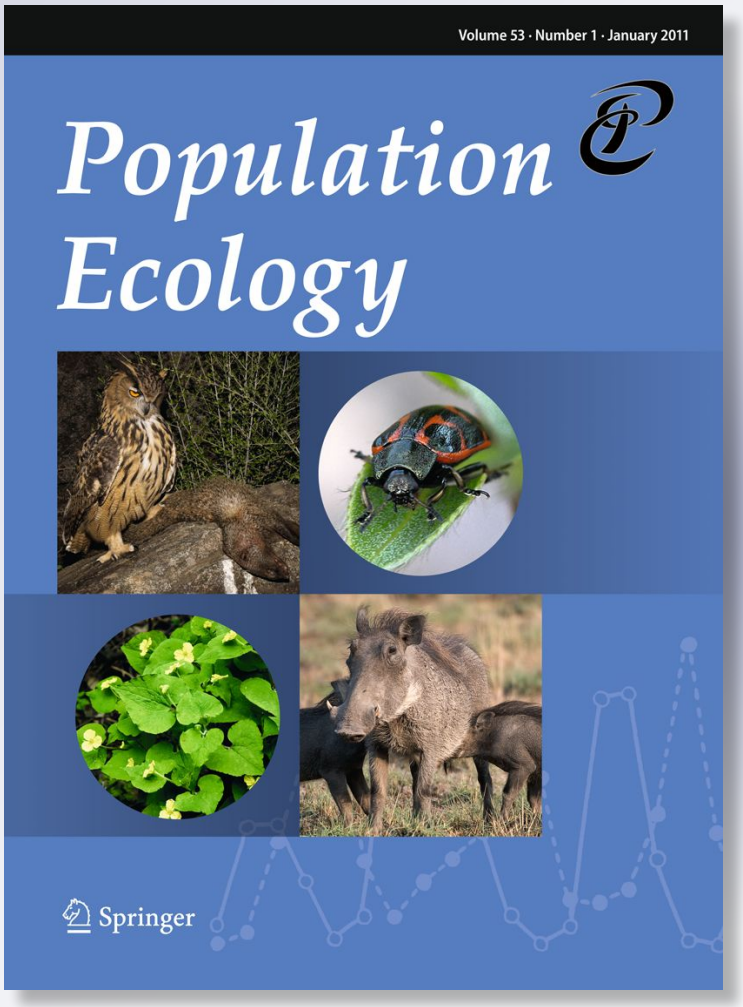
Spatial and temporal dynamics of the male effective population size in bumblebees (Hymenoptera: Apidae)

Stephan Wolf, Theresa Toev, Ruby L. V. Moritz & Robin F. A. Moritz

Population Ecology

ISSN 1438-3896

Popul Ecol
DOI 10.1007/s10144-011-0285-2



Springer

Popul Ecol
DOI 10.1007/s10144-011-0285-2

ORIGINAL ARTICLE

Spatial and temporal dynamics of the male effective population size in bumblebees (Hymenoptera: Apidae)

Stephan Wolf · Theresa Toev · Ruby L. V. Moritz · Robin F. A. Moritz

Received: 16 December 2010 / Accepted: 18 July 2011

Abstract Eusociality and male haploidy of bumblebees (*Bombus spp.*) enhance the deleterious effects of population decline and aggravate the degeneration of population fitness compared to solitary and diploid species. The highly dispersive male sex may be the prime driver to connect otherwise isolated populations. We therefore studied the temporal and spatial structure of the male population of *Bombus terrestris* (Linnaeus 1758) and *Bombus lapidarius* (Linnaeus 1758) using microsatellite DNA markers. We found that the majority of the males in a 1000 m^2 sampling area originated from colonies located outside of the workers foraging range, which was consistent with the genetic distances among colonies. The analyses of temporal population sub-structure based on both colony detection rate over time and the clustering software STRUCTURE consistently suggested one large and temporally unstructured male population. Our results indicate an extended male flight distance for both species. Though the range of queen dispersal remains to be studied, the effective size (N_e) of bumblebees is increased by extended male mating flight ranges (A_m) exceeding worker foraging distance by factor 1.66 ($A_m = 69.75$ km^2) and 1.74 ($A_m = 13.41$ km^2), *B. terrestris* and *B. lapidarius*, respectively. Thus this behaviour may counteract genetic deprivation and its effects. All populations were genetically highly diverse and showed no signs of inbreeding. We discuss the implications of our findings in context of bumblebee population dynamics and conservation. We also highlight the effects and benefits of sampling both workers and males for population genetic studies.

Keywords *Bombus* spp. · Conservation · Genetic diversity · Male flight range · Population genetics · Population structure

S. Wolf · T. Toev · R. F. A. Moritz
Institut für Biologie/Zoologie, Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany

S. Wolf (✉)
Department of Plant and Invertebrate Ecology, Rothamsted Research, Harpenden AL5 2JQ, Hertfordshire, UK
e-mail: stephan.wolf@rothamsted.ac.uk

T. Toev
Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany

R. L. V. Moritz
Institut für Informatik, Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany

R. F. A. Moritz
Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

Introduction

Insect mediated pollination is an essential but potentially endangered ecosystem function as numerous wild and managed pollinators have been reported to have undergone dramatic declines at a large scale (Packer et al. 2005; Biesmeijer et al. 2006). Pollinators' ecological and economical value for long was contrasted by an incomplete understanding of pollinator population dynamics and genetics (e.g., Ghazoul 2005). Only recently this issue has been addressed in various conservation genetic studies especially on social bees (Kearns et al. 1998; Packer et al. 2005; Darvill et al. 2006; Ellis et al. 2006; Goulson et al. 2008a, 2008b; Brown and Paxton 2009; Grixti et al. 2009; Murray et al. 2009; Zayed 2009; Charman et al. 2010). Bumblebees (*Bombus* spp.) are arguably one of the most

Published online: 10 August 2011

efficient pollinators having large worker forces entirely devoted to foraging outnumbering solitary pollinators. However, many aspects in the life history of bumblebees severely limit gene-flow between and within populations reducing the effective population size (N_e) (Chapman and Bourke 2001; Packer and Owen 2001; Murray et al. 2009; Zayed 2009). Monoandry and the annual colony life cycle result in recurrent annual bottlenecks with only few colonies contributing to the next generation gene pool through males and gynes (Müller and Schmid-Hempel 1992; Goulson 2003). Male haploidy further reduces the population's gene pool, again lowering the effective population size (Packer and Owen 2001; Zayed 2009). Indeed, for some declining *Bombus* species low effective population sizes (e.g., *B. muscorum*: Darvill et al. 2006; *B. sylvarum*: Ellis et al. 2006; *B. distinguendus*: Charman et al. 2010) and detrimental inbreeding effects (*B. muscorum*: Darvill et al. 2006) have been reported. Inbreeding is especially detrimental as the sex-determining-locus (sl-*csd*) requires heterozygosity in the female sex, whereas homozygosity leads to the production of unviable or infertile diploid males instead of females (Duchateau and Mariën 1995; Zayed 2004; Darvill et al. 2006; Takahashi et al. 2008; Whitehorn et al. 2009a, b).

As bumblebees have stationary colonies, genetic exchange among sub-populations counteracting population fragmentation can only be realized through dispersing males and queens. Hence, the number of male and queen producing colonies within the mating range determines N_e and the diversity of the next generation's gene pool. However, our knowledge on the dispersal of bumblebee reproductives is limited at best. The few reports on queen dispersal suggest that bumblebee queens can cover large distances during spring migration to suitable nest sites (Mikkola 1984) but seem to compare generally to workers in their dispersal rates (Lepais et al. 2010). The population genetic effects of queen dispersal and the interplay of male and queen dispersal in the late season, meanwhile, remain to be studied.

Male patrolling behaviour of bumblebees, in contrast, already fascinated Darwin (1886) and the spatial dimensions of individual male patrol routes have been studied in great detail in numerous species (e.g., Haas 1949; Svensson 1979). Recently Kraus et al. (2009) showed that the flight distance of *B. terrestris* males exceeds the foraging distance of workers by far at the population level. Hence, males rather than queens may be prime candidates to genetically bridge sub-populations to increase the effective population size. The spatial distribution alone as addressed by Kraus et al. (2009) may, however, be insufficient to assess the full potential of male dispersal if the composition of the male population is also temporally highly diverse. Different colonies might provide males at different points in time or males from more distant colonies might arrive at a later point in time at a certain sampling site than those of nearby colonies. Hence, we here address not only the spatial but also the temporal dynamics of male populations of two bumblebee species, *B. terrestris* and *B. lapidarius* using a time-controlled sampling regime.

Materials and methods

Sampling and species identification

Workers and males of *B. terrestris* and *B. lapidarius* were sampled on a daily basis from June 24th to July 25th, 2008 in the flower-rich urban park "Heide-Süd" in Halle/Saale, Germany on a 1000 m^2 sampling plot (51°29′30 N; 11°56′10 E). Individuals were caught from flowers with an insect net. After initial species identification in the field, bees were sacrificed and stored in Ethanol (70%). Following the identification key of Mauss (1994) all individuals were double-checked for species identity and sex using a microscope. For *B. terrestris* workers, the species identity was confirmed by species-specific mtDNA RFLPs on a sub-sample of $n = 80$ individuals (Murray et al. 2008; Wolf et al. 2009).

DNA analysis

DNA was extracted from one middle leg of each individual following the *Chelex* extraction protocol described by Walsh et al. (1991). Individuals were then genotyped at five microsatellite loci (B10, B100, B11, B124 and B126; Estoup et al. 1993, 1995) following standard PCR protocols (e.g., Kraus et al. 2009) in an automated DNA capillary sequencer (MegaBACE 1000) according to manufacturer's instructions.

Colony assignment

The genetically effective unit in a bumblebee population is the colony. Analyses based on individual workers and males are, thus, prone to biased estimates if colonies unevenly contribute to the overall sample. Hence, individuals sampled in the field need to be assigned to a specific colony (= mother queen) for subsequent population genetic analysis at the colony level. We therefore inferred the genotypes of the putative mother queens from individual workers and males by using the software COLONY 1.3 (Wang 2004). Based on a maximum likelihood approach this software uses both the individual genotypes (both haploid and diploid) and the overall allele frequencies in the sample to infer the minimum number of putative natal colonies giving the two most likely queen genotypes for each inferred colony. For each sample we performed five

replicate COLONY-runs with different random seed numbers selecting the most likely queen genotypes inferred from the run with the highest log probability for further analyses. Sample-size correction of inferred colony numbers (non-sampling error, NSE) was done with the algorithm described by Cornuet and Aries (1980).

Foraging workers

Foraging workers are colony-based and, thus, spatially confined to their foraging range around their mother-colony (e.g., Chapman et al. 2003; Darvill et al. 2004; Knight et al. 2005; Osborne et al. 2008; Wolf and Moritz 2008; Kraus et al. 2009; see also Goulson and Osborne 2009). Consequently, the genetic composition of the foraging population (workers) was assumed to be stable over time and served as a "reference" population referred as to "LQ" (local queens).

Male population

In contrast to workers, males never return to the colony after they have left it. Based on their genotype we checked for males that had been produced by the local queens (= males matching worker-inferred queens LQ). Accordingly we grouped worker inferred local colonies in those contributing both workers and males (LQ_{w+m}, i.e., worker-inferred queens matching with at least one male genotype) and those that contributed only workers (LQ_w).

Males that could not be assigned to a local queen (= "foreign males") were considered to originate from colonies outside the foraging range of its workers. These colonies were inferred from the foreign male genotypes again using COLONY 1.3. and referred to as "foreign queens" (FQ_m). Due to the lack of a priori-information on the potential male-population structure, all males were pooled for queen assignment procedure.

Genetic distance D_a

Based on the inferred queen genotypes (C) we assessed the genetic structure among the three colony types (LQ_w, LQ_{w+m}, FQ_m) by calculating mean pair-wise Nei's standard genetic distances D_a (Nei 1972). In order to correct for different sample sizes (i.e., colony numbers per sub-population) we based our genetic distance calculations on 1000 random sub-samplings within and among the sub-populations (= comparison of two random sub-samples from the same and from different sub-population(s), respectively). We sub-sampled 32 and 21 queen genotypes for *B. terrestris* and *B. lapidarius,* respectively, representing half the number of the queen genotypes in the smallest sub-population. This allowed standardizing the number of queen genotypes over all sub-populations without overlapping sub-sampling within a sub-population. The population genetic structure was visualized by hierarchical clustering (UPGMA) using a dissimilarity matrix generated from mean pair-wise genetic distances (D_a values).

Analysis of temporal structure

In one large and temporally unstructured male population, the repeated sampling represents a cumulative sub-sampling from a constant pool of male providing colonies. Colonies that remained un-detected in the first sampling bouts due to an insufficient sample-size (non-sampling error, NSE) should be traced as the overall sample-size increases. We thus expect a saturation curve approaching the actual total number of colonies in the population with increasing sample size.

In contrast, a temporally strongly structured male population where different colonies sequentially contribute to the male population, sub-sequent sub-samples (weeks I–IV) should not show a saturation curve but rather show a constant increase in the number of colonies detected. We tested both hypotheses (one large population vs. temporally structured population) by comparing the residual variance in the sample set of either population model.

Additionally, we analysed the foreign male genotype data with the model-based clustering algorithm provided by the software package STRUCTURE 2.2 (Pritchard et al. 2000). It uses the genotype data of the males to infer the most likely population structure for a specified number of clusters (k). Assuming genetic admixture and independent allele frequencies between potential clusters, we explored the dimensions of a potential temporal structure by performing ten iterations for $k = 1$ (no turnover) to $k = 56$ (full turnover twice a day). The mean ln-probability (P_{ln}) for each k was used to assess the most likely number of discrete clusters within the overall sample. This analysis was based on individual genotypes rather than on inferred queen genotypes as these most sensitively reflect any genetic structure in the sample. To provide an even more rigorous setting, we divided the male sample into four pools according to the week of sampling and inferred queen genotypes from these pools separately with COLONY. This procedure enhances any potential differences among the four pools by preventing individuals from different sampling bouts to be assigned to one colony. These queen genotypes were pooled and again analysed for the most parsimonious number of clusters (STRUCTURE 2.2).

Effective population size

Effective population size estimates are typically based on worker samples (e.g., Darvill et al. 2006; Ellis et al. 2006)

ignoring male contributing foreign queens. Here, we calculated the effective population size N_e separately for the worker contributing and male contributing population using Wright's (1933) equation for haplo-diploid populations. As monoandry is assumed for both species N_e equals 1.5 times the number of estimated colonies (e.g., Darvill et al. 2006).

Population genetics

The classical population genetic parameters: number of alleles (A_n), observed and expected heterozygosity (H_o, H_e), inbreeding coefficient (F_{IS}) and Hardy–Weinberg equilibrium (HWE) were calculated from inferred queen genotypes using F_{STAT} 2.9.3. (Goudet 1995). Allelic richness (A_r) was calculated using the software HP-rare (Kalinowski 2005) correcting for different sample-sizes (i.e., number of queens). All statistical analyses were performed with Statistica 7.0 (StatSoft).

Male flight range and colony densities

Following the approach of Kraus et al. (2009) we estimated male flight range (A_m) from the total number of male contributing colonies. Not all queens that produce workers also produce males and the local foraging population is thus composed of workers that originated from colonies that only produce workers (LQ_w) and those that produce both workers and males (LQ_{w+m}). Assuming a constant frequency of male contributing colonies in the population, we can estimate the overall number of male contributing colonies from the density of male contributing colonies (d_m) within the foraging range (A_w) of all local colonies ($d_m = LQ_{w+m}/A_w$). Since estimates of the worker flight range vary strongly in the literature (Dramstad 1996; Saville et al. 1997; Kwak et al. 1998; Osborne et al. 1999, 2008; Walther-Hellwig and Frankl 2000; Chapman et al. 2003; Darvill et al. 2004; Knight et al. 2005; Westphal et al. 2006; Wolf and Moritz 2008; reviewed in Goulson and Osborne 2009) we inferred the flight range of the males from all available estimates of worker foraging range and the overall mean the overall mean worker flight range of all these studies (*B. terrestris*: $A_w = 5.76$ km^2, foraging distance $r_w = 1354 \pm 963$ m; *B. lapidarius*: $A_w = 7.71$ km^2, $r_w = 1567 \pm 1151$ m) as follows:

$$A_m = \frac{A_w \times (FQ_m + LQ_{w+m})}{LQ_{w+m}}$$

where A_m is the male flight range (km^2), A_w the mean worker foraging range (km^2), FQ_m the number of male contributing colonies outside of the workers foraging range, LQ_{w+m} is the number of male contributing colonies within the workers foraging range

We used the A_m:A_w ratio to quantify the male flight ranges in relation to that of workers. The estimated mean male flight distance (r_m) is the radius of the population wide mean male flight range estimate A_m, which is assumed to be circular.

Results

Worker population

We genotyped a total of 352 *B. terrestris* workers of which we inferred 198 colonies resulting in a sample size corrected estimate of 273 "local" colonies (NSE = 75) (see Table 1). For *B. lapidarius* we estimated 175 colonies with a non-sampling error of NSE = 34 based on a 287 worker sample. Sub-sample sizes (n), inferred (C), and sample size corrected colony numbers (C_C) and non-sampling error (NSE) for both species are given in Table 1.

Male population

The local male population consisted of 34 and 43 individuals (16.2 and 22.4% of total) for *B. terrestris* and *B. lapidarius*, respectively, which we estimated to originate from 107 (NSE = 40) and 69 (NSE = 27) colonies respectively. Since some male genotypes (23.3–47.4%) matched with more than one queen genotype (putative mother colonies) of which all were treated as local male contributing colonies this estimate is an overestimate.

In both species, only slightly more than one third of the identified local colonies (*B. terrestris*: 39.2%; *B. lapidarius*: 39.4%) did potentially contribute males and the majority of local queens only produced workers [*B. terrestris* LQ_w: $C_C = 171$ (NSE = 40); *B. lapidarius* LQ_w: $C_C = 116$ (NSE = 17)]. Foreign males were estimated to originate from 71 *B. terrestris* (NSE = 6; $n = 176$) and 51 *B. lapidarius* (NSE = 3, $n = 149$) colonies (Table 1). Based on the frequency of male producing colonies in the local population, we estimated 113 *B. terrestris* and 86 *B. lapidarius* foreign colonies that only produced workers [FQ_w, $N_e = 169.5$ and 129, respectively (Table 1)].

Population genetic characterization

Population genetic parameters for both species are summarized in Table 2. In all sub-samples (LQ_w, LQ_{w+m}, FQ_m) we detected high allele numbers (A_n). Using rarefaction allelic richness (A_r) and private allelic richness (pA_r) were standardized to 128 and 80 genes per subpopulation for *B. terrestris* and *B. lapidarius*, respectively. There were no significant differences in the allelic richness (A_r) among the sub-samples, and we detected neither a significant deviation from Hardy–Weinberg equilibrium nor any significant inbreeding.

Table 1 For *B. terrestris* and *B. lapidarius* sample size (*n*; male samples (*m*) given in italic font, worker samples (w) in regular font), number of inferred colonies (*C*), non-sampling error (NSE) and sample-size corrected number of colonies (C_C) are given for worker-derived local colonies (LQ_w/LQ_{w+m}) and male contributing foreign colonies (FQ_m)

Inferred from	Workers (w)			Males (m)					$\frac{LQ_w \times LQ_{w+m}}{FQ_m}$
	Local queens (LQ)			Total	Foreign queens (FQ)				
	Total	LQ_w	LQ_{w+m}		FQ_m	Novel FQ_m over time			FQ_w
						Week I	Week II	Week III	Week IV	
B. terrestris										
n (+*m*)	352 (+*34*)	247	105 (+*34*)	*210*	*176*	*35*	*80*	*31*	*30*	
C	198	131	67	–	65	29	31	5	0	
NSE	75	40	40	–	6	59	3	0	0	
C_C	273	171	107	–	71	88	34	5	0	113
N_e	409.5	256.5	160.5	–		–	–	–	–	**169.5**
mN_e	–	–	**160.5**	–	**106.5**	–	–	–	–	
B. lapidarius										
n (+*m*)	287 (+*43*)	223	107 (+*43*)	*192*	*149*	*43*	*42*	*46*	*18*	
C	141	99	42	–	48	33	11	4	0	
NSE	34	17	27	–	3	43	0	0	0	
C_C	175	116	69	–	51	76	11	4	0	86
N_e	262.5	174	103.5	–		–	–	–	–	**129**
mN_e	–	–	**103.5**	–	**76.5**	–	–	–	–	

For the latter initial male contribution i.e., time of a colonies first detected male contribution is given. Relevant colony numbers are translated into effective population size ($N_e = C_C \times 1.5$). The effective population size calculated from male contributing colonies is referred to as male effective population size (mN_e). We also estimated the number of foreign colonies only producing workers (FQ_w) applying the proportion of local male contributing colonies (LQ_{w+m}) to worker contributing local colonies (LQ_w) to the foreign population

Based on the mean genetic distances (D_a) we found a weak within local population (LQ_w and LQ_{w+m}) separation (*B. terrestris*: $D_a = 0.063 \pm 0.01$; *B. lapidarius*: $D_a = 0.129 \pm 0.03$) but a strong separation of the foreign male population (FQ_m) from the "local" cluster (*B. terrestris*: average of FQ_m–LQ_w and FQ_m–LQ_{w+m}: *B. terrestris*: $D_a = 0.106 \pm 0.02$; *B. lapidarius*: $D_a = 0.254 \pm 0.04$) (Fig. 1). The overall clustering was supported in 97.2% (972 of 1000) and 99.0% (990 of 1000) of the clusters inferred from each of the sub-sampled populations.

Though there were only marginal differences in mean allele richness (A_r) in LQ_{total} and FQ_m, private allele richness (pA_r) is increased in the foreign colonies (albeit only significant for *B. terrestris*: 3.52 (20.9% of A_r) and 1.76 (12.4%), FQ_m and LQ_{total}, respectively; *t* test: $Z = 1.98$, $df = 8$, $P = 0.047$ (*B. lapidarius*: 2.71 (18.7%) and 2.54 (17.8%), FQ_m and LQ_{total}, respectively Mann–Whitney *U* test: ns) (Table 2) indicating the effects of an increased male mating range on the population genetic composition and diversity.

Temporal dynamics of the male population

The observed distribution of foreign males over time closely matched the distribution expected by repeated sampling of one large population. 93.8 and 92.9% of the observed distribution of foreign male contributing colonies over time for *B. terrestris* and *B. lapidarius*, respectively, could be explained by the non-sampling errors associated to the sample size (Tables 1, 3), suggesting one large and temporally unstructured male population.

This is strongly supported by the cluster analyses of individual foreign male genotypes, queen genotypes derived from all foreign males, and queen genotypes derived from weekly pooled male sub-populations (based on four sub-populations according to sampling week). Neither approach suggested any distinct clusters within the foreign male population. The assignment to *k* clusters consistently resulted in a highly significant decrease of the *ln* probability (P_{ln}) as *k* was increased (*B. terrestris*: $P_{ln(k\ =\ 1\text{–}28)}$: $F_{7,72} = 14.9$; $P < 0.001$; *B. lapidarius*: $P_{ln(k\ =\ 1\text{–}28)}$: $F_{7,72} = 6.05$; $P < 0.001$). In all cases $P_{ln(k\ =\ 1)} = -2024.3 \pm 0.77/-1625.0 \pm 9.42$ (*B. terrestris*/*B. lapidarius*) was significantly higher than $P_{ln(k\ =\ 2)} = -2066.29 \pm 17.1/-1660.4 \pm 12.4$ (*B. terrestris*: $t = -7.66$, $df = 18$, $P < 0.001$; *B. lapidarius*: $t = -7.18$, $df = 18$, $P < 0.001$) (Fig. 2). This clearly suggests no temporal structure within the male population as most parsimonious result.

Table 2 Population genetic parameters are given for local worker-only producing colonies (LQ_w), local male and worker producing colonies (LQ_{w+m}), all local colonies (LQ_{total}), and foreign colonies (FQ_m)

	$A_n \pm$ SD	$A_r \pm SD_{(128/80\ genes)}$	$pA_r \pm SD_{(128/80\ genes)}$	$H_o \pm$ SD	$H_e \pm$ SD	F_{IS}
B. terrestris						
LQ_w	18.8 ± 3.56	16.14 ± 3.09	–	0.84 ± 0.05	0.84 ± 0.06	0.007^{ns}
LQ_{w+m}	12.0 ± 3.67	11.94 ± 3.65	–	0.89 ± 0.07	0.77 ± 0.08	-0.156^{ns}
LQ_{total}	19.0 ± 3.32	14.99 ± 2.93	1.76 ± 0.61] *	0.87 ± 0.05	0.81 ± 0.07	-0.074^{ns}
FQ_m	16.8 ± 3.96	16.75 ± 3.97	3.52 ± 1.17] *	0.80 ± 0.05	0.83 ± 0.07	-0.025^{ns}
B. lapidarius						
LQ_w	17.6 ± 3.05	14.38 ± 2.26	–	0.81 ± 0.06	0.85 ± 0.04	0.045^{ns}
LQ_{w+m}	11.6 ± 2.88	11.50 ± 1.83	–	0.91 ± 0.06	0.79 ± 0.04	-0.147^{ns}
LQ_{total}	17.8 ± 2.86	13.67 ± 2.02	2.54 ± 1.78] n.s.	0.84 ± 0.16	0.84 ± 0.04	-0.01^{ns}
FQ_m	14.6 ± 2.88	13.84 ± 2.64	2.71 ± 1.36] n.s.	0.84 ± 0.07	0.84 ± 0.03	0.026^{ns}

We present the average over loci (mean ± SD) of number of alleles per locus (A_n), allelic richness (A_r), private allelic richness (pA_r), observed and expected heterozygosity (H_o and H_e) and Inbreeding coefficient F_{IS} with its significance to deviate from zero. A_r and pA_r are standardized for minimum number of genes within a sub-population per species (*B. terrestris*: 128; *B. lapidarius*: 80) using rarefaction

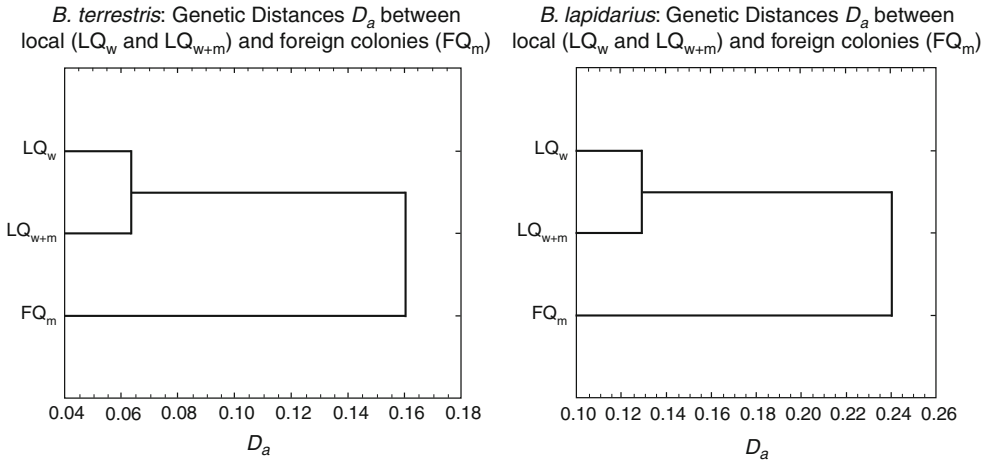


Fig. 1 Allele-frequency derived genetic distances (Nei 1972) among the three types of colonies inferred from *B. terrestris* and *B. lapidarius* samples (LQ_w = only worker contributing, local colonies; i.e., located within flight range of workers around the sample site; LQ_{w+m} = local male contributing colonies; FQ_m = male contributing colonies outside of the workers foraging range). Foreign colonies clearly branch from the local colonies indicating that males may bridge between widely dispersed sub-populations differing in their population genetic profiles. Both trees were supported in 97.2 and 99.0%, *B. terrestris* and *B. lapidarius*, respectively, of the cluster analyses based on individual analyses of 1000 random sub-samples

Effects on the effective population size

Based on the corrected number of colonies derived from the worker samples (C_C = 273 and 175), the effective population sizes were estimated with $N_{e(LQtotal)} = 409.5$ (= 273 × 1.5) and 262.5 (= 175 × 1.5) for *B. terrestris* and *B. lapidarius* respectively. The local male effective population sizes mN_e for the two species calculated from local male contributing queens (LQ_{w+m}) were $mN_{e(LQw+m)} = 160.5$ (*B. terrestris*) and $mN_{e(LQw+m)} = 103.5$ (*B. lapidarius*). This value increased for *B. terrestris* by over 65.0% by including the male contributions of foreign colonies ($mN_{e(total)} = 160.5 + 106.5 = 266.5$). This effect was even stronger in *B. lapidarius* with a 73.9% increase of the genetically effective population size ($mN_{e(total)} = 103.5 + 76.5 = 180.0$).

As male contributing foreign colonies represent only a proportion of the total number of colonies present in the male but not in the worker flight range ($FQ_{total} = FQ_m + FQ_w$) we included FQ_w to the overall effective

Table 3 Male contributing colonies observed (OBS) and expected (EXP) in weekly intervals (weeks)

Week	Cumulative colony numbers		Residuals	Variance	R^2
	OBS	EXP			
B. terrestris					
1	29	27.78	1.22	1.49	
2	60	57.11	2.89	8.38	
3	65	62.05	2.95	8.71	
4	65	65.15	−0.15	0.02	
				6.19	0.938
B. lapidarius					
1	33	29.23	3.77	14.18	
2	44	41.53	2.47	6.12	
3	48	47.19	0.81	0.66	
4	48	48.33	−0.33	0.11	
				7.02	0.929

Observed contributions represent the actually detected colonies, expected contributions reflect the distribution of male contributing colonies over time explained solely by sample-size effects (Non-sampling error calculated for cumulative sub-samples over time in comparison to the total sample size). Residuals (OBS–EXP) and correlation coefficient R^2 strongly indicate that sample-size effects that arose from temporal sub-samples account for over 90% of the temporal distribution of male contributing colonies

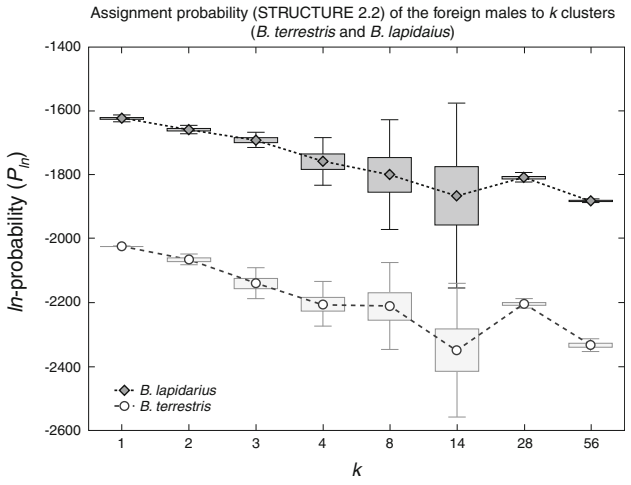


Fig. 2 Mean assignment probability [P_{ln} (*B. lapidarius*: diamonds, *B. terrestris*: *circles*), SD, SE] for foreign male population (FQ_m) clustering into k (1–56) clusters by the software STRUCTURE 2.2. Based on ten iterations for each k corresponding mean P_{ln} in both species were significantly highest for $k = 1$ and resulted in the lowest within assignment variance both indicating no temporal sub-structure in the foreign male population

population size ($N_{e(total)}$). Though these colonies did not contribute males they may well contribute to the queen population available for males.

Including all colonies inferred directly (FQ_m) and indirectly (FQ_w) from our male sample N_e estimates increased substantially by 67.4% ($N_{e(total)} = 685.5$) and 78.3% ($N_{e(total)} = 468$) for *B. terrestris* and *B. lapidarius*, respectively.

Colony density and male flight range

Using the mean of the foraging range estimates of workers for both species (Goulson and Osborne 2009), we estimated the density of male contributing local colonies with 18.6 and 8.9 col/km^2 for *B. terrestris* and *B. lapidarius*, respectively (Table 4).

The flight range of *B. terrestris* males exceeded the foraging range of workers by the factor 1.66 representing a marked increase in the genetically relevant population range estimated from worker samples ($\varnothing\ A_m = 9.75$ km^2; + 69.3%). In *B. lapidarius* male flight distance exceeds the foraging distance by factor 1.74 to $\varnothing\ A_m = 13.41$ km^2, almost twice as much as the worker foraging range. Based on the overall estimates of colony numbers ($LQ_{total} + FQ_m + FQ_w$: *B. terrestris*: 457; *B. lapidarius*: 330) and the male flight ranges, we derived mean overall colony densities of 46.9 col/km^2 (*B. terrestris*) and 24.6 col/km^2 (*B. lapidarius*). Since the male contributing local colonies (LQ_{w+m}) might be overestimated at the expense of foreign male contributing colonies (FQ_m), all above estimates are highly conservative.

Discussion

Our sample site, an urban park, mainly consisting of flower-rich grassland, allowed to sustain large bumblebee populations of over 750 colonies of *B. terrestris* and *B. lapidarius* within the estimated male flight range. Given our conservative estimate of male flight range this may be even an underestimate of the actual number of colonies in the population. The derived colony density for *B. terrestris* (46.9 col/km^2) compares very well with the high density populations assumed for urban park habitats by Chapman et al. (2003) (40 col/km^2). In contrast, the *B. lapidarius* colony density (24.6 col/km^2) in this study is much lower than found in other studies (e.g., 117 col/km^2; Knight et al. 2005). These discrepancies very likely result from differences in the worker foraging distances used as basis for the density estimates. For example, if we recalculate our *B. lapidarius* estimates for a worker foraging distance of 450 m (Knight et al. 2005) the colony density estimates increases to 298.4 col/km^2, more than twice of that reported by Knight et al. (2005).

The flight ranges of both *B. terrestris* and *B. lapidarius* males exceeded the foraging ranges of workers. The majority of males originated from distant colonies, confirming the findings of Kraus et al. (2009) for *B. terrestris*.

Table 4 Density estimates (d_m) for male contributing local colonies (LQ_{w+m}) based on workers foraging range (A_w) are given

	Local colonies			Local and foreign colonies			
	LQ_{w+m}	A_w (km^2)	d_m	Q_m (LQ_{w+m}+FQ_m)	A_m (km^2)	r_m (km)	A_m:A_w
B. terrestris	107	5.76	18.59	178	9.57	1.75	1.66
B. lapidarius	69	7.71	8.94	120	13.41	2.07	1.74

These estimates were used to calculate an overall male flight range (A_m) that could harbor all detected male contributing colonies ($Q_m = LQ_{w+m} + FQ_m$) assuming constant colony density (d_m) and circular flight area. From this circular range male flight distance (r_m) was derived as radius. The dimension of male flight range in comparison to the foraging range of workers is given as proportion A_m:A_w, clearly illustrating males extended flight ranges

This result might also be affected by worker produced males overestimating male-producing foreign queens (FQ_m). Worker reproduction can vary greatly and range for *B. terrestris* from 5% and less (Alaux et al. 2004) to over 80% (Fletcher and Ross 1985; Bourke 1988). However, worker reproduction typically occurs late in the season (Owen and Plowright 1982; Alaux et al. 2004) and since we sampled early in the season with the first appearance of males, only few if any males will be worker offspring (e.g., van Doorn and Heringa 1986). We therefore consider worker produced males to play a minor role and non-local males being produced by queens of distant colonies.

The large male flight range leads to a marked increase in the male effective population size. The population genetic parameters including the estimates of genetic distance and private allelic richness further reveal a diversification of the male gene-pool by foreign males.

Regarding a potential temporal substructure of the male population, we found no evidence that the genetic dissimilarity between the local and the foreign population had been caused by distinct cohorts of males subsequently passing through our sample site. All analyses on the temporal structure clearly suggest a single large male population produced by widely spread colonies.

As low effective population sizes are associated with a high inbreeding risk and genetic depletion (Gerloff et al. 2003; Zayed 2004, 2009; Gerloff and Schmid-Hempel 2005; Darvill et al. 2006; Ellis et al. 2006; Whitehorn et al. 2009a, b), the large mating range of the males may serve as an effective strategy to increase the overall effective population size and counteract the risk of sibling mating. Here future research on the effects of male flight ranges in rare bumblebee species, especially on species with different male mating behaviour (e.g., *B. muscorum*: Darvill et al. 2006, 2007) may be particularly rewarding to further explore the reasons of the decline of some bumblebee species (Goulson et al. 2005, 2008a; Fitzpatrick et al. 2007; Williams and Osborne 2009).

Finally, and probably most importantly extended male flight ranges may also have implications for pollination services. A high pollination potential of male *Bombus impatiens* has been shown by Ostevik et al. (2010). In line with this, S. Wolf and R. F. A. Moritz (unpublished data) demonstrated a high pollination potential of males of these two species, highlighting especially their role as long-distance pollen-vectors for native (e.g., Aguilar et al. 2008), invasive (Chittka and Schürkens 2005) or transgenic plants (Pasquet et al. 2008).

Apart from the biological implications of male dispersal, our results strikingly demonstrate the advantage of using both workers and males for the population genetic characterization of bumblebee populations. Including males to our study allowed the analyses of half of the gene-pool (males) actually available for the next generation after passing through the first selection process, rather than inferring the potential gene contribution to the next generation from worker-inferred queens alone. It also led to a substantial difference in the estimated N_e, increasing by 67.4 and 78.2% for *B. terrestris* and *B. lapidarius*, respectively, potentially affecting the conclusions drawn from such data. As males are easily sampled in sufficient numbers along with workers in the field, they provide an easily assessable and highly valuable resource for population genetic studies on bumblebees allowing detailed insights to population characteristics and dynamics: essential elements for efficient, evidence based bumblebee conservation plans.

Acknowledgments We like to thank Susann Hartung, Julia Müller, Anne-Kathrin Richter, Lydia Segler and Franziska Stöber for valuable help with the field work. We also like to thank H. Michael G. Lattorff (Martin-Luther-Universität Halle-Wittenberg), Elisa Loza-Reyes (Rothamsted Research) and two anonymous referees for helpful suggestions on the manuscript. The project was funded by ALARM (Assessing LArge scale Risks for biodiversity with tested Methods)-Integrated Project, 6th Framework (GOCE-CT-2003-506675).

References

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol Ecol 17:5177–5188

Alaux C, Savarit F, Jaisson P, Hefetz A (2004) Does the queen win it all? Queen–worker conflict over male production in the bumblebee, *Bombus terrestris*. Naturwissenschaften 91:400–403

Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313:351–354

Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. Q Rev Biol 63:291–311

Brown MJF, Paxton RJ (2009) The conservation of bees: a global perspective. Apidologie 40:410–416

Chapman RE, Bourke AFG (2001) The influence of sociality on the conservation of social insects. Ecol Lett 4:650–662

Chapman RE, Wang J, Bourke AFG (2003) Genetic analysis of spatial foraging patterns and resource sharing in bumblebee pollinators. Mol Ecol 12:2801–2808

Charman TG, Sears J, Green RE, Bourke AFG (2010) Conservation genetics, foraging distance and nest density of the scarce Great Yellow Bumblebee (*Bombus distinguendus*). Mol Ecol 19:2661–2674

Chittka L, Schürkens S (2005) Successful invasion of a floral market—an exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature 411:653

Cornuet JM, Aries F (1980) Number of sex alleles in a sample of honeybee colonies. Apidologie 11:87–93

Darvill B, Knight ME, Goulson D (2004) Use of genetic markers to quantify bumblebee foraging range and nest density. Oikos 107:471–478

Darvill B, Ellis JS, Lye GC, Goulson D (2006) Population structure and inbreeding in a rare and declining bumblebee, *Bombus muscorum* (Hymenoptera: Apidae). Mol Ecol 15:601–611

Darvill B, Lye GC, Goulson D (2007) Aggregations of male *Bombus muscorum* (Hymenoptera: Apidae) at mature nests. Incestuous brothers or amorous suitors? Apidologie 38:518–524

Darwin C (1886) Über die Wege der Hummelmännchen In: Krause E (ed) Gesammelte Schriften von Charles Darwin, vol. 2. Leipzig, pp 84–88 (in German)

Dramstad WE (1996) Do bumblebees (Hymenoptera, Apidae) really forage close to their nests? J Insect Behav 9:163–182

Duchateau MJ, Mariën J (1995) Sexual biology of haploid and diploid males in the bumblebee *Bombus terrestris*. Insectes Soc 42:255–266

Ellis JS, Knight ME, Darvill B, Goulson D (2006) Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera: Apidae). Mol Ecol 15:4375–4386

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

Estoup A, Scholl A, Pouvreau A, Solignac M (1995) Monoandry and polyandry in bumblebees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. Mol Ecol 4:89–93

Fitzpatrick U, Murray TE, Paxton RJ, Breen J, Cotton D, Santorum V, Brown MJF (2007) Rarity and decline in bumblebees—a test of causes and correlates in the Irish fauna. Biol Conserv 136: 185–194

Fletcher DJC, Ross KG (1985) Regulation of reproduction in eusocial Hymenoptera. Ann Rev Entomol 30:319–343

Gerloff CU, Schmid-Hempel P (2005) Inbreeding depression and family variation in a social insect, *Bombus terrestris* (Hymenoptera, Apidae). Oikos 111:67–80

Gerloff CU, Ottmer BK, Schmid-Hempel P (2003) Effects of inbreeding on the immune response and body size in a social insect, *Bombus terrestris*. Funct Ecol 17:582–589

Ghazoul J (2005) Pollen and seed dispersal among dispersed plants. Biol Rev 80:413–443

Goudet J (1995) FSTAT (Version 1.2). A computer program to calculate F-statistics. J Hered 86:485–486

Goulson D (2003) Bumblebees—behaviour and ecology. Oxford University Press, Oxford

Goulson D, Osborne JL (2009) Foraging range and the spatial distribution of worker bumblebees. In: Jarau S, Hrncir M (eds) Food exploitation by social insects: ecological, behavioural and theoretical approaches. CRC Press, Taylor & Francis Group, LLC, Boca Raton, pp 97–111

Goulson D, Hanley ME, Darvill B, Ellis JS, Knight ME (2005) Causes of rarity in bumblebees. Biol Conserv 122:1–8

Goulson D, Lye GC, Darvill B (2008a) Decline and conservation of bumblebees. Ann Rev Entomol 53:191–208

Goulson D, Lye GC, Darvill B (2008b) Diet breadth, coexistence and rarity in bumblebees. Biodivers Conserv 17:3269–3288

Grixti JC, Wong LT, Cameron SA, Favret C (2009) Decline of bumblebees (*Bombus*) in the North American Midwest. Biol Conserv 142:75–84

Haas A (1949) Arttypische Flugbahnen von Hummelmännchen. Z Vergl Phys 31:281–307 (in German)

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. Ann Rev Ecol Syst 29:83–112

Knight ME, Martin AP, Bishop S, Osborne JL, Hale RL, Sanderson RA, Goulson D (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Mol Ecol 14:1811–1820

Kraus FB, Wolf S, Moritz RFA (2009) Male flight distance and population substructure in the bumblebee *Bombus terrestris*. J Anim Ecol 78:247–252

Kwak MM, Velterop O, Andel J (1998) Pollen and gene flow in fragmented habitats. Appl Veg Sci 1:37–54

Lepais O, Darvill B, O'Connor S, Osborne JL, Sanderson RA, Cussans J, Goffe L, Goulson D (2010) Estimation of bumblebee queen dispersal distances using sibship reconstruction method. Mol Ecol 19:819–831

Mauss V (1994) Bestimmungsschlüssel für Hummeln. Deutscher Jugendbund für Naturbeobachtungen (DJN), Hamburg (in German)

Mikkola K (1984) Spring migrations of wasp and bumblebee queens across the Gulf of Finland (Hymenoptera: Vespidae and Apidae). Not Entomol 64:125–128

Müller CB, Schmid-Hempel P (1992) Correlates of the reproductive success among field colonies of *Bombus lucorum*, the importance of growth and parasites. Ecol Entomol 17:343–353

Murray TE, Fitzpatrick U, Brown MJF, Paxton RJ (2008) Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. Conserv Genet 9:653–666

Murray TE, Kuhlmann M, Potts SG (2009) Conservation ecology of bees: populations, species and communities. Apidologie 40:211–236

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Osborne JL, Clarke SJ, Morris RJ, Williams IH, Riley JR, Smith AD, Reymolds DR, Edwards AS (1999) A landscape-scale study of bumblebee foraging range and constancy, using Harmonic Radar. J Appl Ecol 36:519–533

Osborne JL, Martin AP, Carreck NL, Swain JL, Knight ME, Goulson D, Hale RJ, Sanderson RA (2008) Bumblebee flight distances in relation to the forage landscape. J Anim Ecol 77:406–415

Ostevik KL, Manson JS, Thomson JD (2010) Pollination potential of male bumblebees (*Bombus impatiens*): movement patterns and pollen transfer-efficiency. J Poll Ecol 2:21–26

Owen RE, Plowright RC (1982) Worker-queen conflict and male parentage in bumblebees. Behav Ecol Sociobiol 11:91–99

Packer L, Owen RE (2001) Population genetic aspects of pollinator decline. Conserv Ecol 5:4–27

Packer L, Zayed A, Grixti JC, Ruz L, Owen RE, Vivallo F, Toro H (2005) Conservation genetics of potentially endangered mutualisms: reduced levels of genetic variation in specialist versus generalist bees. Conserv Biol 19:195–202

Pasquet RS, Peltier A, Hufford MB, Oudin E, Saulnier J, Paul L, Knudsen JT, Herren HR, Gepts P (2008) Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. Proc Natl Acad Sci USA 105:13456–13461

Pritchard JK, Stephens M, Donnelly P (2000) Interference of population structure from multilocus genotype data. Genetics 155:945–959

Saville NM, Dramstad WE, Fry GLA, Corbet SA (1997) Bumblebee movement in a fragmented agricultural landscape. Agric Ecosyst Environ 61:145–154

Svensson BG (1979) Patrolling behaviour of bumblebee males (Hymenoptera: Apidae) in a Subalpine/Alpine area, Swedish Lapland. Zoon 7:67–94

Takahashi J, Ayabe T, Mitsuhata M, Shimizu I, Ono M (2008) Diploid male production in a rare and locally distributed bumblebee, *Bombus florilegus* (Hymenoptera, Apidae). Insectes Soc 55:43–50

van Doorn A, Heringa J (1986) The ontogeny of a dominance hierarchy in colonies of the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). Insectes Soc 33:3–25

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–512

Walther-Hellwig K, Frankl R (2000) Foraging distances of *Bombus muscorum*, *Bombus lapidarius* and *Bombus terrestris* (Hymenoptera, Apidae). J Insect Behav 13:239–246

Wang J (2004) Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979

Westphal C, Steffan-Dewenter I, Tscharntke T (2006) Bumblebees experience landscapes at different spatial scales—possible implications for co-existence. Oecologia 149:289–300

Whitehorn PR, Tinsley MC, Goulson D (2009a) Kin recognition and inbreeding reluctance in bumblebees. Apidologie 40:627–633

Whitehorn PR, Tinsley MC, Brown MJF, Darvill B, Goulson D (2009b) Impacts of inbreeding on bumblebee colony fitness under field conditions. BMC Evol Biol 9:152

Williams PH, Osborne JL (2009) Bumblebee vulnerability and conservation world-wide. Apidologie 40:367–387

Wolf S, Moritz RFA (2008) Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). Apidologie 39:419–427

Wolf S, Rohde M, Moritz RFA (2009) The reliability of morphological traits in the differentiation of *Bombus terrestris* and *B. lucorum* (Hymenoptera: Apidae). Apidologie 41:45–53

Wright S (1933) Inbreeding and Homozygosis. Proc Natl Acad Sci USA 19:411–420

Zayed A (2004) Effective population size in Hymenoptera with complementary sex determination. Heredity 93:627–630

Zayed A (2009) Bee genetics and conservation. Apidologie 40:237–262