MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 53–63 doi: 10.1111/j.1365-294X.2009.04423.x

Cryptic differences in dispersal lead to differential sensitivity to habitat fragmentation in two bumblebee species

B. DARVILL, S. O'CONNOR, G. C. LYE, J. WATERS, O. LEPAIS and D. GOULSON
School of Biological and Environmental Sciences, University of Stirling, Stirling FK9 4LA, UK

Abstract

Habitat loss has led to fragmentation of populations of many invertebrates, but social hymenopterans may be particularly sensitive to habitat fragmentation due to their low effective population sizes. The impacts of fragmentation depend strongly on dispersal abilities, but these are difficult to quantify. Here, we quantify and compare dispersal abilities of two bumblebee species, *Bombus muscorum* and *Bombus jonellus*, in a model island system. We use microsatellites to investigate population genetic structuring, dispersal and spatial patterns in genetic diversity. Populations of both species showed significant structuring, and isolation by distance, but this was markedly greater in *B. muscorum* ($\theta = 0.13$) than in *B. jonellus* ($\theta = 0.034$). This difference could reflect a higher effective population size in *B. jonellus* compared to *B. muscorum*, but this is not consistent with the observed abundance of the two species. We argue that it is more likely that *B. jonellus* has a higher propensity to disperse than *B. muscorum*. This will influence their relative susceptibility to habitat fragmentation and may in part explain differential declines of mainland populations of these and other bumblebee species.

Keywords: *Bombus*, conservation, dispersal, Hymenoptera, inbreeding, island populations, microsatellites

Received 9 June 2009; revision received 2 October 2009; accepted 6 October 2009

Introduction

Over recent decades, many bumblebee species have declined dramatically, both in Europe and North America, primarily as a result of agricultural intensification and associated habitat loss (reviewed in Goulson 2003; Goulson *et al.* 2008). As a consequence, populations of many species are now fragmented and threatened. In the United Kingdom, two species have gone extinct in the last 70 years and several remain only in small isolated populations (Benton 2006), while in North America five species have undergone catastrophic declines with one (*Bombus franklini*) now probably extinct (Colla & Packer 2008). At the same time, a handful of species remain ubiquitous, and it is not clear how these species differ from those that have declined (Goulson *et al.* 2005, 2006). The decline of bumblebees may have serious consequences for agricultural crops and wildflowers (Corbet *et al.* 1991), so it is crucial that we understand the underlying causes. To date, explanations for differential declines have included differences in: tongue length, emergence time, niche breadth, climatic niche space and differential susceptibility to disease (reviewed in Goulson *et al.* 2008).

Whatever the reasons, declines in several species have led to the fragmentation of remaining populations. In some instances, local extinctions have continued, despite the apparent suitability of habitat fragments, and the population as a whole has continued to decline. Within a functioning metapopulation, dispersal is of key importance, as it ensures that local extinctions are followed by re-colonization. However, if habitat fragmentation and loss lead to some habitable patches becoming isolated, not only will suitable patches remain unoccupied, but inbreeding may further accelerate declines. In the absence of occasional immigration, populations lose genetic diversity through bottlenecks and

Correspondence: D. Goulson, Fax: 01786 467843;
E-mail: dave.goulson@stir.ac.uk

drift (Frankham *et al*. 2002; Keller & Waller 2002). Low genetic diversity reduces the long-term viability of populations via inbreeding depression and a lowered capacity to respond to environmental change (Frankham *et al*. 2002; Hansson & Westerberg 2002; Keller & Waller 2002; Reed & Frankham 2003).

Along with other social insects, bumblebees are more susceptible to inbreeding than other invertebrates because the majority of the population are sterile workers, greatly reducing the effective population size (Pamilo & Crozier 1997; Chapman & Bourke 2001). In most bumblebee species, including those studied here, this effect is exacerbated by strict queen monogamy, meaning that colonies are the product of a single diploid queen and haploid male (Chapman & Bourke 2001). In essence, the effective population size is approximated by 1.5 times the number of successful colonies, and may be orders of magnitude lower than the observed number of (largely sterile) workers. This realization prompted recent work, which attempted to determine population structure in a range of species. Initial studies of common and ubiquitous species (*Bombus terrestris*, *Bombus pascuorum* and *Bombus ignitus*) found little or no genetic differentiation over large distances (Estoup *et al.* 1996; Widmer *et al*. 1998; Pirounakis *et al*. 1998; Widmer & Schmid-Hempel 1999; Shao *et al*. 2004; but see Herrmann *et al.* 2007). Recent estimates of male dispersal distances in *B. terrestris* suggest that they may move up to 9.9 km, which may help explain lack of population structure in this species (Kraus *et al.* 2009).

In contrast to common bumblebee species, studies of the rare and declining species *Bombus muscorum* and *Bombus sylvarum* found significant genetic structuring among populations ($\theta = 0.08–0.12$) (Darvill *et al*. 2006; Ellis *et al*. 2006). In *B. sylvarum*, estimates of the effective size of remaining populations were very low (range 21–72). Genetic diversity was considerably lower than in closely related common species and several populations showed signs of recent population bottlenecks. Low frequencies of sterile diploid males (an indicator of inbreeding) were found in both species. However, there was no clear relationship between genetic diversity and either isolation or population size, perhaps because of the confounding effect of recent bottlenecks, or because populations had not yet reached mutation–drift equilibrium (both species have declined greatly in recent years) (Frankham *et al*. 2002).

Although we are beginning to understand the potential for (and importance of) population substructuring and inbreeding in bumblebees, we have little idea whether all species are equally at risk. Molecular markers have recently revealed significant differences in the nesting density and foraging range of a number of bumblebee species (Darvill *et al*. 2004; Knight *et al.* 2005). It is conceivable that species may also vary in their dispersal ability, which would greatly influence their ability to cope with habitat fragmentation.

Here, we compare two bumblebee species, *Bombus muscorum* (L.) and *Bombus jonellus* (Kirby) in a model island system (Hebrides, UK). On the mainland, *B. muscorum* has undergone rapid and widespread declines, having disappeared from the majority of its former range. It belongs to the subgenus *Thoracobombus*, a group which seems to be especially sensitive to habitat fragmentation. In the UK, there are five *Thoracobombus* species, and four of these have been accorded UK Biodiversity Action Plan (BAP) status. In contrast, *B. jonellus* is widespread where suitable habitat allows throughout the mainland and the Hebrides, and does not appear to have declined (although once again, data on historical abundance are lacking) (Benton 2006; Macdonald & Nisbet 2006). It belongs to the subgenus *Pyrobombus*, of which none of the four UK species have BAP status. We thus predict that *B. muscorum* has a lower propensity or ability to disperse than *B. jonellus*, rendering it more sensitive to habitat fragmentation.

Although *B. muscorum* has declined greatly, the populations in the Hebrides appear to be large and stable (so far as is known) (Benton 2006; Macdonald & Nisbet 2006). The persistence of *B. muscorum* and other declining bumblebee species in the Hebrides is generally attributed to the low-intensity farming systems that have survived there, in contrast to the intensification of farming practices on the mainland (Goulson *et al*. 2006). Hence within the island system both *B. muscorum* and *B. jonellus* are relatively abundant and they are generally sympatric on the study islands, allowing a direct comparison between species. For each species, we quantify population genetic structuring and the relationship between genetic diversity and isolation.

Methods

Sample collection

During the summers (June–September) of 2003–2005, individuals of *Bombus muscorum* and *Bombus jonellus* were collected from islands in the Inner and Outer Hebrides (Scotland, UK), aiming for a range of island sizes and varying levels of isolation (Fig. 1). Previously published genotypic data for *B. muscorum* (Darvill *et al.* 2006) were supplemented by additional populations (181 new individuals) to strengthen the comparison between the two species. Efforts were made to collect samples from the west coast of mainland Scotland, but despite extensive searching, *B. muscorum* was very scarce or absent, and samples of this species were not collected. To minimize impacts upon populations, most

Fig. 1 A map of the study area (Hebrides, NW Scotland) showing the locations of the islands from which samples were collected.

workers were sampled using nonlethal tarsal samples following Holehouse *et al.* (2003), with some destructive sampling for planned studies of parasite prevalence. Workers were caught from numerous locations within each population (where possible >200 m from one another) to minimize the probability of sampling individuals from the same colony. Samples were preserved in pure ethanol and stored at ambient temperature. On most islands visited, *B. muscorum* appeared to be the most abundant bee species, occurring in most habitats, so that samples could be obtained quickly, while *B. jonellus* was largely confined to upland areas and was generally scarce. In total, 1061 *B. muscorum* (965 ♀ & 96 ♂) and 762 *B. jonellus* (758 ♀ & 4 ♂) were genotyped (Table 1).

Molecular methods

DNA was extracted using the HotShot protocol (Truett *et al.* 2000). Workers were genotyped at nine microsatellite loci in each species: (*B. muscorum* = B132, B131, B118, B96, B10, B11, B124, B126; *B. jonellus* = B132, B100, B131, B96, B10, B11, B124, B126, B121) (Estoup *et al.* 1995, 1996) following Darvill *et al.* (2006). Genotypes were manually scored in a conservative manner. Repeat PCRs were carried out on any samples that had failed to amplify or were uncertainly scored, multiple times if necessary. Any individuals that failed to amplify or did not yield a consensus genotype after repeated rechecks were excluded from the final data set.

Statistical methods

Both data sets were first checked for unexpected mutation steps, large gaps in the data or unusually sized alleles using MSA (Dieringer & Schlotterer 2003). *Bombus muscorum* samples collected in 2003 were not genotyped at B131, and where appropriate this locus was excluded from subsequent analyses. Tests for genotypic linkage disequilibrium and departure from Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP version 3.4 (Raymond & Rousset 1995). Sequential Bonferroni corrections (Rice 1989) were applied to minimize type I errors. Where deviations from HWE or apparent linkage disequilibrium suggested the presence of sisters within a population sample, KINSHIP v 1.3.1 (Goodnight & Queller 1999) was used to assign workers to colonies and remove all but one representative from each nest, following the method of Darvill *et al.* (2004).

Genetic population structure was assessed with *F*-statistics (Wright 1951), using Weir & Cockerham's (1984) estimators (F, f and θ), as implemented in FSTAT version 2.9.3 (Goudet 2001). *F*-statistics were calculated for all populations, and pairwise θ for all pairs of populations. Mean values and standard errors were obtained by jackknifing over samples and loci. Both global θ and R_{ST} (Slatkin 1995; Rousset 1996; Goodman 1997) were calculated (although the latter is strictly appropriate only to microsatellites mutating in a stepwise fashion). Significance levels of both global and pairwise θ values were determined by permuting alleles (100 000 permutations) using MSA, applying strict Bonferroni corrections (Rice 1989). Rigorous comparisons between species must account for the effect of genetic variation on the range of possible θ values (Hedrick 2005). Standardized equivalents (G'_{ST}) of genetic differentiation were therefore calculated in FSTAT following Meirmans (2006). Expected heterozygosity and allelic richness were also calculated in FSTAT.

In a nonlinear system, genetic isolation by distance (IBD) is expected to increase with the logarithm of physical separation (Rousset 1997; Hardy & Vekemans 1999). IBD in each species was therefore examined by regression of pairwise estimates of genetic distance $\theta/(1-\theta)$ against the corresponding logarithms of geographic separation (Rousset 1997). A Mantel test (Mantel 1967) was used to assess the significance of any correlation, performing 50 000 permutations in ISOLATION BY DISTANCE (Bohonak 2002). To test for inter-

Table 1 The sample size, average (unbiased) heterozygosity (H_E) and allelic richness of each of the populations (±SE). For *Bombus muscorum*, allelic richness and H_E were calculated using all loci except B131. Allelic richness values are standardized to the smallest sample size (which for both species is 25)

	Bombus muscorum				*Bombus jonellus*			
Population	Sample size	Sampling period	Allelic richness	H_E	Sample size	Sampling period	Allelic richness	H_E
Barra	50 ♀	2003	3.10 ± 0.66	0.393 ± 0.113	82 ♀	2003	8.63 ± 1.70	0.766 ± 0.048
Mingulay	49 ♀	2003	2.99 ± 0.64	0.374 ± 0.115	32 ♀	2003 & 2005	7.50 ± 1.31	0.696 ± 0.048
Muldoanich	25 ♀	2003	3.63 ± 0.90	0.421 ± 0.103	—	2003	—	—
Pabbay	37 ♀	2003	3.33 ± 0.68	0.399 ± 0.118	36 ♀	2003 & 2005	7.55 ± 1.33	0.729 ± 0.046
Sandray	58 ♀	2003	3.05 ± 0.63	0.367 ± 0.111	25 ♀	2003	9.00 ± 1.80	0.763 ± 0.054
Monachs*	44 ♀	2005	2.22 ± 0.39	0.305 ± 0.092	—	—	—	—
Uists*	87 ♀	2005	3.32 ± 0.66	0.404 ± 0.113	92 ♀	2005	9.30 ± 1.98	0.755 ± 0.054
Outer Hebrides average			3.09 ± 0.17	0.380 ± 0.014			8.40 ± 0.37	0.742 ± 0.013
Colonsay	67 ♀	2004	3.21 ± 0.50	0.416 ± 0.086	28 ♀	2004	10.24 ± 2.31	0.756 ± 0.064
Lunga	36 ♀	2004	3.43 ± 0.56	0.507 ± 0.108	38 ♀	2004	9.90 ± 2.07	0.742 ± 0.076
Staffa	52 ♀	2004	3.33 ± 0.51	0.484 ± 0.091	46 ♀	2004	8.19 ± 1.70	0.697 ± 0.082
Iona*	50 ♀	2005	4.22 ± 0.91	0.554 ± 0.087	—	—	—	—
Canna	62 ♀	2003 & 2004	3.11 ± 0.57	0.433 ± 0.086	44 ♀	2003 & 2004	9.58 ± 2.01	0.758 ± 0.070
Coll	70 ♀	2003	3.46 ± 0.69	0.499 ± 0.091	44 ♀	2003 & 2005	9.05 ± 1.86	0.738 ± 0.073
Eigg	64 ♀	2003 & 2004	3.30 ± 0.51	0.533 ± 0.094	36 ♀	2004	9.87 ± 2.09	0.757 ± 0.066
Muck	52 ♀	2003 & 2004	2.91 ± 0.42	0.425 ± 0.088	47 ♀	2003 & 2004	8.81 ± 1.79	0.751 ± 0.056
Rum	42 ♀	2004	2.91 ± 0.48	0.451 ± 0.077	32 ♀	2004	10.00 ± 2.10	0.749 ± 0.079
Tiree	119 ♀	2003	3.27 ± 0.55	0.499 ± 0.086	54 ♀	2003	8.18 ± 1.71	0.715 ± 0.076
Skye	—	—	—	—	43 ♀	2005	10.03 ± 2.10	0.745 ± 0.068
Mull	—	—	—	—	37 ♀	2004 & 2005	10.68 ± 1.93	0.769 ± 0.057
Inner Hebrides average			3.32 ± 0.12	0.480 ± 0.015			9.50 ± 0.24	0.743 ± 0.006
Overall Hebrides average			3.23 ± 0.09	0.437 ± 0.015			9.16 ± 0.23	0.743 ± 0.005
Mainland UK†	71 ♀ 23 ♂	2003	4.01 ± 0.06	0.509 ± 0.013	42 ♀ 0 ♂	2004 & 2005	10.02 ± 1.98	0.755 ± 0.071

*For *B. muscorum*, these are new populations for which genetic data have not previously been published. The remaining populations of *B. muscorum* were sampled for Darvill *et al.* (2006).

†For *B. muscorum*, these figures represent average values for the two Southern UK samples genotyped in Darvill *et al.* (2006).

specific differences in the degree of IBD (both with θ and G'_{ST}), a Monte Carlo analysis was performed using POPTOOLS (version 2.6.9; CSIRO, http://www.cse.csiro.au/poptools). The observed average (interspecific) difference in interisland genetic distances was compared to the equivalent value from data randomized by shuffling (without replacement). A *P*-value was estimated by calculating the number of times the real interspecific differences exceeded that of the randomized data in 10 000 randomizations.

To examine geographic patterns of genetic substructuring and relatedness, Bayesian genetic clustering was performed using Structure version 2.2.3 (Pritchard *et al.* 2000) independently for each species, using the Admixture and Correlated Allele models (Falush *et al.* 2003). We first ran Structure with a number of clusters (*K*) varying from 1 to 10 with five runs for each *K*, with 10 000 burn in periods and 50 000 Markov chain Monte Carlo (MCMC) repetitions. We computed the posterior probabilities of *K* following Bayes' Rule according to the Structure manual (Pritchard *et al.* 2007) to identify the most probable *K* for the data. We then ran Structure with the same models setting *K* as previously determined and using 100 000 burn in periods and 200 000 MCMC repetitions, which constituted the final clustering results. The clustering result for each population was individualized and mapped using R (R Development Core Team 2005) to help with interpretation of geographic patterns. When appropriate, allele frequency divergence among clusters (net nucleotide distance as estimated by Structure) was used to draw trees representing the genetic distances among groups using the R package APE (Paradis *et al.* 2004).

Possible loss of genetic variation through bottlenecking (or founder effects) was tested for using Bottleneck version 1.2.02 (Piry *et al.* 1999). Three mutation models have been proposed for microsatellites: stepwise (SMM), infinite allele (IAM) and two-phase (TPM). As the debate is ongoing, tests were conducted using all three models, allowing for either 70% or 90% stepwise mutations within the TPM. A total of 100 000 iterations were performed in each case.

Results

Hardy–Weinberg and linkage disequilibrium

Bombus muscorum. Due to inherently low levels of genetic diversity, it was not possible to reliably test for the presence of sisters within the data set due to low statistical power. Nevertheless, global tests neither per locus nor per population detected any significant deviation from HWE. Significant linkage disequilibrium ($P < 0.05$) was found between four pairs of loci, B132–B131, B96–B126, B131–B96 and B131–B118 when testing each locus pair across all populations. Tests within each population found significant linkage disequilibrium in just two populations for these locus pairs (Staffa at B132–B131, B96–B126 and the Monachs at B131–B96 and B131–B118). A global test across all populations excluding both Staffa and the Monachs found no significant linkage disequilibrium, so subsequent analyses were carried out both with and without these two populations.

Bonbus jonellus. Global tests per locus detected highly significant deviations from HWE for locus B131. Indeed for 11 individuals no alleles amplified at this locus, despite re-extraction and amplification. It is therefore likely that null-alleles occur at this locus, and it was excluded from subsequent analyses. Global tests per locus and per population (excluding B131) again found significant deviations from HWE, as did tests for linkage disequilibrium. KINSHIP was therefore used to remove sisters from each population, leaving only one representative per nest. Subsequent tests showed no significant deviation from HWE or evidence of linkage disequilibrium.

Genetic diversity and differentiation between populations

Bombus muscorum was significantly more genetically depauperate than *B. jonellus*, both for expected heterozygosity (H_E) and allelic richness (Mann–Whitney tests; both $P < 0.001$).

Bombus muscorum. Overall, population structuring was moderately high, with $\theta = 0.128 \pm 0.025$ SE ($P < 0.0001$) (excluding Staffa and Monachs, $\theta = 0.120 \pm 0.026$ SE, $P < 0.0001$), and with $R_{ST} = 0.1024$ (excluding Staffa and Monachs, $R_{ST} = 0.1005$). The global G'_{ST} value was 0.262. The locus B11 proved to be monomorphic in all but two populations, and produced a global G'_{ST}-value of −0.081, which was inconsistent with other loci. It is possible that this new metric is unduly influenced by loci with very low variability. In the absence of locus B11, the global G'_{ST}-value for *B. muscorum* was 0.301.

Genetic differentiation between populations (pairwise θ) was highly significant ($P < 0.01$) for 132 of 153 comparisons, significant ($P < 0.05$) in three cases and non-significant for the remaining 18. With the exception of Coll and Tiree, pairwise comparisons between all Inner Hebridean islands and between all Inner and Outer Hebridean islands were significant (Table 2). The majority of Outer Hebridean islands were not significantly differentiated from one another (17 of 28

Table 2 Pairwise genetic distances between all populations for (upper triangle) *Bombus jonellus* and (lower triangle) *Bombus muscorum*. Significant θ values ($P < 0.05$) are shown in bold

	Outer Hebrides							Inner Hebrides												
	Barra	Mingulay	Muldoanich	Pabbay	Sandray	Monach	Uists	Coll	Canna	Eigg	Colonsay	Lunga	Muck	Mull	Rum	Skye	Staffa	Tiree	Iona	Mainland
Outer Hebrides																				
Barra	—	**0.028**	—	0.008	0.003	—	**0.014**	**0.038**	**0.041**	**0.031**	**0.040**	**0.036**	**0.041**	**0.042**	**0.041**	**0.038**	**0.069**	**0.059**	—	**0.037**
Mingulay	0.016	—	—	0.016	**0.037**	—	**0.051**	**0.072**	**0.074**	**0.056**	**0.067**	**0.051**	**0.061**	**0.071**	**0.071**	**0.063**	**0.096**	**0.098**	—	**0.058**
Muldoanich	0.012	**0.037**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pabbay	0.008	0.003	0.032	—	0.013	—	**0.017**	**0.065**	**0.068**	**0.055**	**0.064**	**0.053**	**0.067**	**0.067**	**0.065**	**0.063**	**0.101**	**0.082**	—	**0.056**
Sandray	−0.002	0.023	0.031	0.008	—	—	**0.020**	**0.049**	**0.051**	**0.044**	**0.053**	**0.050**	**0.052**	**0.049**	**0.053**	**0.050**	**0.087**	**0.075**	—	**0.054**
Monach	**0.032**	**0.067**	**0.061**	**0.051**	**0.037**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Uists	0.004	0.012	0.027	0.010	0.014	**0.058**	—	**0.051**	**0.054**	**0.037**	**0.050**	**0.049**	**0.060**	**0.047**	**0.050**	**0.049**	**0.083**	**0.066**	—	**0.050**
Inner Hebrides																				
Coll	**0.228**	**0.239**	**0.195**	**0.219**	**0.244**	**0.277**	**0.231**	—	0.004	0.003	0.006	0.003	**0.014**	0.002	0.004	0.002	0.007	0.008	—	0.008
Canna	**0.137**	**0.122**	**0.129**	**0.104**	**0.142**	**0.235**	**0.111**	**0.148**	—	0.005	0.009	0.006	**0.014**	0.001	—0.004	0.004	**0.017**	**0.014**	—	0.011
Eigg	**0.158**	**0.146**	**0.133**	**0.146**	**0.182**	**0.225**	**0.133**	**0.065**	**0.077**	—	0.004	0.005	0.005	0.004	0.002	0.001	**0.020**	**0.022**	—	0.008
Colonsay	**0.175**	**0.177**	**0.138**	**0.162**	**0.178**	**0.219**	**0.173**	**0.130**	**0.148**	**0.117**	—	0.007	0.007	0.002	0.011	0.003	**0.018**	**0.025**	—	0.004
Lunga	**0.133**	**0.127**	**0.138**	**0.114**	**0.149**	**0.189**	**0.113**	**0.121**	**0.111**	**0.067**	**0.137**	—	0.006	0.007	0.005	0.000	**0.017**	**0.018**	—	−0.002
Muck	**0.178**	**0.175**	**0.181**	**0.160**	**0.198**	**0.209**	**0.143**	**0.094**	**0.113**	**0.051**	**0.166**	**0.060**	—	0.012	0.014	0.007	**0.029**	**0.034**	—	0.009
Mull	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.002	0.003	0.012	**0.016**	—	0.011
Rum	**0.230**	**0.211**	**0.202**	**0.199**	**0.244**	**0.295**	**0.186**	**0.083**	**0.052**	**0.053**	**0.136**	**0.126**	**0.119**	—	—	0.001	**0.019**	**0.014**	—	0.013
Skye	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**0.015**	**0.018**	—	0.000
Staffa	**0.141**	**0.153**	**0.143**	**0.127**	**0.154**	**0.208**	**0.149**	**0.111**	**0.111**	**0.091**	**0.128**	**0.034**	**0.083**	—	**0.125**	—	—	**0.025**	—	**0.018**
Tiree	**0.205**	**0.222**	**0.172**	**0.204**	**0.224**	**0.246**	**0.211**	0.005	**0.141**	**0.069**	**0.143**	**0.120**	**0.085**	—	**0.090**	—	**0.102**	—	—	**0.021**
Iona	**0.084**	**0.084**	**0.055**	**0.083**	**0.102**	**0.147**	**0.080**	**0.070**	**0.057**	**0.033**	**0.083**	**0.068**	**0.082**	—	**0.063**	—	**0.081**	**0.067**	—	—
Mainland	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

comparisons). The populations from North and South Uist were genetically indistinguishable ($\theta = 0.008$) and were pooled for subsequent analyses.

Bombus jonellus. Significant population structuring was also found in this species, with global $\theta = 0.034 \pm 0.006$ SE ($P < 0.0001$) and $R_{ST} = 0.0392$. However, global values of both θ and R_{ST} were significantly lower in *B. jonellus* than those observed in *B. muscorum* [for θ, two-tailed Mann–Whitney, $P = 0.002$ (excluding Monachs and Staffa, $P = 0.005$); and for R_{ST}, two-tailed Mann–Whitney, $P = 0.027$ (excluding Monachs and Staffa, $P = 0.074$)]. The global G'_{ST}-value for *B. jonellus* was 0.176.

Pairwise θ values were nonsignificant ($P > 0.05$) for 54 of 153 comparisons, were significant ($P < 0.05$) in five cases and were highly significant ($P < 0.01$) for the remaining 94. Unlike in *B. muscorum*, comparisons between Inner Hebridean islands found no significant differentiation between populations in the majority of cases (40 of 58). However, all comparisons between Inner and Outer Hebridean islands were significant. Within the Outer Hebridean island group, 8 of 15 pairwise comparisons were nonsignificant. As with *B. muscorum*, populations from North and South Uist were genetically indistinguishable ($\theta = -0.001$) and were pooled prior to subsequent analyses.

Isolation by distance

For both species, there was a highly significant relationship between genetic distance ($\theta/1-\theta$) and the natural logarithm of physical separation (Mantel test, $P < 0.00002$, $R^2 = 0.369$ and $P < 0.00002$, $R^2 = 0.298$ for *B. muscorum* and *B. jonellus* respectively). The extent of this relationship differed significantly between species, with *B. jonellus* populations retaining genetic cohesion over greater distances [for both θ (Fig. 2a) and G'_{ST} (Fig. 2b), $P < 0.0001$ based on Monte Carlo analysis]. Both species were not present on all of the islands sam-

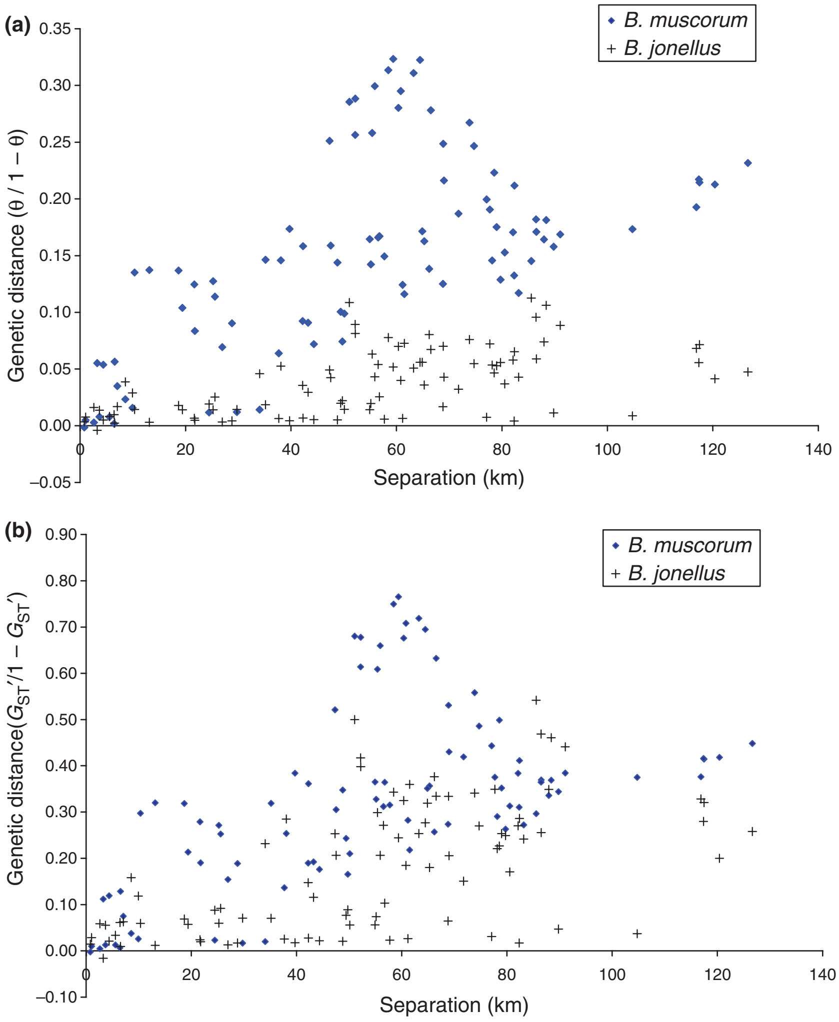

Fig. 2 The physical separation of populations and the corresponding genetic distances between them, for both *Bombus muscorum* and *Bombus jonellus*. Values derived from (a) θ are shown for reference, along with those from (b) the standardized measure G'_{ST}. For *B. muscorum*, the largely monomorphic locus B11 gives negative values, which reduce overall G'_{ST} values. In the absence of this locus, interspecific differences are intermediate between (a) and (b). Populations for which only one species was sampled are excluded from this figure (Mull, Iona, Skye, Mainland, Monachs and Muldoanich).

pled so, for clarity, only islands on both of which were found are included in Fig. 2.

Some *B. muscorum* populations as little as 3.2 km apart were significantly differentiated from one another, as were the majority of populations separated by more than 10 km. All discrete populations more than 34 km apart were significantly differentiated, irrespective of stepping-stone populations. In contrast, in *B. jonellus* no populations <7.1 km apart were significantly differentiated and some populations separated by as much as 104 km remained genetically undifferentiated.

Bayesian genetic clustering

The Bayesian genetic clustering indicated that seven clusters best explained the genetic structure for *B. muscorum* [$\Pr(K = 7) = 0.92$ followed by $\Pr(K = 8) = 0.08$] while two clusters best explained the genetic structure for *B. jonellus* [$\Pr(K = 2) \sim 1$]. Using the hierarchical ΔK method (Evanno *et al.* 2005) led to the same conclusions (data not shown).

For *B. muscorum*, the Outer Hebrides populations are composed of two clusters genetically well differentiated from the five clusters founded in the Inner Hebrides populations (Fig. 3a). These two genetic clusters are intermixed in the Outer Hebrides populations with the exception of the Monachs population (yellow, Fig. 3a). The Inner Hebrides populations are composed of five genetic clusters that showed a clear geographical pattern. The Tiree and Coll populations shared the same genetic cluster that appeared to be divergent from the remaining four clusters (red, Fig. 3a). The four other clusters were closely genetically related and shared by nearby islands: Canna and Rum (purple), Eigg and Muck (blue), Lunga and Staffa (orange, Fig. 3a). Colonsay composed its own genetic cluster (azure) while the Iona population seemed to be a mixture of different clusters (Fig. 3a). Finally, genetic divergence between the clusters was correlated with the degree of geographic isolation among the populations (insert Fig. 3a).

For *B. jonellus*, the Outer Hebrides populations were composed of one cluster (blue, Fig. 3b) and the Inner Hebrides populations were composed by another (red, Fig. 3b). There was not further genetic substructuring within each of the island systems. The genetic divergence between these two clusters was equivalent to the genetic distance between closely related genetic clusters of *B. muscorum* populations in the Inner Hebrides.

Population bottlenecks

Under the IAM, 14 of 17 *B. muscorum* populations (all except Pabbay, Sandray and Muldoanich) showed signs of bottlenecking (Wilcoxon Test, one-tailed for heterozygote excess, $P < 0.05$). Using the TPM (default settings in which 70% of mutations are stepwise), only six of the populations (Coll, Eigg, Muck, Rum, Iona and Monachs) showed bottlenecking. More stringent settings (90% stepwise) showed only Eigg as significant ($P = 0.0039$), although Rum ($P = 0.055$) and the Monachs ($P = 0.078$) approached significance. Using the SMM, only Eigg showed significant signs of recent bottlenecking ($P = 0.004$). Populations of *B. muscorum* from the Inner Hebrides showed a greater probability of having passed through a recent bottleneck than populations from the Outer Hebrides (Mann–Whitney test, $P = 0.001$, $P = 0.013$ and $P = 0.026$ for the IAM, TPM and SMM respectively). No such trend was in evidence for *B. jonellus* (Mann–Whitney tests, all $P > 0.05$), however, 9 of 17 *B. jonellus* populations showed evidence of bottlenecking under the IAM (all except Colonsay, Lunga, Mainland, Mingulay, Muck, Mull, Skye and Staffa). The TPM model (70% stepwise) suggested that

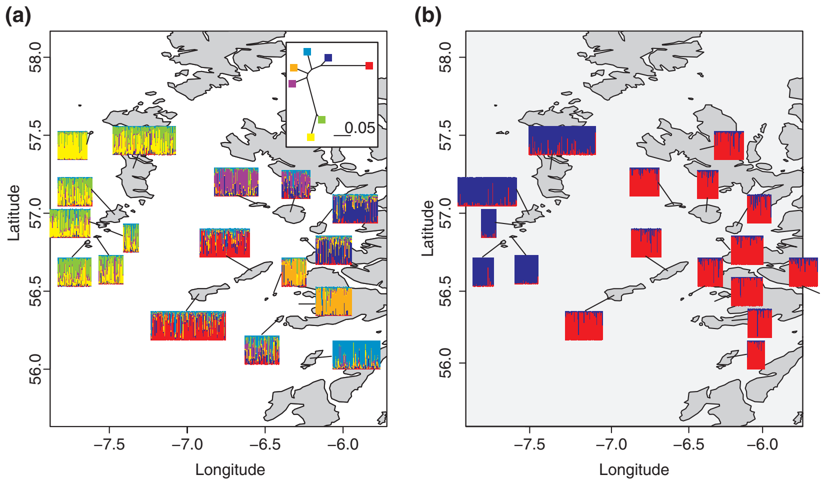

Fig. 3 Structure clustering results obtained for seven clusters identified in *Bombus muscorum* (a) and two clusters in *Bombus jonellus* (b). Each individual is represented by a thin vertical line partitioned into seven or two coloured segments proportional to its membership in the corresponding genetic cluster. The insert in (a) represents the genetic tree computed based on the net nucleotide distance computed by Structure between the seven genetic clusters of *B. muscorum*.

only three populations (Coll, Barra and Sandray) had been recently bottlenecked, and no populations appeared to have passed through a recent bottleneck under more stringent settings in the TPM (90% stepwise) or when following the SMM.

Discussion

Hardy–Weinberg and linkage disequilibrium

The absence of deviations from HWE or patterns of linkage suggest that the chosen loci (except the discarded B131) were suitable for use in this study. Due to low levels of genetic diversity, it was not possible to attempt the removal of sisters from the *Bombus muscorum* data set, but nevertheless problems were not broadly evident. This suggests low frequencies of sampled sisters in most populations. Sampling was spread over a wide area where possible, greatly facilitated by the range of habitats used by *B. muscorum*. Previous studies of population structure in more common bumblebee species have generally found low numbers of sister pairs in samples of workers taken from single sites, with the majority of bees being unrelated (Darvill *et al.* 2004; Knight et al. 2005). Hence we conclude that there are likely to be only small number of sister pairs in our *B. muscorum* data set. The linkage disequilibrium observed in *B. muscorum* populations on Staffa and the Monachs probably reflects the presence of sisters amongst the sampled workers, as these were the smallest (and among the most isolated) islands visited. However, other explanations are possible, notably: a recent population bottleneck; or severe inbreeding (Beebee & Rowe 2004).

Population structure

The two species differed significantly in overall heterozygosity (H_E). Genetic differentiation was evident in both species, but was much higher for *B. muscorum* than for *Bombus jonellus*. Pairwise comparisons and genetic clustering similarly showed that *B. jonellus* populations retain genetic cohesion over much greater distances. There are three likely explanations for the observed difference, none of which are mutually exclusive. First, interspecific differences in effective population sizes could lead to different rates of genetic drift. Second, substantial differences in gene flow between (source) mainland populations and the islands could lead to apparent differences between species. Finally, the difference in population structuring could reflect a difference in dispersal range and/or dispersal propensity between species. Clearly, more frequent long-range dispersal will reduce population structuring. In the absence of gene flow, small populations should diverge more rapidly (through drift) than equivalent larger populations. However, the effects of sufficient migration (>1 successful migrant per generation) are manifest irrespective of population size, so the observed spatial genetic structure (IBD and genetic clustering) points to the importance of dispersal.

If population size were contributing towards observed differences, we would expect *B. jonellus* to be consistently and significantly more abundant. It is hard to quantify the relative population sizes of the two species with the available data. Anecdotal field observations suggest that worker *B. muscorum* is considerably more abundant than *B. jonellus* on most islands, and is very often more abundant than any other bumblebee species (in marked contrast to the situation on the UK mainland). It also occurs in a broad range of habitats, including machair, gardens and moorland, while *B. jonellus* is largely confined to moorland. However, it is conceivable that this impression is biased by the larger size of *B. muscorum*, and the tendency of *B. jonellus* to inhabit more remote upland areas. Effective population size depends on the number of nests, not the number of sterile workers, so relative nest size will affect the relationship between worker abundance and effective population size. However, nest sizes of the two species are thought to be similar (Benton 2006). Weak evidence that *B. muscorum* populations may be lower than those of *B. jonellus* can be inferred from our genetic data: *B. muscorum* exhibits more evidence for recent bottlenecking, and has less genetic variation overall (although comparing genetic variation across species using a limited number of loci is unreliable). Overall, we conclude that *B. muscorum* probably has a larger effective population size that *B. jonellus* on most islands; it certainly seems unlikely that effective population sizes in *B. muscorum* are sufficiently low compared to *B. jonellus* to explain the marked disparity in population structuring between the two species.

The observed IBD suggests that gene flow between islands is relatively more important than gene flow from the mainland. Nevertheless, occasional gene flow from the mainland in *B. jonellus* but not *B. muscorum* could explain the observed differences in genetic structure between the species. Both *B. muscorum* and *B. jonellus* are still present on Skye, Mull and the adjacent mainland but, following recent declines in the former, *B. jonellus* (although uncommon) is now marginally more abundant in these locations. The presence of stepping-stone populations down the coast may in part explain low genetic distances between some *B. jonellus* populations, notably Colonsay and Canna. These islands are 105 km apart, and in *B. jonellus* are separated by a genetic distance (θ) of just 0.009 and represent one genetic cluster, whereas *B. muscorum* shows a distance of 0.148 with marked population substructure.

It is interesting to speculate as to why *B. jonellus* might disperse further than *B. muscorum*. As a heathland species, it has probably always had a somewhat fragmented distribution, with colonization of new habitats requiring dispersal over lowland areas between ranges of hills or mountains. It is noteworthy that *B. jonellus* belongs to the subgenus *Pyrobombus* (Dalla Torre), of which three other species occur in the UK: *Bombus pratorum*, *Bombus monticola* and *Bombus hypnorum*. Bumblebees have been studied in detail in the UK since the beginning of the 20th century, and colonization events are rare. However, in recent years *B. hypnorum* arrived from continental Europe (Goulson & Williams 2001) and both *B. monticola* and *B. pratorum* have colonized Ireland (Fitzpatrick et al. 2007). It is possible that long-range dispersal is a trait common to this subgenus. No member of this genus is endangered in the UK.

In contrast, *B. muscorum* is generally a lowland species, and in the UK frequently occurs along the coastline (Benton 2006). Long range dispersal may historically have conferred little advantage, and might have increased the probability of movement into unsuitable habitat. It is notable that the subgenus *Thoracobombus* to which *B. muscorum* belongs contains five species in the UK, and four of these are formally recognized as endangered (they have been accorded UK BAP status). It may be that *Thoracobombus* as a group tend to have low dispersal abilities, rendering them susceptible to habitat fragmentation. Bumblebees are frequently observed several kilometres offshore from ferries, yachts or at lightships, but *Thoracobombus* are seldom or never observed (Mikkola 1984; Goulson 2003; B.D., personal observation). Obtaining a better understanding of the relative dispersal abilities of endangered insect species is clearly important if we are to devise appropriate conservation strategies to ensure their survival.

Acknowledgements

We thank the Leverhulme Trust (Grant no. F/00180/M) for providing funding to support this work, and the C.B. Dennis Trust for funding J. Waters. Thanks are also due to the RSPB, SNH, Scottish Wildlife Trusts, National Trust for Scotland and Hebridean Trust for allowing access to field sites; and Mike Edwards and Murdo MacDonald for advice.

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Ben Darvill is interested in research which has practical relevance for the conservation of bumblebees. He has worked extensively on population genetics, population structure and aspects of inbreeding in bumblebees. Steph O'Connor's research is largely focussed on factors affecting the survival of bumblebee nests. She is currently handler of the world's only bumblebee sniffer dog, which has been trained to locate bumblebee nests. Gillian Lye is finishing a PhD on nest site choice of bumblebees. She is also involved in population genetic studies, including assessing the viability of source populations for bumblebee reintroduction attempts. Joe Waters conducted research on niche differentiation in a cryptic bumblebee complex before leaving academia. Olivier Lepais is a population geneticist with a particular interest in the role of hybridisation in the evolution of oak species. Dave Goulson works on a range of aspects of the ecology, behaviour and conservation of bumblebees.