moritz 2008). The estimates range from a few hundred metres to several kilometres and it is unclear to what extent the variance among these studies reflects differences among the studied populations (different habitats, urban or agricultural) or different experimental approaches (field or genetic based). Also, abundance and density of flowering plants is an important factor influencing foraging distances (Wolf & Moritz 2008). If forage is sparse, bees must fly much farther for rewarding food plants than in a setting with high plant density (Heinrich 1976; Dramstad 1996). However, since our estimate of the flight distance of males is relative to

the ranges of the workers, the resulting relative differences are valid independent of the absolute values.

Our data show that the flight range of males significantly exceeds the foraging range of workers in *B. terrestris*. Given that drones visit flowers for energy intake and thus may well displace pollen grains, drones may function as important long-range pollen vectors, supplementing the pollination service of workers. This would not be the first case where the biology of the males has been overly neglected in social insect research (Koeniger & Baer 2004), and it would not be surprising if the impact of male bumblebees on long-distance pollination might exceed that of workers by far.

Besides this pollination perspective, our results also imply that colony number estimates based on worker samples alone might underestimate the actual number of colonies in a given population severely. In our data, up to 50% of colonies in the 2004 population would have remained undetected using the colony estimate based on the worker sample alone.

Conclusions

We showed that male bumblebees considerably increase the estimates of the census population size (number of colonies) compared with estimates which are based on worker samples alone. Furthermore, male bumblebees may enhance the area of the pollination effective size of bumblebee populations since their flight ranges exceed those of workers. This increased flight range of males may be important for both gene flow between distant or fragmented plant populations and gene flow among bumblebee populations themselves.

Acknowledgements

We wish to thank the Botanical Garden Halle for permission to catch bumblebees, the national park authorities of Cabrera (Parque Nacional Archipiélago de Cabrera), especially J.E. Moreno Perez and J.H. Amengual, for facilitating the sampling on the Island of Cabrera. This study was part of the EU – Project ALARM (Assessing Large Scale Environmental Risks for Biodiversity with Tested Methods), Pollinator Module, EU Contract No.: 506675).

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Received 12 April 2007; accepted 28 August 2008

Handling Associate Editor: Stuary Piertney