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RESEARCH ARTICLE

Population structure, dispersal and colonization history of the garden bumblebee *Bombus hortorum* in the Western Isles of Scotland

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Abstract New methods of analysing genetic data provide powerful tools for quantifying dispersal patterns and reconstructing population histories. Here we examine the population structure of the bumblebee *Bombus hortorum* in a model island system, the Western Isles of Scotland, using microsatellite markers. Following declines in other species, *B. hortorum* is the only remaining long-tongued bumblebee species found in much of Europe, and thus it is of particular ecological importance. Our data suggest that populations of *B. hortorum* in western Scotland exist as distinct genetic clusters occupying groups of nearby islands. Population structuring was higher than for other bumblebee species which have previously been studied in this same island group ($F_{st} = 0.16$). Populations showed significant isolation by distance. This relationship was greatly improved by using circuit theory to allow dispersal rates to differ over different landscape features; as we would predict, sea appears to provide far higher resistance to dispersal than land. Incorporating bathymetry data improved the fit of the model further; populations separated by shallow seas are more genetically similar than those separated by deeper seas. We argue that this probably reflects events following the last ice age when the islands were first colonized by this bee species (8,500–5,000 ybp), when the sea levels were lower and islands separated by shallow channels would have been joined. In the absence of significant gene flow these genetic clusters appear to have since diverged over the following 5,000 years and arguably may now represent locally adapted races, some occurring on single islands.

Keywords Microsatellite · Isolation by distance · Bathymetry · Circuit theory

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Introduction

Oceanic islands have been frequently used as model systems to examine the effects of isolation on population and community structure. The Western Isles of Scotland have become a model system for studying and comparing the ecology, population structure and dispersal ability of bumblebee species (Darvill et al. 2006, 2007, 2010; Waters et al. in press; Redpath et al. 2010; Charman et al. 2010). Aside from the value of using island populations where the distribution of species is readily quantified, this area is of particular interest because it supports a high diversity of bumblebee species, including a number of species such as *B. muscorum* and *B. distinguendus* which are in rapid decline elsewhere.

Dispersal ability is likely to correlate with ability to persist in fragmented habitat patches, so quantifying dispersal is valuable in informing conservation strategies. Bumblebees may be particularly susceptible to habitat fragmentation for several reasons. As social insects, the majority of the population do not produce offspring, so the effective population size (*Ne*) is likely to be orders of magnitude lower than the actual number of bees. Bumblebee colonies are generally founded by a single queen, and most species are monoandrous (Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000), so each colony represents just one breeding female and her haploid mate. Hence it has been argued that they may be

particularly prone to metapopulation collapse (Goulson et al. 2008a).

Gene flow between populations can only be effected by queens or males, but quantifying their movement directly is difficult; it has been achieved for queens of *B. lapidarius* and *B. pascuorum* (estimated to be able to disperse at least 5 and 3 km from their natal site, respectively, Lepais et al. 2010) and males of *B. terrestris*, which can disperse up to 9.9 km (Kraus et al. 2009). Dispersal can also be inferred indirectly from patterns of genetic structuring between populations. Using this approach, our previous work has demonstrated that bumblebee species exhibit large differences in their dispersal abilities which cannot readily be predicted; *B. muscorum* exhibits very limited dispersal while *B. jonellus* regularly travels >30 km across water barriers. *Bombus distinguendus* would appear to be intermediate between the two (Charman et al. 2010). Dispersal ability is likely to closely link to the ability of species to cope with habitat fragmentation, so these findings are of direct value to those devising conservation strategies for bumblebees. Dispersal is of particular importance to bumblebees because their social nature renders their effective population size much lower than that of comparable solitary species (most members of the population are sterile workers), potentially making them highly susceptible to inbreeding. Genetic studies of fragmented populations of declining bumblebee species suggest that effective population sizes are indeed often very low (Ellis et al. 2006; Charman et al. 2010), and that in some species this may be driving loss of genetic variation (Ellis et al. 2006; Takahashi et al. 2008; Lozier and Cameron 2009).

Bombus hortorum is an unusual bumblebee in that it has a very long tongue, adapted for feeding on very deep flowers which other bees are unable to feed from. Most long and medium-tongued bumblebee species have declined markedly in western Europe in the last 60 years (Goulson et al. 2008a; Williams and Osborne 2009). Long-tongued bumblebees appear to be particularly associated with Fabaceae as a source of pollen, and with flower-rich grassland habitats which tend to be rich in Fabaceae (Goulson et al. 2005, 2006, 2008b). It is the disproportionate loss of this habitat which is the likely cause of the decline of many longer-tongued bumblebees. *B. hortorum* appears to be among the most specialized of bumblebees, at least with regard to pollen collection, usually gathering >90% of its pollen from Fabaceae and often almost all of this from one species, *Trifolium pratense* (Rasmont and Mersch 1988). *T. pratense* has declined in abundance in the UK and probably elsewhere in Europe due to the widespread loss of species-rich grasslands and abandonment of crop rotations including clover leys (Carvell et al. 2006), yet *B. hortorum* remains one of the six most common bumblebee species throughout western and northern Europe (albeit often the least abundant of the six). The persistence of *B. hortorum* while most long-tongued species have declined, and despite declines in its main foodplant, has not been explained.

The aim of the present study was to investigate the contemporary and historical factors influencing the population structure of *B. hortorum* in this island system using microsatellite markers. Darvill et al. (2010) found significant isolation by distance in both *B. jonellus* and *B. muscorum*, but the model fits were poor. We use circuit theory to estimate how the fit of isolation by distance models can be improved by incorporating landscape factors, and also to quantify recent and historical migration rates between islands, and effective population size. Given that *B. hortorum* is widespread and abundant throughout much of Europe, and appears to be one of a small number of bumblebee species able to cope with habitat degradation and fragmentation, we predict that it should have greater powers of dispersal and lower population structuring than the more scarce species studied previously in this system.

Methods

Sampling

During the summers (June–September) of 2003–2005, individuals of *B. hortorum* were collected from fourteen islands in the Inner and Outer Hebrides (Scotland, UK), and from one site on the Scottish mainland. Although all islands were visited in more than one year, samples were not collected in all years from all islands. Samples were mostly non-lethal, following Holehouse et al. (2003), although some voucher specimens were taken. Workers were caught from numerous locations within each island (where possible >200 m from one another) to minimize the probability of sampling individuals from the same colony. This distance has been found sufficient to avoid frequent sampling of sisters in other bumblebee species (Darvill et al. 2010). When encountered, male bumblebees were also caught in order to assess the frequency of diploid males, and were destructively sampled to conclusively assess their sex (the presence of diploid males may provide an indication of inbreeding in haplodiploid species, Zayed and Packer 2005). Samples were preserved in pure ethanol and stored at ambient temperature. In total, 600 bees (561 ♀ and 39 ♂) were genotyped.

Molecular methods

DNA was extracted using the HotShot protocol (Truett et al. 2000). All samples were genotyped at nine microsatellite loci (B100, B118, B132, B11, B10, B96, B126, B124 and

B121), identified by screening the large number of microsatellites developed by Estoup et al. (1995, 1996) for *B. terrestris* for ones that amplified well in *B. hortorum*. Polymerase Chain Reactions (PCR) were carried out on samples using the QIAGEN Multiplex PCR kit. Multiplex PCRs were run for combinations of the loci B100(HEX)-B118(FAM)-B132(NED), B11(FAM)-B10(HEX)-B96(NED) and B126 (FAM)-B124(HEX)-B121(NED) (fluorescent markers indicated in parentheses). PCR reactions were 10 µl in volume and consisted of approximately 1 µl Q-solution, 5 µl PCR Master Mix, 1 µl primer solution (3 × 0.2 µM of each primer, forward primers labelled with NED, HEX or FAM dyes, Applied Biosystems), 1 µl template DNA and 2 µl HPLC H_2O. Samples were denatured at 95°C for 15 min, and this was followed by thirty-four 210 s cycles consisting of a denaturing step at 94°C for 30 s, an annealing step at 49°C for 90 s and an extension step at 72°C for a further 90 s. This was then followed by a final extension step at 72°C for 10 min.

PCR products were visualized on an ABI 3730 capillary DNA sequencer (The Sequencing Service, University of Dundee) with a 1:80 dilution before the run and using a GeneScan 500 LIZ internal size standard (Applied Biosystems). Fragments were sized using STRand software (Veterinary Genetics Laboratory, University of California at Davis, http://www.vgl.ucdavis.edu/informatics/strand.php) and raw alleles sizes were binned into discrete classes using the MsatAllele package v 1.01 (Alberto 2009) for R (R Developement Core Team 2009).

Samples for which amplification was not successful, or scoring was uncertain, were re-run and re-extraction of DNA was carried out if necessary. Any individuals that failed to amplify after repeated attempts were excluded from the final dataset.

Data analysis

Identification and removal of sibling workers

Colony v 2.0 (Jones and Wang 2009) was used within each island sample to assign workers to colonies and remove all but one representative (chosen at random) from each colony for subsequent analyses, ensuring the observed pattern of population genetic structure was not confounded by family structure. This programme uses maximum likelihood methods to assign sibship or parent-offspring relationships, and has been found to be the most reliable method available for assigning sibship in bumblebees (Lepais et al. 2010). Assigning sisterships in haplodiploid species is unusually reliable as full sisters share 75% of genes by descent. Genotyping error was accounted for in the analysis (Wang 2004) by setting the error rate at 2% (allele drop out 0.5% and other errors 1.5%). This procedure may regroup a small fraction of unrelated individuals into a common colony (Lepais et al. 2010) leading to the rejection of a higher number of individuals than necessary and giving a conservative estimate of the number of colonies for subsequent analysis. For each island sample the total number of workers and colonies were recorded (Table 1).

Genetic parameters

Genepop v 4.0 (Rousset 2010) was used to test for genotypic linkage desequilibrium between pairs of loci, and the Hardy–Weinberg Equilibrium (HWE) of each locus within an island. Bonferroni corrections for multiple tests were used to minimize type I errors (Rice 1989) and the presence of null alleles was checked using Micro-checker (Van Oosterhout et al. 2004).

FSTAT v 2.9.3 (Goudet 1995) was used to compute the observed and unbiased expected heterozygosity (Ho and He respectively, Nei 1987) and inbreeding coefficient (Fis) over all loci within each island. Allelic richness was obtained by rarefaction (El Mousadik and Petit 1996) for four diploid individuals (the smallest sample size). Statistical differences in these genetic parameters were tested between four population groups (Table 1). Statistical significance was assessed using 1,000 randomisations of individuals between groups as implement in Fstat. Genetic differentiation and the inbreeding coefficient (Fst and Fis, Weir and Cockerham 1984) and their 95% confidence intervals were computed using 10,000 bootstraps over loci using Genetix v 4.05 (Belkhir et al. 2004). Pair-wise genetic differentiation between populations were estimated by Fst (Weir and Cockerham 1984) and tested for significance using the exact G test implemented in Genepop.

Genetic clustering

Genetic population structure was calculated using Bayesian genetic clustering in Structure v 2.3.2 (Pritchard et al. 2000). The Admixture and Correlated Allele Frequency models (Falush et al. 2003) were used and the software was run with the number of clusters (K) varying from 1 to 15, with eight runs for each K value using 10,000 burn in periods and 50,000 Markov Chain Monte Carlo (MCMC) repetitions. LnP(D) values were examined for each K and outlier runs that yielded a relatively low likelihood were identified. To identify such outlier runs, outlier detection methods were implemented in the extreme values R package (van der Loo 2010) under the assumption that LnP(D) values within K were drawn from the normal distribution. The posterior probabilities of K were computed following the Bayes' Rule as indicated in the Structure manual (Pritchard et al. 2009) to identify the most likely number of genetic groups, including all runs or

Table 1 Genetic diversity indices

	N workers	N colonies	A	Ar	He	Ho	Fis	Fis 95% CI
Outer Hebrides								
Barra	105	45	52	3.0	0.57	0.53	0.054	−0.056–0.136
Benbecula	21	11	40	3.5	0.65	0.53	0.187	−0.105–0.316
NUist	25	16	39	3.2	0.63	0.65	−0.032	−0.194–0.056
S. Uists	46	26	46	3.4	0.65	0.58	0.112	−0.037–0.212
Group 1				3.3[a]	0.61[a]	0.57[a]	0.071[a]	
Inner group 1								
Canna	19	16	47	3.8	0.69	0.58	**0.171**	**0.031–0.227**
Eigg	52	33	73	4.2	0.77	0.65	**0.157**	**0.070–0.216**
Muck	6	5	40	4.6	0.80	0.67	0.183	−0.333–0.245
Rum	34	20	54	4.3	0.79	0.69	**0.121**	**0.015–0.178**
Group 2				4.2[a]	0.76[ab]	0.65[a]	0.152[a]	
Inner group 2								
Coll	49	22	43	3.1	0.57	0.55	0.049	−0.092–0.141
Tiree	35	19	58	3.6	0.69	0.60	0.132	−0.056–0.239
Colonsay	47	33	67	4.1	0.76	0.70	0.079	−0.021–0.153
Group 3				3.6[a]	0.68[a]	0.63[a]	0.083[a]	
Mainland side								
Arran	8	8	55	5.5	0.87	0.84	0.034	−0.247–0.034
Mainland	14	12	87	6.2	0.93	0.75	0.194	−0.019–0.277
Mull	25	20	99	5.8	0.89	0.68	**0.248**	**0.126–0.316**
Skye	39	27	91	5.3	0.86	0.81	0.060	−0.026–0.099
Group 4				5.7[b]	0.89[b]	0.76[b]	0.139[a]	
Overall	525	313	173	4.2	0.74	0.65	0.110	0.082–0.136

N number of individuals analysed (after removal of siblings), *A* number of alleles observed, *Ar* allele number obtained by rarefaction of a minimum sample size of four diploid individuals, *He* expected heterozygosity estimated without bias, *Ho* observed heterozygosity, *Fis* inbreeding coefficient and 95% confidence intervals estimated by 10,000 bootstraps of the individuals within population

[a, b] Population groups with *different letters* indicate significant difference in genetic diversity parameters as tested by 10,000 permutations in Fstat. Fis values in *bold* differ significantly from zero

excluding the outliers runs. Structure was then run for an additional long run using the best K value and 50,000 burn in periods following by 100,000 MCMC repetitions to obtain the final results. The matrix of genetic distance between the inferred clusters as provided by the Structure output was used to build the corresponding neighbour joining (NJ) tree using the APE package (Paradis et al. 2004).

Spatial pattern of genetic structure

The presence of a pattern of isolation by distance (IBD) was tested using a Mantel test (Legendre and Legendre 1998; implemented in the R package VEGAN, Dixon 2003) assessing the correlation between geographical distance (and its decimal logarithm) and linearised genetic differentiation (expressed as Fst/(1 − Fst), Rousset 1997) between populations (model M1 and M2, Table 2). This relationship assumes that the populations are separated by a homogeneous landscape which is unlikely to be the case (most obviously because the study area contains both land and sea).

To test whether landscape factors might influence dispersal, circuit theory (McRae 2006) was used to compute the resistance to dispersal between each pair of populations using CIRCUITSCAPE (McRae and Beier 2007). The idea behind this method is to consider the landscape matrix between populations as an electronic circuit. We might expect bees to be less likely to disperse over sea than land, and also mountains could readily act as barriers to movement of a lowland species such as *B. hortorum* (note: the area contains substantial mountains rising to ∼1200 m). Each cell in the raster map representing the landscape is modelled by an electric resistance, and the ease with which individuals (or genes) disperse between two populations is modelled by the way the electric current flows between

Table 2 The influence of landscape characteristics on population genetic structure

Mantel test				Mantel partial			
Model	Test	*r*	*P* value	Model	Test	*r*	*P* value
M1	Gt ~ Go	0.199	0.0988	–	–	–	–
M2	Gt ~ log10(Go)	0.288	0.0218	MP2	Gt ~ log10(Go)/Go	0.272	0.0034
M3	Gt ~ Res1	0.605	<0.0001	MP3	Gt ~ Res1/log10(Go)	0.586	<0.0001
M4	Gt ~ Res2	0.587	<0.0001	MP4	Gt ~ Res2/Res1	−0.027	0.5132
M5	Gt ~ Res3	0.691	<0.0001	MP5	Gt ~ Res3/Res1	0.439	0.0030
M6	Gt ~ Res4	0.653	<0.0001	MP6	Gt ~ Res4/Res3	−0.070	0.6104

Gt genetic distance expressed as Fst/(1 − Fst), *Go* geographic distance in meters, *log10(Go)* log of the geographic distance, *Res1* Resistance[land] = 1 and Resistance[water] = 100, *Res2* Resistance[land] = 1 + (altitude/100) and Resistance[water] = 100, *Res3* R[land] = 1 and R[water] = absolute value (ocean depth − 10), *Res4* R[land] = 1 + (altitude/100) and R[water] = absolute value (ocean depth − 10). See text for more details. For the Mantel tests: matrix1 ~ matrix2 means the correlation between matrices 1 and 2. For Mantel Partial tests: matrix1 ~ matrix2/matrix3 means partial correlation between matrices 1 and 2 conditioned on matrix3. *r*: Mantel statistic, *P* value: *P* value obtained by 10,000 permutations

these two points in the circuit (McRae 2006). The resistance value of each cell in the raster map is determined according to the physical characteristics of the studied landscape. The global bathymetry and elevation data at 30 arc seconds resolution (SRTM30 PLUS; Becker et al. 2009) was used as geographic data projected onto the UTM geographic coordinate system using LANDSERF 2.3 GIS software (J. Wood; http://landserf.org/). A resistance value for each cell in the landscape was then assigned and exported onto the resulting map as an ASCII grid raster consisting of a grid of 388 × 650 cells.

Firstly, the impact of the ocean in restricting bumblebee dispersal was addressed. A resistance value of 1 was assigned to land and a value of 100 to water (matrix Res1). These arbitrary values account for the fact that dispersal over water is presumed to be much more difficult for bumblebees than dispersal over land. The effect of the topology on genetic structure was then tested by adding to 'land resistance' a value equal to the altitude in meters divided by 100, representing the landmass and resulting in land cells with a resistance from 1 to 12 (matrix Res2). Finally, the effect of the ocean depth was assessed by including information on bathymetry in the resistance map. A resistance equal to the absolute value of the actual sea depth in meters plus 10 was assigned (so that the value of resistance for the cell representing the ocean was at least 11), while the land was either assigned a resistance of one (matrix Res3) or computed from the altitude as described above (matrix Res4). The resistance values used were arbitrary; they do not represent measurable quantities but instead are conceptual values. CIRCUITSCAPE (McRae and Beier 2007) was used to compute pair-wise resistance between populations considered as focal points, with an eight neighbour cell connection scheme. The resulting pair-wise resistance matrix was then correlated with the previously described genetic differentiation matrix using a Mantel test to check the model of isolation by resistance (IBR) using the different resistance matrices. Competing models were further compared using a partial Mantel test (Legendre and Legendre 1998; package VEGAN) as the model with the best support should show a significant positive partial correlation with genetic distance after controlling for each of the competing models (Cushman et al. 2006; McRae and Beier 2007).

Estimation of recent and long term migration rates

Two groups each compromising four populations were selected to estimate recent and long term migration rates, since it has been shown that the accuracy of migration rates estimated by BayesAss (Wilson and Rannala 2003) decreases with the number of studied populations (Faubet et al. 2007). Four populations were selected along a transect from the mainland to the Outer Hebrides, with each population belonging to a different genetic cluster to maximise the resolution of the analyses which depended on the amount of genetic differentiation (Wilson and Rannala 2003; Beerli 2008). Within genetic clusters the largest populations available were used. Population group A comprised the northern populations of Skye, Rum, Eigg and South Uist while population group B comprised the southern populations of Mull, Colonsay, Coll and Barra.

Recent immigration leads to genotypic linkage disequilibrium which is used by BayesAss to infer the inbreeding coefficient, assign individuals to populations and to estimate recent migration rates within a Bayesian framework. BayesAss v 1.3 was run following the recommendation of Faubet et al. (2007) concerning convergence issues: 10 replicated MCMC runs were applied, with different seed numbers using 21,000,000 iterations with a sampling frequency of 2,000 and a burn in of 1,000,000. The delta values were set to 0.20, 0.10 and 0.20 for allele

frequency, migration rate and inbreeding respectively for population set A, and 0.25, 0.12 and 0.15 for allele frequency, migration rate and inbreeding respectively for population set B, in order to get an acceptance ratio between 0.4 and 0.6 as recommended in the BayesAss manual. The distribution of the log likelihood of the results was then inspected and the run with the higher log likelihood was selected to estimate recent migration rates (m). Close inspection of the estimations for other runs with a high likelihood yielded comparable estimates (data not shown).

The genealogical data inherent in genetic data such as microsatellites can be used to infer the long term migration rate and effective population size using coalescent methods. Such an approach was used as implemented in Migrate 3.0.3 (hosted at the CBSU Web Computing Interface http://cbsuapps.tc.cornell.edu/migrate.aspx, Beerli 2006) to estimate the long term immigration rate M (expressed as $M = m/\mu$, with m the migration rate and μ the mutation rate per site per generation) and the mutation scaled effective population size Θ ($\Theta = 4N_e\mu$). The parameter M describes the relative impact of immigration and mutation in bringing new allele variants into the population (Beerli 2008), and assumes an island model in equilibrium conditions. A preliminary run with default parameters was used to adjust the initial values. A first run was then conducted using the Brownian motion mutation model, with starting values for M and Θ generated from Fst calculations, and exponential prior distribution for both parameters (Θ: min = 0, mean = 2.5 and max = 5.0; M: min = 0, mean = 25 and max = 50). The Metropolis–Hastings algorithm for the proposed distribution and the mutation rate were calculated from the data for each locus. A long chain of 10,000,000 visited genealogies was created by recording one in every 100 after a burn in of 50,000. The M and Θ estimates were then used as starting values for the next identical run to test the reliability of the analysis and improve the posterior distribution. The analysis was repeated using the new Migrate 3.1.3 version; results were concordant so those from version 3.0.3 are shown here. The same procedure was used for both population sets.

Results

Genotyping and sibling worker removal

A total of 600 individuals were genotyped for eight microsatellite loci. Thirty individuals that failed to amplify for more than three loci were excluded from further analyses. None of the 39 males were diploid (diploid males might indicate inbreeding depression). Sibs among the remaining 531 workers were identified using Colony version 2.0.0.1 (Jones and Wang 2009). Throughout all the sampled islands, 319 distinct colonies were found; one randomly selected worker per colony was kept for subsequent analyses (Table 1). Finally, four populations containing less than five individuals (6 individuals in total) were removed from the analysis. The final dataset used for subsequent analyses consisted of 313 individual workers representing 313 colonies (Table 1) and originating from 15 different island populations (Fig. 1).

Genetic parameters

Departure from Hardy–Weinberg was significant after Bonferroni correction, in 4 out of a total of 120 tests (Eigg B11, B96, B126 and Mull B124). Microchecker detected the potential presence of a null allele in only 9 out of 120

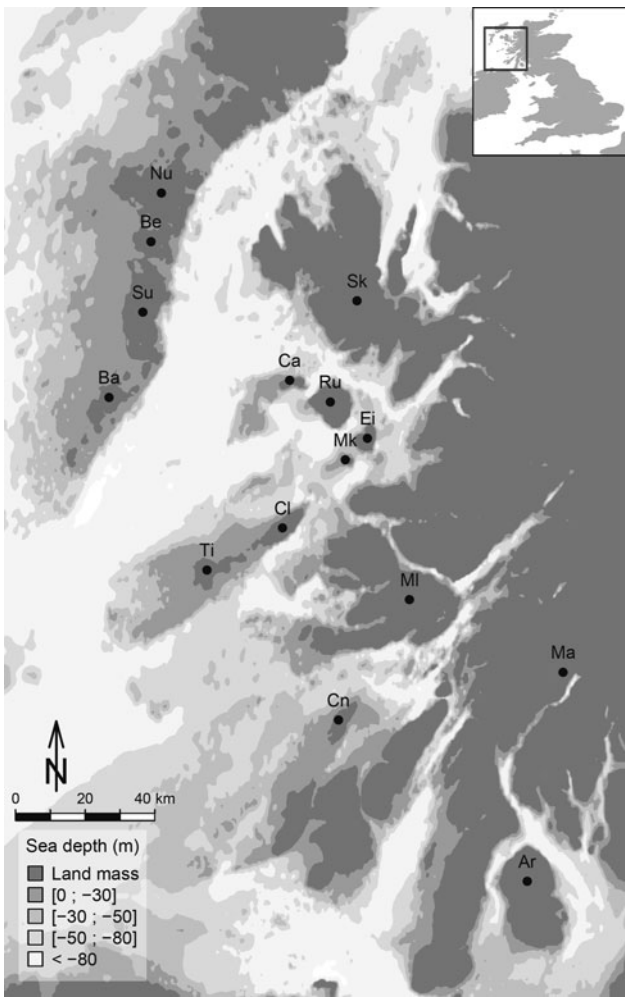

Fig. 1 Location and geographical context of the analysed populations. *Grey* levels indicate oceanic depths (see legend). Each point represents a sampled population (*Ar* Arran, *Ba* Barra, *Be* Benbecula, *Ca* Canna, *Cl* Coll, *Cn* Colonsay, *Ei* Eigg, *Ma* Mainland, *Mk* Muck, *Ml* Mull, *Nu* North Uists, *Ru* Rum, *Sk* Skye, *Su* South Uists, *Ti* Tiree). Insert: location of the study area within United Kingdom

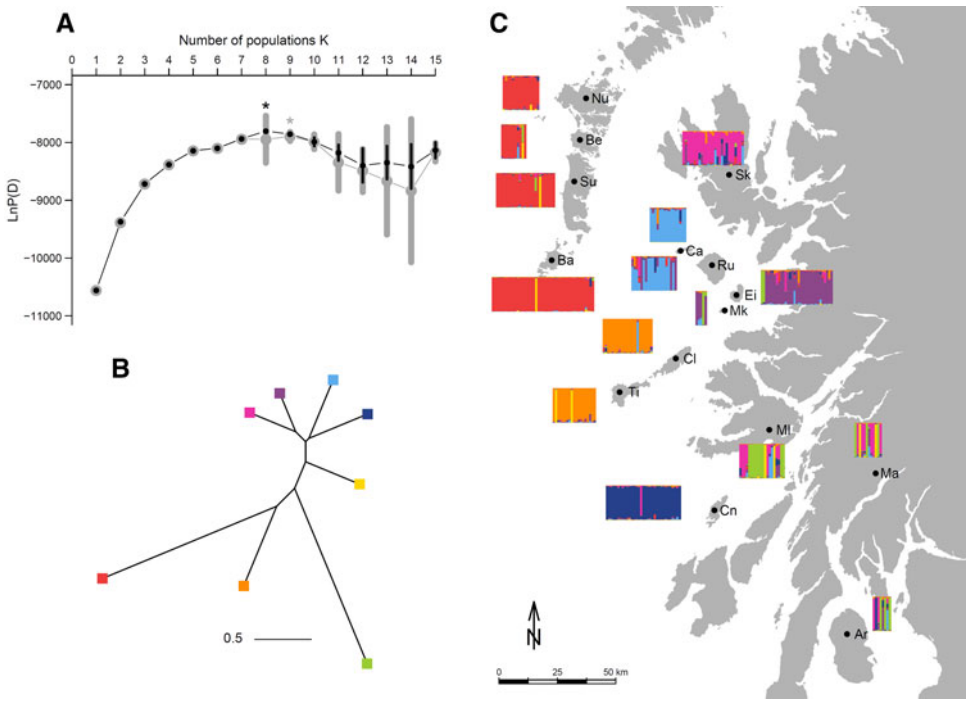

Fig. 2 Genetic clustering results. **a** Determination of the number of clusters (K). A star denotes the most likely number of clusters according to the Pritchard Bayes formula. *Grey*: including all eight repetitions for each K number, *black*: filtered out for runs with outlier likelihood values. **b** Neighbour joining genetic tree computed based on the net nucleotide distance provided by Structure between the eight genetic clusters. **c** Structure results obtained for eight clusters. Each individual is represented by a thin vertical line partitioned into eight coloured segments proportional to its membership in the corresponding genetic cluster. Location codes: *Ar* Arran, *Ba* Barra, *Be* Benbecula, *Ca* Canna, *Cl* Coll, *Cn* Colonsay, *Ei* Eigg, *Ma* Mainland, *Mk* Muck, *Ml* Mull, *Nu* North Uists, *Ru* Rum, *Sk* Skye, *Su* South Uists, *Ti* Tiree

tests (B100 in Canna and Coll, B11 in Eigg, B10 in Mull, B124 in Eigg and South Uists, B96 in Mainland, B121 in Mull and B126 in Mull). No evidence of linkage disequilibrium was detected so all loci were considered independent.

The overall level of genetic diversity was moderate to high with expected heterozygosity ranging from 0.57 to 0.93 across populations (Table 1) and the number of alleles obtained by rarefaction (from four diploid individuals) ranging from 3.0 to 6.2. Both estimators of genetic diversity consistently showed a significantly higher genetic diversity for populations located close to the mainland, while island populations further from the mainland showed a lower genetic diversity (Table 1). Although Outer Hebrides populations appeared to harbour the lowest genetic diversity, there was no significant difference from the other island populations (Table 1). The inbreeding coefficient (Fis) varied among populations, with only four populations showing a confidence interval that did not include 0 (Table 1). There were no significant differences in Fis between the four population groups (Table 1).

Overall genetic differentiation was relatively high with Fst = 0.16 (confidence interval: 0.13–0.20) and ranged from −0.014 to 0.330 in pair-wise comparisons between populations (mean: 0.140, median: 0.138, standard deviation: 0.081). Only 10 out of 105 pair-wise comparisons were not significant, mostly among Outer Hebrides populations (Barrra–North Uists, Barra–South Uists, North Uists–South Uists) or between populations located near to the mainland (Arran–Mainland, Arran–Muck, Arran–Mull, Muck–Mainland, Muck–Mull, Muck–Eigg and Mainland–Muck).

Genetic clustering

Among the eight repeated analyses for each of the 15 possible numbers of genetic groups (K = 1–15), a maximum of two runs per K value did not converge or became stuck in a local sub-optimal solution (these runs were detected based on their outlier likelihood value). When all runs were included in the analysis, the most likely number of genetic groups (K) was nine (Fig. 2a, grey points). When outlier runs were removed, the most likely K value was eight (Fig. 2a, black points). This discrepancy was due to a highly improbable run for one repetition leading to a lower mean likelihood at K = 8. We thus used K = 8 as the best clustering solution. Six of the clusters represent different panmictic units corresponding to groups of nearby islands or single islands (Fig. 2c: Outer Hebrides

populations in red, Coll and Tiree in orange, Canna and Rum in cyan, Eigg and Muck in purple, Colonsay in blue, Skye in pink). Conversely, populations located close to or on the mainland showed a mixture of mostly two different clusters in various proportions (green and pink in Arran, Mainland and Mull). The yellow genetic cluster is only represented by a few individuals scattered in different populations in the southern part of the studied area (Fig. 2c: Mainland, Mull, Tiree, Barra and South Uists). These individuals may originate from an unsampled population, probably south of our sampling area (such as Jura, Islay or Ireland). Globally, the genetic structure appears spatially organised as isolated external islands (Outer Hebrides and Coll/Tiree) corresponding to divergent clusters, while more internal islands (northern Inner Hebrides) contain closely related genetic clusters (Fig. 2b, c). There are, however, some exceptions to this general pattern such as Colonsay appearing more genetically close to the latter group of islands despite being situated quite far away, and there is also a cluster found on or close to the mainland which appears relatively divergent without being found in an isolated population (green). Finally, a close inspection of the Structure results suggest some recent migration (at most two generations ago) between closely situated islands, notably between the three islands Skye, Rum and Eigg and to a lesser extent a few migrants between more distant islands (for instance, one immigrant found on Colonsay and Coll, and a few on Eigg and Canna).

Spatial pattern of genetic structure

There was a low but significant pattern of isolation by distance (IBD) (Table 2; Fig. 3a). The pattern is noisy in part because some genetically similar populations belonging to the same genetic cluster are geographically far apart, e.g. North Uist and Barra (squares in Fig. 3a). The isolation by resistance model (IBR) that accounts for the geographical configuration of the islands (matrix Res1) results in a far better model (Table 2, models M3 and MP3). Notably, populations from the same genetic cluster are separated by a small resistance distance because they are segregated mostly by land (for example all of the Outer Hebridean populations). A model including the effects of topology in the resistance matrix (Res 2) did not provide a better fit (Table 2, models M4 and MP4), suggesting that mountains do not act as barriers to dispersal within the study area. However, including bathymetry did improve the fit of the model (matrix Res3, model M5, Fig. 3c). This model is significantly better than the previous best model M3 (Mantel Partial MP5, Table 2) and the corresponding correlation is very close, especially when looking at comparisons between populations from the same genetic clusters (squares in Fig. 3c). The good fit of this model indicates that populations are more similar when separated by shallow water (which would have been land during past periods of lower sea level), while populations tend to be more genetically dissimilar when separated by deep water. Finally, the model M6 which adds the effect of topology to the previous model, does not enhance the explanation of genetic structure (Table 2).

Recent estimates of migration rates

Analyses of recent migration rates by BayesAss show a very restricted pattern of migration between nearby islands (Table 3). There appears to have been significant movement from Rum and Eigg to Skye, from Eigg to Rum (Table 3, population set A) and from Colonsay and Coll to Mull (Table 3, population set B). The estimated direction of migration is the opposite of that expected, where mainland populations would act as source population and island populations as sinks due to their expected difference in population size. These estimates also depart from the Structure results which indicate recent migration, for example from Skye to Rum and Eigg. Nevertheless, the pattern of migration is geographically consistent in detecting migration between nearby islands, principally within the trio of Skye, Rum and Eigg, providing results that are in accordance with the Structure analysis. Overall, the low migration rates indicate that water represents a strong dispersal barrier between most of the islands.

Long term estimates of migration rates and effective population size

A clear pattern of a decrease in mutation-scaled effective population size (Θ) toward the West was observed within the two population groups (Table 4). Notably, populations connected to the mainland (Skye and Mull) showed a Θ value about one order of magnitude higher than island populations. The overall pattern of mutation-scaled immigration rate (M) is relatively similar to the recent estimates, in that migration rates were found to be higher in Population Set A than in Population Set B (Tables 3, 4). However, in contrast to recent migration rate estimates, evidence was not found of strongly asymmetrical patterns of migration between Skye, Rum and Eigg (Table 4). Overall, M values which estimate the relative importance of immigration and mutation in generating new allele variants in the population are smaller than 1, indicating that mutation and genetic drift are the principal factors shaping the observed population genetic structure on most islands.

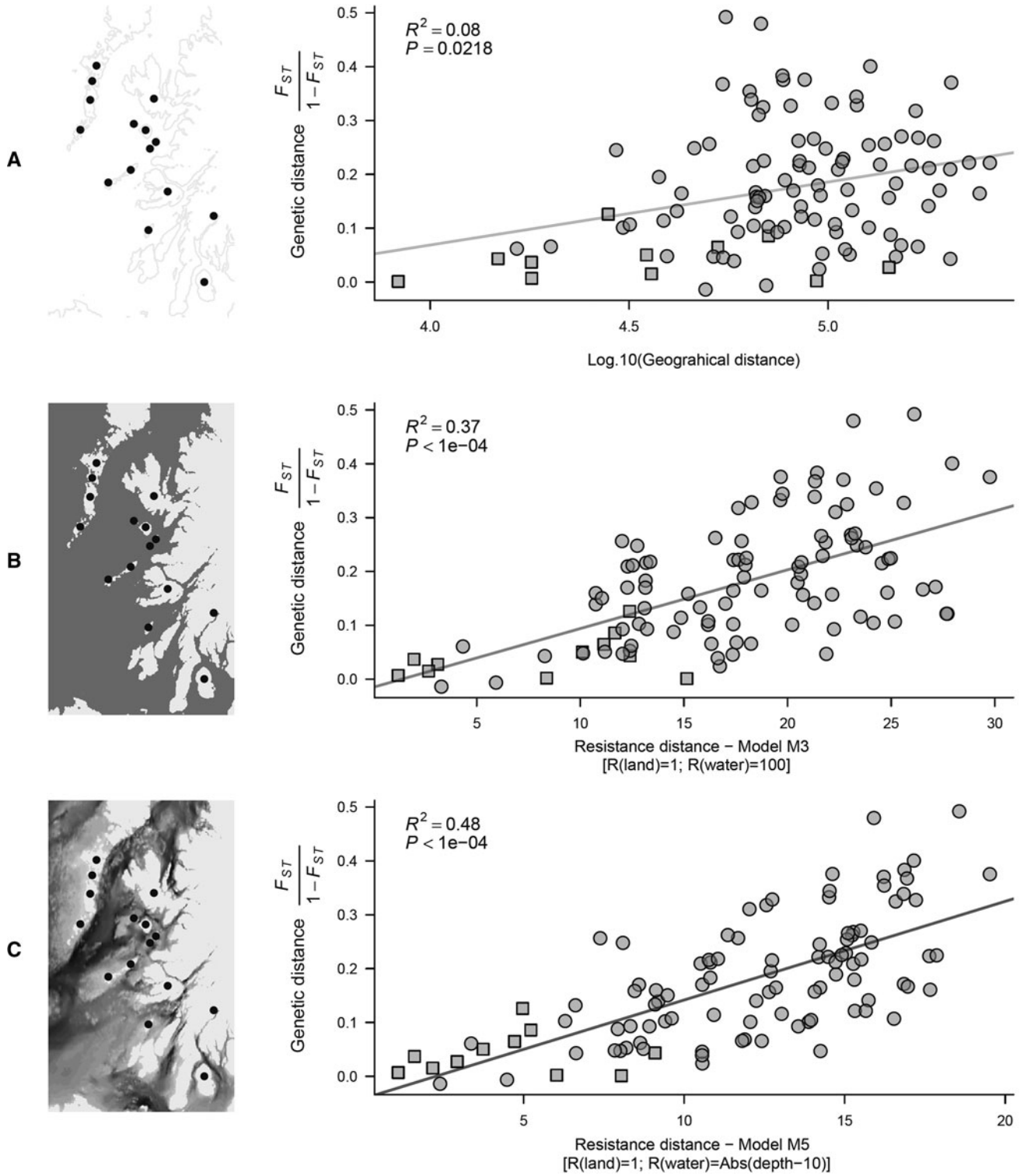

Fig. 3 Isolation by distance (**a**) and isolation by resistance models (**b**, **c**) between insular *B. hortorum* populations. Correlation between linearised genetic differentiation (Fst/(1 − Fst)) and the logarithm decimal of the geographic distance (**a**) or resistance distance for the model M3 (**b**) and M5 (**c**). *Square* points indicate pair-wise comparisons between populations from the same genetic cluster while circles represent pair-wise comparisons between populations from different genetic clusters. Maps on the left in **b** and **c** illustrate the resistance values of each cell in the raster map with *light grey* indicating low resistance (value of 1 for land) and *dark grey* indicating high resistance to dispersal (except *black* points that indicate the location of the populations). In **a** the geographical distance between points was used considering the landscape as a homogeneous space (represented in *white*, except for the coastal outline illustrate in *light grey* for visibility)

Table 3 Recent migration rate (*m*) estimated by BayesAss

Into\From	Skye	Rum	Eigg	S. Uist
Population set A				
Skye	**0.76**	**0.19**	**0.03**	**0.02**
	(0.68–0.87)	(0.06–0.30)	(0.00–0.14)	(0.00–0.08)
Rum	0.01	**0.92**	**0.06**	0.01
	(0.00–0.04)	(0.80–0.99)	(0.00–0.17)	(0.00–0.05)
Eigg	0.01	0.01	**0.97**	0.01
	(0.00–0.05)	(0.00–0.03)	(0.93–1.00)	(0.00–0.02)
S. Uists	0.01	0.01	0.01	**0.98**
	(0.00–0.04)	(0.00–0.03)	(0.00–0.03)	(0.94–1.00)
Into\From	Mull	Colonsay	Coll	Barra
Population Set B				
Mull	**0.85**	**0.11**	**0.03**	0.01
	(0.78–0.93)	(0.04–0.19)	(0.00–0.09)	(0.00–0.05)
Colonsay	0.00	**0.99**	0.00	0.00
	(0.00–0.02)	(0.96–1.00)	(0.00–0.02)	(0.00–0.02)
Coll	0.00	0.01	**0.98**	0.01
	(0.00–0.02)	(0.00–0.03)	(0.950–1.000)	(0.00–0.03)
Barra	0.01	0.00	0.00	**0.99**
	(0.00–0.03)	(0.00–0.02)	(0.00–0.02)	(0.96–1.00)

Immigrant source populations are listed in the first *row* (From) and receiving populations are listed in the first *column* (Into). Numbers refer to migration rates (off-diagonal), non migration rates (diagonal) and their 95% confidence intervals (in *brackets* underneath). Rates with upper confidence intervals higher that 0.05 are highlighted in *bold*. Non informative mean and 95% confidence interval expected in cases of insufficient signal in the data: 0.83 (0.67–0.99) for the non-migration rate and 0.06 (0.00–0.22) for the migration rate. Our narrower confidence intervals indicate that relevant signal about recent migration rates is contained in our data

Discussion

Our data demonstrate that *B. hortorum* populations in western Scotland exhibit marked population structuring with little or no gene flow in recent times between all but the most geographically proximate islands. Previously, Darvill et al. (2006) and Darvill et al. (2010) examined the genetic structuring of populations of *B. muscorum* and *B. jonellus* in this same island system. They concluded that these two species differ markedly in dispersal ability, with *B. muscorum* being relatively sedentary while *B. jonellus* regularly dispersed over sea barriers of 30 km or more. Our study suggests that *B. hortorum* is more sedentary than either of these two species (Fst values: *B. hortorum*, 0.16; *B. muscorum,* 0.13; *B. jonellus*, 0.034). This is perhaps surprising. We might expect sedentary species to be more susceptible to habitat loss and fragmentation than more dispersive species, and indeed Darvill et al. (2010) advance this argument to explain why, at a broader geographic scale, *B. muscorum* has declined more than *B. jonellus* (*B. muscorum* has declined greatly in the UK, while *B. jonellus* remains widespread although often scarce). *B. hortorum* is ubiquitous throughout the UK and much of Europe, appearing to have been affected little by the habitat loss which has had dramatic effects on some other bumblebee species. It is thus unexpected to find evidence that it is a highly sedentary species.

Despite their apparently sedentary nature, genetic diversity within *B. hortorum* populations appears to remain high, even on small islands and remote island groups such as the Outer Hebrides (for comparison, H_e values: *B. hortorum*, 0.57–0.93; *B. muscorum,* 0.31–0.53; *B. jonellus*, 0.70–0.77; *B. distinguendus* 0.361–0.439, Darvill et al. 2010; Charman et al. 2010). This suggests that these populations have remained large and stable for a considerable period of time. Application of coalescent methods (using Migrate) suggests that high genetic diversity within populations is largely driven by mutation on all but the most closely connected islands, with a small and often negligible influence of immigration. It is perhaps surprising that populations of this social insect on, in some cases, quite small islands could be large enough to avoid loss of genetic diversity through drift in the absence of significant migration. This may be aided by the relatively small colony size of *B. hortorum* compared to other bumblebees (Sladen 1912) which means that the breeding individuals make up a larger proportion of the population (for a given bee density, the effective population size is larger).

Table 4 Long term mutation-scaled immigration rate ($M = m/\mu$) and population size ($\Theta = 4N_e\mu$) estimated by Migrate

Population set A	Skye	Rum	Eigg	S. Uist
Θ	14.1 (7.7–22.4)	1.8 (0.6–3.1)	3.4 (1.8–5.4)	0.9 (0.0–1.9)
M Into\From	Skye	Rum	Eigg	S. Uists
Skye		**0.6** (0.0–1.0)	**0.4** (0.1–0.9)	0.1 (0.0–0.3)
Rum	0.3 (0.0–1.3)		**1.5** (0.8–2.9)	0.3 (0.0–0.9)
Eigg	**0.6** (0.2–1.2)	**1.2** (0.6–2.1)		**0.4** (0.1–0.9)
S. Uists	0.0 (0.0–1.3)	0.2 (0.0–0.7)	**0.8** (0.3–1.9)	
Population Set B	Mull	Colonsay	Coll	Barra
Θ	15.6 (8.9–27.4)	1.5 (0.3–2.7)	0.6 (0.0–1.6)	0.7 (0.0–1.7)
M Into\From	Mull	Colonsay	Coll	Barra
Mull		0.0 (0.0–0.2)	0.0 (0.0–0.1)	0.0 (0.0–0.2)
Colonsay	**0.4** (0.1–1.2)		0.1 (0.0–0.6)	0.2 (0.0–0.6)
Coll	0.0 (0.0–0.9)	0.2 (0.0–1.3)		0.0 (0.0–0.6)
Barra	**1.4** (0.4–2.2)	0.0 (0.0–0.3)	0.1 (0.0–0.6)	

Immigrant source populations are listed in the first row (From) and receiving populations are listed in the first column (Into). 90% confidence intervals are provided underneath in *brackets*. Migration rates whose confidence intervals do not include 0 are highlighted in *bold*

Analysis of genetic clusters suggests that there are eight distinct genetic groups present. Six of these correspond neatly with groups of islands (e.g. the Outer Hebrides) or single islands (e.g. Colonsay). One cluster (green on Fig. 2) is found mainly on or close to the mainland. The remaining cluster (yellow on Fig. 2) is thinly scattered among populations at the southern edge of our sample area, and it seems likely that these represent occasional immigrants from an unsampled population (perhaps from Jura, Islay or Ireland). Overall, the picture is consistent with high population structuring and low dispersal apart from between closely neighbouring populations, with slightly greater genetic diversity and dispersal in the east close to the mainland. The presence of small numbers of individuals which appear to be from distant populations (and of individuals from the yellow cluster of unknown origin) appears to contradict this conclusion, and ought to erode the strong genetic structuring we describe. We suggest that these may represent stray workers blown off course when foraging, or perhaps wandering workers from nests which had been destroyed and which, having no reproductive potential, do not contribute to gene flow.

As we would expect for a sedentary species, we found significant isolation by distance (populations further apart tended to be more genetically distinct). The value of incorporating variable resistance of the landscape to dispersal in such models is clearly illustrated by our data, whereby assuming that sea provides a greater barrier to dispersing bees than land significantly improved the fit of the model. Intriguingly, factoring in bathymetry also significantly improved the model. It is hard to conceive how current day ocean depth could influence bee dispersal (it makes no difference to a flying bee how deep the water beneath it is), so this strongly suggests that the patterns of genetic structuring we observe today are influenced by colonisation and dispersal events which occurred thousands of years ago. The relative sea level during the Holocene was highly variable around Britain (Ritchie et al. 2001; Jordan et al. 2010), and so it is difficult to determine exact sea levels for any given period. However, globally, the sea level following the last glacial maximum 12,000 ybp was $\sim$120 m below current levels, so it seems likely that many of the islands we sampled would have been connected to one another and to the mainland at that time. Evidence of the presence of hazel and birch pollen in the Outer Hebrides occurs in paleontological records from around 9,500 ybp, and of *Calluna* from around 8,500 ybp (Ritchie et al. 2001), and it is likely that colonisation by bumblebees would have occurred approximately at this time. Relative sea level also varied considerably between 12,000 and 5,000 ybp, at which time levels reached their current depth (Ritchie et al. 2001; Jordan et al. 2010). Thus the pattern we observe in the present-day population structure of *B. hortorum* is still influenced by colonization processes which occurred 5000–8,500 ybp. This finding accords with our estimates of migration rates between islands, which are negligible for most island pairs. Genetic signatures from events which occurred thousands of generations ago would have long since been obscured if bee movement was frequent.

Recent studies of other taxa have found similar patterns; for example Esselstyn and Brown (2009) found that sea level fluctuations during the Pleistocene had an impact on the present-day phylogenetic diversity of shrew taxa in the Philippine Archipelago. They reported that islands separated by shallow water channels appear to harbour similar biological communities, while those separated by deep water channels maintain more distinctive biota.

From an applied perspective, our data suggest that *B. hortorum* in the Western Isles occurs as a number of distinct genetic clusters which have been largely isolated

from one another for long time periods, and which may well be locally adapted, particularly since the islands vary substantially in topography, climate, and the habitats present. Some of these clusters appear to occur on single islands (e.g. Colonsay), and some appear to have small population sizes (e.g. the Outer Hebridean cluster). The Hebrides support a number of rare and endangered species and habitats (e.g. the flower-rich machair grasslands) which are threatened by changing farming practices and depopulation (Redpath et al. 2010). Given the role that *B. hortorum* plays as the main pollinator of deep-flowered plants, the conservation of these local island races perhaps deserves particular attention. More broadly, although *B. hortorum* remains ubiquitous across much of Europe, our evidence that it is a relatively sedentary species suggests that it could be susceptible to decline if suitable habitat becomes more fragmented than at present.

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