Molecular Ecology (2003) **12**, 2801–2808 doi: 10.1046/j.1365-294X.2003.01957.x

Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators

R. E. CHAPMAN, J. WANG and A. F. G. BOURKE
Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK

Abstract

Conservation biologists, evolutionary ecologists and agricultural biologists require an improved understanding of how pollinators utilize space and share resources. Using microsatellite markers, we conducted a genetic analysis of space use and resource sharing at several spatial scales among workers of two ecologically dissimilar bumble bee species (*Bombus terrestris* and *B. pascuorum*) foraging in an urban landscape (London, UK). At fine scales, the relatedness of workers visiting small patches of flowers did not differ significantly from zero. Therefore, colonies shared flower patches randomly with other colonies, suggesting that worker scent-marks deterring visits to unrewarding flowers have not evolved as signals benefiting nestmates. To investigate space use at intermediate scales, we developed a program based on Thomas & Hill's maximum likelihood sibship reconstruction method to estimate the number of colonies utilizing single sites. The average number of colonies (95% confidence limits) sending workers to forage at sites of ≈ 1 ha in area was 96 colonies (84–118) in *B. terrestris* and 66 colonies (61–76) in *B. pascuorum*. These values are surprisingly high and suggested that workers travelled far from their colonies to visit the sites. At the landscape scale, there was little or no genetic differentiation between sites. We conclude that urban habitats support large bumble bee populations and are potentially valuable in terms of bumble bee conservation. In addition, bumble bee-mediated gene flow in plants is likely to occur over large distances and plant–bumble bee conservation requires landscape-scale action.

Keywords: *Bombus*, foraging ecology, microsatellite, plant–pollinator relationship, relatedness, social insect

Received 6 May 2003; revision received 10 July 2003; accepted 10 July 2003

Introduction

Knowledge of how pollinators use space and share resources at different spatial scales is required for effective plant–pollinator conservation (Kearns *et al*. 1998; Schulke & Waser 2001; Gathmann & Tscharntke 2002; Steffan-Dewenter *et al*. 2002; Steffan-Dewenter & Kuhn 2003; Dick *et al*. 2003). It is also important for determining patterns of gene flow among pollinated plants (Proctor *et al*. 1996; Cresswell 1997; Schulke & Waser 2001; Cresswell *et al*. 2002), including genetically modified crops (Rieger *et al*. 2002), and the evolutionary basis of communication of resource quality and location among pollinators (Goulson *et al*. 1998, 2000; Stout *et al*. 1998; Williams 1998; Dornhaus & Chittka 1999). However, very little is known about large-scale spatial foraging patterns or resource sharing at any scale in insect pollinators, including bumble bees (Osborne *et al*. 1999; Schulke & Waser 2001; Steffan-Dewenter *et al*. 2002). Bumble bee species are targets for conservation because many wild flowers and commercial crops largely depend on them for pollination and because several bumble bee species are undergoing severe declines (Williams 1982; Matheson *et al*. 1996; Kearns *et al*. 1998). Urban habitats are potentially important for bumble bee conservation because of the presence of flower-rich gardens and parks (Matheson *et al*. 1996; Benton 2000; Goulson *et al*. 2002). Urban habitats mimic many agricultural landscapes in the fragmented distribution of their resources (Samways 1994).

Bombus terrestris and *B. pascuorum* are common European bumble bees, but *B. terrestris* has large colonies and short-tongued workers that visit a general range of flowers, whereas *B. pascuorum* has smaller colonies and long-tongued workers that specialize on visiting flowers with deep corollae

Correspondence: A. F. G. Bourke. Fax: +44-20-7586-2870; E-mail: andrew.bourke@ioz.ac.uk

(Alford 1975; Prys-Jones & Corbet 1991). Workers of both species deposit scent-marks on flowers that temporarily deter revisitation by themselves, conspecific workers and workers of other bumble bee species (Goulson *et al*. 1998; Stout *et al*. 1998; Williams 1998). Receivers of these signals benefit by avoiding depleted flowers, yet kin selection theory (Hamilton 1964) predicts that selection should not favour the production of costly signals of resource quality benefiting unrelated conspecifics or members of other species. It has, therefore, been suggested, but not demonstrated, that these signals have evolved to benefit either the scent-marking forager herself or her nestmates (Stout *et al*. 1998; Williams 1998).

Studies of foraging bumble bees involving mark–recapture or tracking using harmonic radar have shown that individual workers and colonies tend to be area-constant (favour repeatedly foraging in the same area over periods of hours or days) (Heinrich 1976; Dramstad 1996; Thomson 1996; Saville *et al*. 1997; Osborne *et al*. 1999; Walther-Hellwig & Frankl 2000; Osborne & Williams 2001). They have also shown that workers may fly, on average, hundreds of metres from their nests to forage and some workers may fly 1–2 km (e.g. mean flight distances of 663 m and 275 m, and maxima of 1750 m and 631 m, respectively, in two studies of *B. terrestris*, Osborne *et al*. 1999; Walther-Hellwig & Frankl 2000; see also Dramstad 1996 and Dramstad *et al*. 2003). However, because they are labour-intensive, mark–recapture and radar-tracking measurements of foraging distance have been collected from relatively few colonies. They are also likely to be truncated because, in mark–recapture (Walther-Hellwig & Frankl 2000), observers' search effort falls with increasing distance from the nest, and, in radar-tracking (Osborne *et al*. 1999), workers become undetectable behind obstacles or beyond 700 m. By contrast, genetic analyses permit unbiased, population-wide inferences about average patterns of space use by many colonies simultaneously. They also allow censusing of the number of colonies visiting individual sites.

To characterize space use and resource sharing by bumble bees foraging in an urban landscape, we analysed variation at up to six polymorphic microsatellite DNA loci (Estoup *et al*. 1995, 1996) in 531 *B. terrestris* and 458 *B. pascuorum* workers collected at flowers in 11 sites in London, UK. We conducted our investigation at three spatial scales. First, at a fine scale, we tested the hypothesis that workers visiting the same flower patch are nestmates. Second, at an intermediate scale, we estimated from the genetic data the numbers of colonies sending foraging workers to single sites and the foraging ranges of these workers. Third, at the landscape scale, we analysed worker mixing across sites by estimating between-site genetic differentiation. Darvill *et al*. (submitted) independently investigated space use and resource sharing in *B. terrestris* and *B. pascuorum* at a rural site in Hampshire, UK. Our study and that of Darvill *et al*. are novel because they provide the first genetic estimates of the numbers of colonies visiting sites and of workers' foraging range. Two previous genetic studies estimated the number of colonies contributing to male mating aggregations in social insects (Baudry *et al*. 1998; Paxton 2000), but to our knowledge only the current study and that of Darvill *et al*. (submitted) have estimated the number of colonies represented in a sample of foraging workers. Studies of other invertebrates have also used genetic markers as the basis of indirect census methods (e.g. to estimate the number of foundresses in oak gall-wasps: Atkinson *et al*. 2002). Our work differs from and is complementary to that of Darvill *et al*. (submitted) in that it estimates colony number using a maximum likelihood method, includes an investigation of fine-scale resource sharing, and samples an urban rather than an agricultural environment.

Materials and methods

Field collection and sampling

Between 21 June and 27 July 2001, we collected 74–100 workers of each species at five (*Bombus terrestris*) or four (*B. pascuorum*) sites from sampling areas of mean 0.8 ha (Table 1). The mean distance between sites (Table 1) was 14.7 km (range, 5–27 km). Within each site, up to 10 conspecific workers arriving to forage at each of 10 patches of flowers (mean ± SD area = 0.76 ± 0.25 m^2, mean distance apart = 57.4 m) were collected in order of their arrival (mean per patch collection times for *B. terrestris* and *B. pascuorum* were 41 and 31 min, respectively). Henceforth, we refer to the collecting sites (Table 1) as 'sites' and to individual patches of flowers as 'patches'. Data from 10 workers collected at each of an additional six sites (Table 1) were used only to estimate between-site genetic differentiation. All collections took place between 09.45 and 18.30. *B. terrestris* workers were distinguished from similar *B. lucorum* workers by a buff 'tail' or a distinct buff line between the 'tail' and the neighbouring black abdominal band (Prys-Jones & Corbet 1991; Benton 2000). Each worker was caught in a plastic tube, chilled and later frozen at –80 °C.

Molecular genetic methods

Workers were genotyped at a mean 5.8 microsatellite loci (range, 3–6) at the loci *B10*, *B11*, *B96*, *B100*, *B124* and *B126* (*B. terrestris*) and *B96*, *B118*, *B124*, *B126*, *B131* and *B132* (*B. pascuorum*) (Estoup *et al*. 1995, 1996). DNA was extracted from an entire middle leg using proteinase K digestion in 1× TE buffer (500 µl TE [10 mM Tris–Cl, pH 7.4 and 10 mM EDTA] + 10 µl of 20 mg/mL proteinase K) at 55 °C overnight and then heated to 99 °C for 10 min to

Table 1 Collection details and estimated number of bumble bee colonies visiting sites in London, UK. Flowers making up sampling patches were the following: for *Bombus terrestris*, *Ballota nigra*, *Campanula* sp., *Centaurea nigra*, *Cirsium arvense*, *Deutzia* sp., *Epilobium* sp., *Galega officinalis*, *Geranium pratense*, *Hebe* sp., *Lavendula* sp., *Lotus corniculatus*, *Rubus fruticosus*, *Solanum dulcamara*, *Trifolium repens*, *T. pratense*; for *Bombus pascuorum*, *B. nigra*, *C. nigra*, *G. officinalis*, *Lamium album*, *Lathyrus pratensis*, *L. corniculatus*, *R. fruticosus*, *S. dulcamara*, *S. nigrum*, *T. repens*, *T. pratense*, *Vicia cracca*. Numbers of colonies are given without correction for unsampled colonies, from sites where 74–100 workers were collected

				B. terrestris		*B. pascuorum*	
Site (postal code)	Grid reference	Habitat type	Sampling area (ha)	No. of workers	No. of colonies	No. of workers	No. of colonies
Nunhead Cemetery SE15	TQ355755	Cemetery	0.85	100	58	100	55
Barnes Common SW13	TQ225759	Public park	0.91	99	66	100	60
Regent's Park NW1	TQ277833	Public park	0.66	74	30	100	53
Millennium Village SE10	TQ399792	Public park	0.70	100	61	98	40
Thames Barrier Park E16	TQ412798	Public park	0.88	98	69	0	n/a
Tolworth Roundabout KT9	TQ198650	Wasteground	0.30	10	n/a	10	n/a
Hanwell Cemetery W7	TQ159800	Cemetery	0.28	10	n/a	10	n/a
Woodgrange Park E12	TQ418851	Cemetery	0.18	10	n/a	10	n/a
St James Lane N10	TQ288895	Garden	0.14	10	n/a	10	n/a
Beddington Park CR0	TQ290654	Public park	0.28	10	n/a	10	n/a
Grove Park SE9	TQ415725	Public park	0.14	10	n/a	10	n/a
Totals				531		458	

n/a, not applicable.

denature the proteinase K prior to polymerase chain reaction (PCR). PCR amplifications were performed using standard protocols (Morin *et al*. 1998). Amplification products were visualized on an ABI PRISM™ 373 automated sequencer and allele sizes were scored using an internal size standard (GeneScanTAMRA 500, Applied Biosystems). The mean numbers of alleles per locus and mean heterozygosities were, respectively, 14.5 (range, 10–20) and 65.5% for *B. terrestris* and 12.5 (range, 9–16) and 67% for *B. pascuorum*.

Across both species, 734 of a total of 5711 genotypes were retyped (using repeat PCRs), from which frequencies of erroneous genotypes were calculated as 4.6% (*B. terrestris* heterozygotes), 23.1% (*B. terrestris* homozygotes), 0% (*B. pascuorum* heterozygotes) and 6.2% (*B. pascuorum* homozygotes). Errors among *B. terrestris* heterozygotes were unlikely to have affected the results because *B. pascuorum*, with no such errors, yielded very similar findings. Most errors among *B. terrestris* homozygotes were due to allelic drop-out (87% involved apparent homozygotes that retyping showed to be heterozygotes). These were also unlikely to have biased the results. First, only a minority (34.5%) of *B. terrestris* genotypes were homozygous, so the overall contribution to the error rate from erroneous homozygotes was 8.0%. Second, relatedness, F_{ST} and colony number estimates from the site accounting for most (56%) of these errors (Nunhead Cemetery) were qualitatively identical to those from a site with no such errors (Regent's Park). Third, simulations (see subsection, 'Estimation of number of colonies utilizing single sites') showed that these errors caused colony number to be underestimated from our datasets and hence to be conservative.

Estimation of linkage disequilibrium and inbreeding

Tests for linkage disequilibrium and for the presence of inbreeding ($F_{IS} > 0$) were carried out on subsamples of workers from each of the sites from which more than 10 workers were collected using GENEPOP 3.1b (Raymond & Rousset 1995), available at http://wbiomed.curtin.edu.au/genepop/. Twenty workers were randomly selected 10 times from each site-sample. This subsampling procedure was designed to minimize the inclusion in the comparisons of nonindependent genotypes due to the presence of relatives in the site-samples. Bonferonni correction was applied for multiple tests.

Estimation of worker relatedness within patches

Because *B. terrestris* and *B. pascuorum* colonies are headed by a single, once-mated queen (Prys-Jones & Corbet 1991; Estoup *et al*. 1995; Schmid-Hempel & Schmid-Hempel 2000), nestmate workers are full sisters with an expected relatedness (for outbred haplodiploids) of 0.75. Hence the expected relatedness of workers within patches is 0 if workers within sites are randomly distributed over patches, but is 0.75 if single colonies monopolize patches. We therefore calculated regression relatedness (Queller & Goodnight

1989) within patches using RELATEDNESS 5.0.8 (http://gsoft.smu.edu/Gsoft.html) and tested for differences from 0 and 0.75 using *t*-tests. Bonferonni correction was again applied for multiple tests.

Estimation of number of colonies utilizing single sites

Given that workers from the same *B. terrestris* or *B. pascuorum* colony share their mother and father (Prys-Jones & Corbet 1991; Estoup *et al*. 1995; Schmid-Hempel & Schmid-Hempel 2000), the minimum number of full sisterhoods present in a sample of workers equals the minimum number of colonies represented in the sample. Using allele-sharing criteria for haplodiploid full sisters, we developed a program (COLONY 1.0, available from the authors) adapting the maximum likelihood sibship reconstruction method of Thomas & Hill (2000) to reconstruct worker sibships from each site at which > 10 workers were collected (Table 1). To check the method's power in datasets resembling ours, we simulated the case of a sample of 100 individuals typed at 6 loci each having 10 co-dominant alleles following a uniform frequency distribution, assuming allelic drop-out rates of (i) 0% and (ii) 20% per locus. For (i), when the 'actual' colony size followed a truncated Poisson distribution with $m = 0.9$ and $m = 15$ (where m is the mean size of the workforce representing each colony in the sample), the estimated numbers of colonies were 0.92 ± 0.03 and 1.0 ± 0.0 (mean $\pm$ SD, $n = 100$ replicates) of their actual values, respectively. Therefore, the method's power increased with increasing m, but, for the case of $m = 0.9$, which mimics our data (with many colonies represented in each site sample by a few workers each; see Results), it slightly underestimates colony number. This is because the number of unrelated individuals that, by chance, have multilocus genotypes consistent with full sisterhood increases as colony number increases. For (ii) with $m = 0.9$, the method returned a more conservative estimate (0.85 ± 0.04 of the actual colony number, $n = 100$ replicates) than in (i). Therefore, observed levels of allelic drop-out in our datasets (see 'Molecular genetic methods') caused an additional underestimate of colony number. For larger families ($m >> 0.9$), allelic drop-outs resulted in overestimates of colony number because larger families were split. Effects of other types of error in the genetic data (e.g. scoring or data entry errors) were not investigated. Although such errors might have been present in the data, they were likely to have been rare because, of 139 genotypes scored from 24 additional workers collected from known nests (two *B. terrestris* nests and one *B. pascuorum* nest collected in London; data not shown), only 2 were inconsistent with monogyny and monandry and hence likely to have been scoring or data entry errors. When calculating the average number of *B. terrestris* colonies visiting sites, we excluded the Regent's Park site to equalize the sample sizes (98–100 workers) of included sites (Table 1).

Estimation of foraging distance

The density at which bumble bee nests occur naturally in any environment is unknown at the landscape scale. For scarcer species in nonurban landscapes, the per species density of mature nests successfully producing female sexuals has been estimated at ≈ 1–2 per km^2 (M. Edwards, pers. commun.). We assumed a range of nest densities of 2–40 nests per km^2, given that urban bumble bees are likely to occur at relatively high densities (Goulson *et al*. 2002) and not all nests produce female sexuals. This range also approximates the range (2–7 nests per ha per species) measured in two site-scale studies of nest density (Cumber 1953; Harder 1986), correcting for the fact that areas with many nests were selected for investigation in these studies (Cumber 1953; Harder 1986) and the likelihood that such areas are relatively rare across landscapes (Matheson *et al*. 1996). If nests are distributed at density d randomly with respect to foraging sites, the radius r of a circle centred on a site (considered as a point) and enclosing K colonies is $\sqrt{(K/\pi d)}$. Because 50% of colonies will occur in the annulus whose outer and inner borders are at radii r and $(\sqrt{0.5})r$, respectively, from the centre of the circle, median foraging distance was estimated as $(\sqrt{0.5})[\sqrt{(K/\pi d)}]$.

Estimation of between-site genetic differentiation

We estimated levels of between-site genetic differentiation (F_{ST}) using FSTAT (http://www.unil.ch/izea/softwares/fstat.html). To achieve balanced sampling and minimize the inclusion of related workers, we included in the analysis all 10 workers per site from the 6 sites in which only 10 workers were sampled and 10 randomly selected workers per site from the remaining sites. Bonferonni correction was again applied for multiple tests. To investigate the relationship of genetic differentiation and geographical distance, we regressed pairwise F_{ST} on geographical distance, testing the significance of the relationship using a Mantel test with 20 000 permutations.

Results

Linkage disequilibrium and inbreeding

There was no evidence for significant linkage disequilibrium between loci in either species ($n = 10 \times 15$ possible pairwise comparisons between each of the six loci in each species; all $P > 0.0003$, corresponding to table-wide $P = 0.05$). There was also no evidence for significant inbreeding in any site [*Bombus terrestris*: global $F_{IS} = 0.062$ (range, –0.025 to 0.115), $n = 5$ sites, all $P > 0.14$; *B. pascuorum*: global

Table 2 Inbreeding coefficients (F_{IS}) calculated from bumble bee worker samples at sites in London, UK (Table 1), from which 74–100 workers were collected, tested for significant difference from zero. n/a, not applicable

	Bombus terrestris			*Bombus pascuorum*		
Site	F_{IS}	Range	Mean *P*-value	F_{IS}	Range	Mean *P*-value
Nunhead Cemetery	0.1144	0.0432 to 0.2428	0.2560	0.0459	–0.0422 to 0.1145	0.4089
Barnes Common	0.1154	0.0357 to 0.1887	0.2552	0.0744	0.0158 to 0.1728	0.2761
Regent's Park	–0.0251	–0.1808 to 0.0272	0.1436	0.0669	0.0025 to 0.1380	0.2971
Millennium Village	0.1001	0.0443 to 0.2090	0.2163	–0.0028	–0.0972 to 0.0755	0.2823
Thames Barrier Park	0.1093	0.0425 to 0.1948	0.2621	n/a	n/a	n/a

F_{IS} = 0.052 (range, –0.003 to 0.074), n = 4 sites, all $P > 0.28$; Table 2].

Worker relatedness within patches

Mean relatedness of workers visiting patches was 0.008 in *B. terrestris* (range = –0.065 to 0.216, n = 50 patches with mean 9.4 workers each from 5 sites) and 0.012 in *B. pascuorum* (range = –0.068 to 0.147, n = 40 patches with mean 9.9 workers each from 4 sites). In both species, within-patch relatedness was never significantly greater than 0 (*B. terrestris*: all $P > 0.001$, corresponding to table-wide $P = 0.05$; *B. pascuorum*: all $P > 0.0012$, corresponding to table-wide $P = 0.05$) and was always significantly less than 0.75 (*B. terrestris*: all $P < 0.001$; *B. pascuorum*: all $P < 0.0012$).

Number of colonies utilizing single sites

The average minimum number of colonies visiting sites was estimated at 63 colonies per site in *B. terrestris* (n = 4 sites with 99.2 workers genotyped per site) and 52 colonies per site in *B. pascuorum* (n = 4 sites with 99.5 workers genotyped per site) (Table 1). These values are conservative because many colonies visiting sites may have remained unsampled and because our method tended to underestimate colony number from our datasets (see 'Estimation of number of colonies utilizing single sites'). To investigate the scale of the former effect, we pooled datasets from all sites within each species. The observed frequency distributions of the sizes of the workforces from different colonies present in the entire sample did not differ significantly from a truncated Poisson distribution (*B. terrestris*: $\chi^2 = 6.0$, df = 3; *B. pascuorum*, $\chi^2 = 10.4$, df = 5; both $P > 0.05$). We therefore used fitted Poisson distributions to estimate the frequency of colonies unrepresented in the sample and hence the average overall numbers of colonies visiting a site. The resulting overall averages (95% confidence limits) were 96 colonies (84–118) per site for *B. terrestris* and 66 colonies (61–76) per site for *B. pascuorum*.

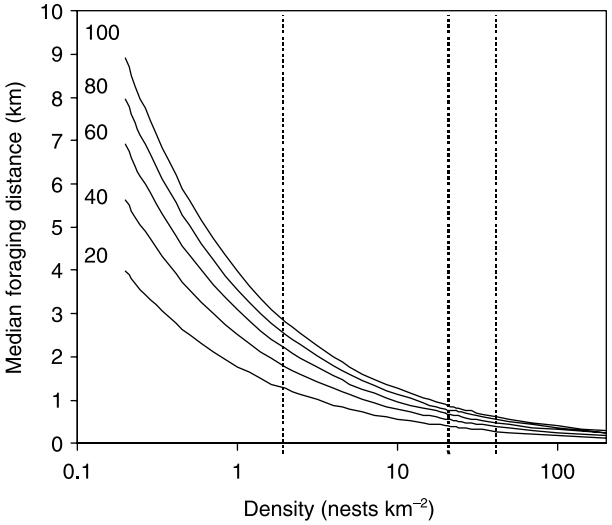

Fig. 1 Estimated median foraging distance as a function of nest density for workers visiting a site attracting K = 20–100 colonies; dotted vertical lines show nest densities of 2, 20 and 40 nests per km^2.

Foraging distance

We combined our estimates of average colony number visiting sites (96 for *B. terrestris* and 66 for *B. pascuorum*) with estimates of nest density (see 'Estimation of foraging distance') to estimate the foraging distances of workers visiting the sites. We estimated median foraging distances to be 0.62–2.8 km for *B. terrestris* and 0.51–2.3 km for *B. pascuorum* (Fig. 1). The corresponding maximum foraging distances were 0.87–3.9 km for *B. terrestris* and 0.72–3.2 km for *B. pascuorum* (Fig. 1).

Between-site genetic differentiation

There was no significant genetic differentiation among sites in *B. terrestris* and slight but significant differentiation in *B. pascuorum* (global F_{ST} [95% confidence limits] = 0.000

[–0.012 to 0.012] and 0.009 [0.003–0.015], respectively). In *B. terrestris*, pairwise differentiation was not significant between any sites (pairwise F_{ST} = –0.0003 to 0.046, n = 55 comparisons between 11 sites, all $P > 0.0009$, corresponding to table-wide $P = 0.05$). In *B. pascuorum*, there was significant pairwise differentiation between only a single pair of sites (pairwise F_{ST} = –0.007 to 0.039, n = 45 comparisons of 10 sites, significant $P = 0.001$). There was no significant correlation between the degree of genetic differentiation among sites and their geographical distance apart in either species (Mantel tests: *B. terrestris*, $r^2 = 6.52\%$; *B. pascuorum*, $r^2 = 0.1\%$; both $P > 0.05$).

Discussion

We conducted a genetic analysis of space use and resource sharing by workers of two bumble bee species (*Bombus terrestris* and *B. pascuorum*) foraging in an urban environment (London, UK). We found that individual colonies did not monopolize flower patches but, instead, workers from different colonies mixed at random within patches. In addition, we found that, surprisingly, many colonies visit, and share forage resources within, individual sites. Hence it is likely that workers foraged far from their nests. Finally, we found no or little genetic differentiation across sites at the landscape scale. Our findings regarding genetic differentiation suggest extensive recent or current gene flow among bee populations across sites, implying that either foraging workers fly far and hence mix across sites, or queens disperse far prior to colony foundation (Mikkola 1984; Stenström & Bergman 1998), or both. They are also consistent with weak or absent genetic differentiation reported in *B. terrestris* and *B. pascuorum* at regional scales (Estoup *et al*. 1996; Widmer & Schmid-Hempel 1999). Our finding that no significant inbreeding was detectable in the study samples is also consistent with previous genetic studies of wild populations of these and other bumble bee species (Owen & Plowright 1980; Estoup *et al*. 1996; Widmer & Schmid-Hempel 1999). Overall, our analyses demonstrate that workers from many colonies mix extensively at the scale of both patches and sites, and suggest that bumble bee workers routinely fly far from their nests (hundreds of metres to several kilometres) to forage. These findings have several implications.

The first implication stems from the finding that, although they are area-constant (Thomson 1996; Saville *et al*. 1997; Osborne *et al*. 1999; Osborne & Williams 2001), individual foragers and colonies clearly share flower patches with unrelated workers from other colonies. This is consistent with lack of recruitment of nestmates to specific locations in bumble bees (Dornhaus & Chittka 1999) and implies that repellent scent-marks left by workers on flowers (Goulson *et al*. 1998; Stout *et al*. 1998; Williams 1998), if they are costly to produce, have not evolved to benefit nestmates, as nestmates would not benefit preferentially from them. They are therefore most likely to have evolved to benefit the individual depositing them (by reducing the chances of its accidentally revisiting an unrewarding flower), or to be nonadaptive (Goulson *et al*. 1998; Stout *et al*. 1998; Williams 1998).

A second implication of our findings is that, although our estimates of foraging range are reliant on uncertain estimates of colony density, bumble bee workers forage even further than previous estimates using mark–recapture or radar-tracking suggested (Osborne *et al*. 1999; Walther-Hellwig & Frankl 2000). Our estimates were also larger than those measured (by translocation experiments) for a range of nonsocial bee species (maxima of 150–600 m: Gathmann & Tscharntke 2002), similar to those deduced (by decoding workers' waggle dances) for honey bees, *Apis mellifera*, foraging over agricultural and wooded landscapes (median foraging distance of 1.2 km: Steffan-Derwenter & Kuhn 2003), but smaller than those deduced (also by decoding workers' waggle dances) for honey bees foraging over moorland (median foraging distance of 6.1 km: Beekman & Ratnieks 2000). Nonetheless, our results suggest that plant gene flow via pollen borne by bumble bee workers is likely to occur over large distances (up to several kilometres), and that flower patches several kilometres from bumble bee nesting areas are likely to receive visits by foraging workers. This knowledge should inform decisions concerning both the conservation of fragmented populations of endangered wild flowers pollinated by bumble bees (Schulke & Waser 2001) and the location of stands of genetically modified crops (Rieger *et al*. 2002). Moreover, if viable pollen is exchanged between workers within the nest as in honey bees (Free & Williams 1972; DeGrandi-Hoffman *et al*. 1986), pollen could be carried by bumble bee workers over distances that are even greater than an individual worker's maximum foraging distance. Extensive mixing of bumble bees from different colonies at flower patches and sites also implies that the potential for horizontal transmission of bumble bee parasites, which are known to be numerous and to have important effects on their hosts' life history (Schmid-Hempel 1998), is large.

The significant difference between the average numbers of colonies visiting single sites in *B. terrestris* and *B. pascuorum*, and hence the difference in their estimated foraging distances, support the suggestion that *B. pascuorum* and species sharing its ecological traits are more localized foragers than *B. terrestris* and similar species (Hedtke & Schricker 1996; Walther-Hellwig & Frankl 2000). Darvill *et al*. (submitted) likewise found that *B. terrestris* had a greater foraging range than *B. pascuorum* and so reached the same conclusion. Note that our comparison of the foraging ranges of *B. terrestris* and *B. pascuorum* assumes similar nest densities in these two species: if

B. pascuorum nests occurred at lower densities, then foraging distances similar to those of *B. terrestris* would also result in fewer *B. pascuorum* colonies being represented at single forage sites, as observed (Table 1; Fig. 1). However, Darvill *et al*. found *B. pascuorum* nests to occur at higher densities than those of *B. terrestris* in an agricultural landscape.

Finally, although for ease of collecting we initially chose sampling sites for the large numbers of workers they attracted, it is clear that resource-rich forage sites in urban areas can serve populations of many colonies from a large surrounding area. This confirms the potential importance of urban sites for bumble bee conservation (Benton 2000). This conclusion is reinforced by the finding of Darvill *et al*. (submitted) that fewer colonies of either species visited sites of similar size in an agricultural landscape, suggesting that these bumble bee species occur at higher density in urban and suburban habitats than in agricultural habitats. Colonies visiting sites in our study were also likely to be utilizing multiple neighbouring forage sites. For all these reasons we conclude that, across all types of environment, conservation strategies for bumble bees and wild flowers dependent on them for pollination should involve coordinated action at a scale larger than that of single sites, namely a landscape scale.

Acknowledgements

We thank A. Bhole for help with genotyping, L. Chittka, S.A. Corbet, M. Edwards, S.J. Goodman, R.L. Hammond and S.C. Thomas for advice, T. Chapman, J. Field, D. Goulson, R.L. Hammond, W.C. Jordan, C. Lopez Vaamonde, G.M. Mace, C.B. Müller and J.J.M. Pereboom, as well as G. Stone and an anonymous referee, for comments on the manuscript, B. Darvill, M.E. Knight and D. Goulson for sharing unpublished work, and site managers for collecting permissions. This work was supported by a Natural Environment Research Council studentship held by R.E.C.

References

Alford DV (1975) *Bumblebees*. Davis-Poynter, London.

Atkinson RJ, McVean GAT, Stone GN (2002) Use of population genetic data to infer oviposition behaviour: species-specific patterns in four oak gallwasps (Hymenoptera: Cynipidae). *Proceedings of the Royal Society of London, Series B*, **269**, 383–390.

Baudry E, Solignac M, Garnery L *et al.* (1998) Relatedness among honeybees (*Apis mellifera*) of a drone congregation. *Proceedings of the Royal Society of London, Series B*, **265**, 2009–2014.

Beekman M, Ratnieks FLW (2000) Long-range foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology*, **14**, 490–496.

Benton T (2000) *The Bumblebees of Essex*. Lopinga Books, Wimbish.

Cresswell JE (1997) Spatial heterogeneity, pollinator behaviour and pollinator-mediated gene flow: bumblebee movements in variously aggregated rows of oil-seed rape. *Oikos*, **78**, 546–556.

Cresswell JE, Osborne JL, Bell SA (2002) A model of pollinator-mediated gene flow between plant populations with numerical solutions for bumblebees pollinating oilseed rape. *Oikos*, **98**, 375–384.

Cumber RA (1953) Some aspects of the biology and ecology of bumble-bees bearing upon the yields of red-clover seed in New Zealand. *New Zealand Journal of Science and Technology*, **34**, 227–240.

DeGrandi-Hoffman G, Hoopingarner R, Klomparens K (1986) Influence of honey bee (Hymenoptera: Apidae) in-hive pollen transfer on cross-pollination and fruit set in apple. *Environmental Entomology*, **15**, 723–725.

Dick CW, Etchelecu G, Austerlitz F (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology*, **12**, 753–764.

Dornhaus A, Chittka L (1999) Evolutionary origins of bee dances. *Nature*, **401**, 38.

Dramstad WE (1996) Do bumblebees (Hymenoptera: Apidae) really forage close to their nests? *Journal of Insect Behavior*, **9**, 163–182.

Dramstad WE, Fry GLA, Schaffer MJ (2003) Bumblebee foraging — is closer really better? *Agriculture, Ecosystems and Environment*, **95**, 349–357.

Estoup A, Scholl A, Pouvreau A, Solignac M (1995) Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. *Molecular Ecology*, **4**, 89–93.

Estoup A, Solignac M, Cornuet J-M, Goudet J, Scholl A (1996) Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Molecular Ecology*, **5**, 19–31.

Free JB, Williams IH (1972) The transport of pollen on the body hairs of honeybees (*Apis mellifera* L.) and bumblebees (*Bombus* spp. L.). *Journal of Applied Ecology*, **9**, 609–615.

Gathmann A, Tscharntke T (2002) Foraging ranges of solitary bees. *Journal of Animal Ecology*, **71**, 757–764.

Goulson D, Hawson SA, Stout JC (1998) Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. *Animal Behaviour*, **55**, 199–206.

Goulson D, Hughes WOH, Derwent LC, Stout JC (2002) Colony growth of the bumblebee, *Bombus terrestris*, in improved and conventional agricultural and suburban habitats. *Oecologia*, **130**, 267–273.

Goulson D, Stout JC, Langley J, Hughes WOH (2000) Identity and function of scent marks deposited by foraging bumblebees. *Journal of Chemical Ecology*, **26**, 2897–2911.

Hamilton WD (1964) The genetical evolution of social behaviour I, II. *Journal of Theoretical Biology*, **7**, 1–52.

Harder LD (1986) Influences on the density and dispersion of bumble bee nests (Hymenoptera: Apidae). *Holarctic Ecology*, **9**, 99–103.

Hedtke C, Schricker B (1996) Heimfinden von *Apis mellifera* und 4 *Bombus*-Arten im Vergleich. *Apidologie*, **27**, 320–323.

Heinrich B (1976) The foraging specializations of individual bumblebees. *Ecological Monographs*, **46**, 105–128.

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant–pollinator interactions. *Annual Review of Ecology and Systematics*, **29**, 83–112.

Matheson A, Buchmann SL, O'Toole C, Westrich P, Williams IH, eds. (1996) *The Conservation of Bees*. Academic Press, London.

Mikkola K (1984) Migration of wasp and bumble bee queens across the Gulf of Finland (Hymenoptera: Vespidae and Apidae). *Notulae Entomologicae*, **64**, 125–128.

Morin PA, Mahboubi P, Wedel S, Rogers J (1998) Rapid screening and comparison of human microsatellite markers in baboons: allele size is conserved, but allele number is not. *Genomics*, **53**, 12–20.

Osborne JL, Clark SJ, Morris RJ *et al.* (1999) A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *Journal of Applied Ecology*, **36**, 519–533.

Osborne JL, Williams IH (2001) Site constancy of bumble bees in an experimentally patchy habitat. *Agriculture, Ecosystems and Environment*, **83**, 129–141.

Owen RE, Plowright RC (1980) Abdominal pile color dimorphism in the bumble bee, *Bombus melanopygus*. *Journal of Heredity*, **71**, 241–247.

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

Proctor M, Yeo P, Lack A (1996) *The Natural History of Pollination*. Harper Collins, London.

Prys-Jones OE, Corbet SA (1991) *Bumblebees*, Richmond Publishing, Slough.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Raymond M, Rousset F (1995) GENEPOP Version 1.2.: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rieger MA, Lamond M, Preston C, Powles SB, Roush RT (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science*, **296**, 2386–2388.

Samways MJ (1994) *Insect Conservation Biology*. Chapman & Hall, London.

Saville NM, Dramstad WE, Fry GLA, Corbet SA (1997) Bumblebee movement in a fragmented agricultural landscape. *Agriculture, Ecosystems and Environment*, **61**, 145–154.

Schmid-Hempel P (1998) *Parasites in Social Insects*. Princeton University Press, Princeton, NJ.

Schmid-Hempel R, Schmid-Hempel P (2000) Female mating frequencies in *Bombus* spp. from Central Europe. *Insectes Sociaux*, **47**, 36–41.

Schulke B, Waser NM (2001) Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*. *Oecologia*, **127**, 239–245.

Steffan-Dewenter I, Kuhn A (2003) Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society of London, Series B*, **270**, 569–575.

Steffan-Dewenter I, Münzenberg U, Bürger C, Thies C, Tscharntke T (2002) Scale-dependent effects of landscape context on three pollinator guilds. *Ecology*, **83**, 1421–1432.

Stenström M, Bergman P (1998) Bumblebees at an alpine site in northern Sweden: temporal development, population size, and plant utilization. *Ecography*, **21**, 306–316.

Stout JC, Goulson D, Allen JA (1998) Repellent scent-marking of flowers by a guild of foraging bumblebees (*Bombus* spp.). *Behavioral Ecology and Sociobiology*, **43**, 317–326.

Thomas SC, Hill WG (2000) Estimating quantitative genetic parameters using sibships reconstructed from marker data. *Genetics*, **155**, 1961–1972.

Thomson JD (1996) Trapline foraging by bumblebees. I. Persistence of flight-path geometry. *Behavioral Ecology*, **7**, 158–164.

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

Widmer A, Schmid-Hempel P (1999) The population genetic structure of a large temperate pollinator species, *Bombus pascuorum* (Scopoli) (Hymenoptera: Apidae). *Molecular Ecology*, **8**, 387–398.

Williams CS (1998) The identity of the previous visitor influences flower rejection by nectar-collecting bees. *Animal Behaviour*, **56**, 673–681.

Williams PH (1982) The distribution and decline of British bumble bees (*Bombus* Latr.). *Journal of Apicultural Research*, **21**, 236–245.

This work formed part of Roselle Chapman's PhD research, supervised by Andrew Bourke, on the conservation and foraging ecology of urban bumble bees. Andrew Bourke's research focuses on the behavioural ecology, genetics and conservation biology of social insects, especially ants and bumble bees. Jinliang Wang wrote the COLONY program and is interested in developing population and quantitative genetics models and methods of analysis of empirical data to address issues in evolutionary and conservation biology.
