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# Conservation genetics, foraging distance and nest density of the scarce Great Yellow Bumblebee (*Bombus distinguendus*)

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## Abstract

**The conservation genetics of bees is of particular interest because many bee species are in decline, so jeopardizing the essential ecosystem service of plant pollination that they provide. In addition, as social haplodiploids, inbred bees may be vulnerable to the extra genetic load represented by the production of sterile diploid males. Using microsatellite markers, we investigated the genetic structure of populations of the Great Yellow Bumblebee (*Bombus distinguendus* Morawitz) in the UK, where this species has undergone a precipitous decline. By means of a mixture of analytical methods and simulation, we also extended—and then applied—genetic methods for estimating foraging distance and nest density in wild bees. *B. distinguendus* populations were characterized by low expected heterozygosity and allelic richness, inbreeding coefficients not significantly different from zero, absence of detected diploid males, absence of substantial demographic bottlenecking, and population substructuring at large (*c.* 100+ km) but not small (10s of km) spatial scales. The minimum average effective population size at our sampling sites was low (*c.* 25). In coastal grassland (machair), the estimated modal foraging distance of workers was 391 m, with 95% of foraging activity occurring within 955 m of the nest, and estimated nest density was 19.3 nests km$^{-2}$. These findings show that *B. distinguendus* exhibits some genetic features of scarce, declining or fragmented populations. Moreover, *B. distinguendus* workers appear to forage over above-average distances and nests remain thinly distributed even in current strongholds. These considerations should inform future conservation actions for this and similar species.**

*Keywords*: declining species, foraging range, microsatellite, pollinator, population biology, social insect

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## Introduction

Populations in decline potentially exhibit a suite of genetic phenomena brought about by the fragmentation of formerly continuous ranges to create groups of sub-populations. These phenomena include loss of genetic diversity, inbreeding (mating with relatives), increased population substructuring and reduced effective population size (e.g. Frankham *et al.* 2002). Because they may in turn accelerate population decline (Spielman *et al.* 2004), it is important to establish the extent of these phenomena in groups of conservation concern. One such group is represented by the pollinating insects, in which global declines are of widespread concern because of the essential ecosystem service of plant pollination that they provide (Biesmeijer *et al.* 2006).

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Bees (Hymenoptera: Apoidea) represent a major group of pollinating insects, and for this reason there is growing interest in the genetic consequences of population decline and habitat fragmentation in wild bee populations. Evidence for increased inbreeding, reduced effective population size or reduced genetic diversity has been found in declining or fragmented populations of bumblebees (Darvill *et al.* 2006; Ellis *et al.* 2006; Takahashi *et al.* 2008a; Lozier & Cameron 2009), euglossine bees (Roubik *et al.* 1996; Takahashi *et al.* 2001; Zayed *et al.* 2004) and halictine bees (Zayed & Packer 2001). Such phenomena are of particular interest in social haplodiploids, which, under inbreeding, may suffer from an additional genetic load created by complementary sex determination. This load occurs through increased homozygosity at the sex-determining locus that, in turn, leads to the production of sterile, diploid males (Pamilo & Crozier 1997; Chapman & Bourke 2001; Zayed & Packer 2005).

At the same time as interest in the conservation genetics of bees has increased, new genetic techniques have been developed with which to estimate foraging distance, the numbers of colonies represented by individuals occurring at particular sites and nest density in wild bees. Foraging distance (or foraging range, i.e. the distance flown by a worker bee from the nest to floral resources) is critical for bees' provision of pollination services because it determines how far and how often pollen travels across landscapes (Dick 2001; Schulke & Waser 2001; Pasquet *et al.* 2008). Knowledge of foraging distance must also be important in reserve management for declining bees. Direct methods of measuring foraging distance (i.e. methods using marked foragers) show that bees may forage many hundreds of metres and even several kilometres from their nests (Osborne *et al.* 1999, 2008a; Walther-Hellwig & Frankl 2000; Pasquet *et al.* 2008; Wolf & Moritz 2008). However, these methods exhibit various biases and are labour-intensive and manipulative, making them hard to apply to large samples of wild colonies. By contrast, genetic methods of estimating foraging distance (Darvill *et al.* 2004; Knight *et al.* 2005), although indirect, measure average foraging distance across large numbers of colonies in the wild. The number of colonies represented by individuals occurring at particular sites can be estimated by grouping individuals into families on the basis of their multilocus genotypes (Chapman *et al.* 2003; Darvill *et al.* 2004; Wang 2004). This way, researchers have estimated the numbers of colonies sending workers to foraging sites in bumblebees (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005) and sending males to male aggregations in bumblebees, stingless bees and wild honey bees (Darvill *et al.* 2007; Kraus *et al.* 2008, 2009; Jaffe *et al.* 2009). Combining such methods with data on foraging distance has yielded estimates of nest density (Darvill *et al.* 2004; Knight *et al.* 2005, 2009). Since foraging distance, the numbers of colonies represented at particular sites and nest density are not well known in wild bees (Gathmann & Tscharntke 2002; Greenleaf *et al.* 2007; Osborne *et al.* 2008b; Zurbuchen *et al.* 2010), these methods are potentially highly informative.

In temperate regions, bumblebees (*Bombus* spp.) form a key group of wild pollinators (Goulson 2003). In common with other bees, some species of bumblebee have exhibited steep population declines in recent decades, both in Europe and North America (Goulson *et al.* 2008; Grixti *et al.* 2009). Accordingly, population-genetic studies of bumblebees have moved from characterizing baseline parameters in widespread species (e.g. Estoup *et al.* 1996; Widmer & Schmid-Hempel 1999; Chapman *et al.* 2003; Shao *et al.* 2004) to focus on the genetic effects of population decline and habitat fragmentation (Darvill *et al.* 2006; Ellis *et al.* 2006; Herrmann *et al.* 2007; Takahashi *et al.* 2008a; Lozier & Cameron 2009), dispersal ability (Darvill *et al.* 2010; Lepais *et al.* 2010) and genetic properties of invasive populations of nonnative bumblebees (Schmid-Hempel *et al.* 2007). Similarly, genetic methods for estimating foraging distance, numbers of colonies represented at particular sites and nest density have been particularly applied to bumblebees (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005, 2009; Herrmann *et al.* 2007; Kraus *et al.* 2009).

In this study we use genetic methods to estimate population-genetic parameters, foraging distance and nest density in a declining species of bumblebee, the Great Yellow Bumblebee, *Bombus (Subterraneobombus) distinguendus* Morawitz. The general biology of *B. distinguendus* has been reviewed by Benton (2006) and Charman (2007). Worldwide, *B. distinguendus* occurs across northern Europe and Asia from the British Isles to eastern Siberia. In recent decades, *B. distinguendus* has undergone range contractions in a number of different countries and regions where it has been studied, including Belgium, France, Germany, Scandinavia and the UK (Løken 1973; Benton 2006; Charman 2007). In the UK, *B. distinguendus* has retreated north and west over the second half of the twentieth century. Having been thinly but widely distributed, it is now found only on islands off north-west and northern Scotland and in a few sites on the mainland of northern Scotland (Benton 2006). As a result, *B. distinguendus* is listed as Nationally Scarce in the UK and is a Priority Species within the UK Biodiversity Action Plan (http://www.ukbap.org.uk/). As in other bumblebee species, the cause of this decline is believed to be loss of foraging habitat brought about by agricultural intensification (Benton 2006; Goulson *et al.* 2008). In the present study, we add to genetic studies of other

scarce or declining bumblebee populations (Darvill *et al.* 2006; Ellis *et al.* 2006; Takahashi *et al.* 2008a; Lozier & Cameron 2009) in several ways. First, since *B. distinguendus* has declined in the UK with unusual severity, we investigate the conservation genetics of an extreme case among declining bees. Second, we extend existing methods for estimating foraging distance and nest density from genetic data in bees (Darvill *et al.* 2004; Knight *et al.* 2005). Finally, we present the first genetic estimates of foraging distance and nest density for a scarce and declining bumblebee species.

## Materials and methods

### *Sample collection*

A total of 646 *Bombus distinguendus* individuals (549 workers and 97 males) were sampled for DNA in July–September over the 3 years 2003–2005 from sites on islands in the Outer and Inner Hebrides and in the Orkney Islands, Scotland, UK (Table 1; Fig. 1). Sampling was conducted non-lethally by capturing each bee with a net or tube and removing the tarsal tip of a mid- or hind-leg (Holehouse *et al.* 2003). To prevent any bee being sampled for DNA more than once, sampled bees were paint-marked before release, which occurred at or near (within 5 m of) the point of capture. Tarsal samples were stored in the field in 100% ethanol and frozen at the end of each field day.

The worker samples fell into three groups depending on the method of collection. In the first group (375 workers, all worker samples except Howmore and Milton 2005; Table 1), sites for sampling were selected non-randomly (i.e. sites appearing rich in floral resources were preferentially visited) and sampling was conducted by walking freely within each site and capturing workers visiting flowers. The size of the sampled areas was in the range 0.5–10 ha (some samples from The Reef, Tiree, came from a larger area, but 89% of samples from this site were from an area of 4 ha). In the second group (seven workers, Howmore sample; Table 1), workers were collected from the entrance of a *B. distinguendus* nest that was discovered in tussocky dune grassland at Howmore, South Uist. In the third group (167 workers, Milton 2005 sample; Table 1), workers were sampled along a transect for the purpose of estimating their foraging distance (Darvill *et al.* 2004; Knight *et al.* 2005). This transect was 1 km long and was placed along a coastal path running north–south adjacent to mixed low-intensity farmland (crofted

**Table 1** Details of *Bombus distinguendus* individuals sampled for genotyping and estimated number of colonies and effective population sizes represented by worker samples

| Island | Site | Location (latitude, longitude) | Sampling dates | No. workers | No. males | No. colonies (range)* | Minimum $N_e$ † |
|---|---|---|---|---|---|---|---|
| North Uist | Balranald | 57°36′03″N, 7°31′57″W | 8–9 August 2003 | 20 | 1 | 10 | 15.0 |
| Benbecula | Borve | 57°25′51″N, 7°23′12″W | 16–19 August 2004 | 15 | 4 | 9 (9–10) | 13.5 |
| South Uist | Garrynamonie | 57°06′60″N, 7°23′25″W | 9–22 August 2004 | 27 | 1 | 13 | 19.5 |
| | Howmore | 57°18′04″N, 7°23′27″W | 12–23 July 2005 | 7‡ | 0 | 1 | n/a |
| | Milton | 57°12′45″N, 7°25′15″W | 17–21 August 2004 | 29 | 0 | 15 (14–15) | 22.5 |
| | Milton | 57°12′45″N, 7°25′15″W | 20 July–23 August 2005 | 167# | 0 | 42 (41–43) | 63.0 |
| | Ormiclate | 57°15′30″N, 7°25′22″W | 6–7 August 2003 | 38 | 9 | 18 | 27.0 |
| | Stilligarry | 57°19′34″N, 7°23′40″W | 13–14 August 2004 | 20 | 0 | 10 (10–11) | 15.0 |
| | Stoneybridge | 57°17′04″N, 7°25′12″W | 1–5 August 2003 | 82 | 18 | 27 | 40.5 |
| Coll | Red Rock | 56°35′33″N, 6°38′31″W | 11–31 August 2003 | 47 | 0 | 17 (17–18) | 25.5 |
| | Totronald | 56°36′45″N, 6°37′09″W | 31 August 2003 | 0 | 1 | n/a | n/a |
| Tiree | The Reef | 56°30′34″N, 6°51′46″W | 24–29 August 2003 | 72 | 16 | 27 (26–28) | 40.5 |
| Mainland, Orkney | Brodgar | 59°01′02″N, 3°20′30″W | 30 August–2 September 2005 | 15 | 0 | 7 | 10.5 |
| | The Loons | 59°06′05″N, 3°17′09″W | 4–5 September 2003 | 10 | 41 | 8 | 12.0 |
| | Mill | 58°57′31″N, 2°50′57″W | 2 September 2003 | 0 | 1 | n/a | n/a |
| | Newark Bay | 58°55′25″N, 2°45′05″W | 3 September 2003 | 0 | 5 | n/a | n/a |
| Totals/mean (range) | | | | 549 | 97 | | 25.4 (12.0–63.0) |

n/a, not applicable.

*No. colonies estimated from worker data using COLONY 1.3; range added if separate runs returned more than one result.

†Minimum effective population size, calculated as 1.5 × no. colonies (see Materials and methods).

‡Sample of workers collected from the entrance of a *B. distinguendus* nest.

#Sample of workers collected at intervals along a transect for estimation of foraging distance (see Materials and methods).

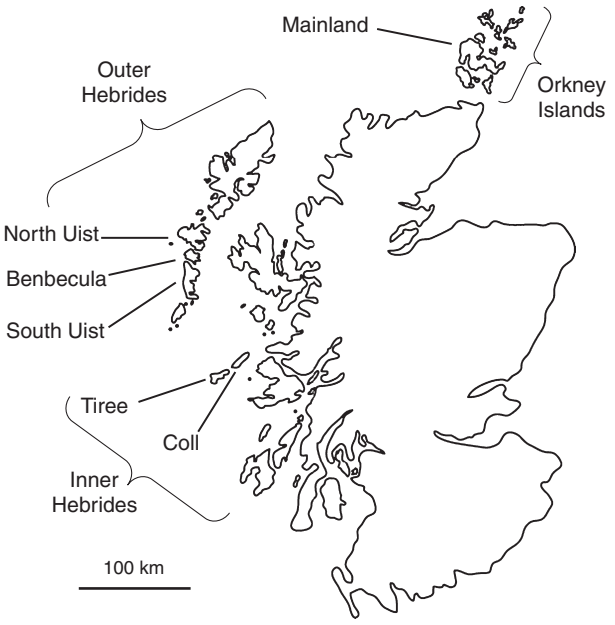


**Fig. 1** Map of Scotland, UK, showing islands where *Bombus distinguendus* workers and males were sampled.

machair). Workers were sampled at flowers within 50 m of six points at 0, 200, 400, 600, 800 and 1000 m, respectively, from the southern end of the transect (0 m point). The capture location of each sampled bee (to within 5 m) was recorded with a Global Positioning System (GPS) device (Garmin International, Inc., Olathe, KS, USA). Thirty workers were sampled at each point, with the exception of the northernmost point, from which 17 workers were sampled. When paint-marked prior to release, each worker from the transect sample was given a mark specific to one of the six sampling points. Across all islands visited, males visiting flowers were sampled opportunistically during either the collection of the worker samples or visits to other suitable sites (Table 1).

### *Molecular methods*

DNA was extracted from the tarsal samples by adding 60 μL of a heavily vortexed mixture of 0.07 g Chelex 100 resin, 1 mL water and 6μg Proteinase-K to each rinsed, macerated sample. This mixture was then incubated at 60 °C for 2 h. The extraction was stopped by denaturing at 96 °C for 15 min. Samples were genotyped at eight microsatellite loci, B10, B11, B96, B100, B118, B124, B126 and B132 (Estoup *et al.* 1995, 1996), which a pilot study of Scottish *B. distinguendus* (Bourke AFG & Hammond RL, unpublished) had identified as being polymorphic with allele sizes that could be unambiguously determined. PCR amplification was carried out in a 20 μL reaction mixture containing 2 μL Chelex supernatant, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 200 μM dNTPs, 2.5 mM $MgCl_2$ (2 mM in the case of loci B11, B124 and B126), 250 nM of each labelled primer (150 nM in the case of loci B11, B124 and B126) and 0.5 units Invitrogen Taq. In nearly all cases, locus B124 was multiplexed with B126 and locus B10 was multiplexed with B100, reactions for all other loci being conducted singly. Reactions were conducted in a Gene Amp 2700 PCR System with a touchdown PCR thermal profile, i.e. 5 min at 95 °C to denature DNA, followed by 19 cycles of 30 s at 95 °C, 30 s at the annealing temperature and 30 s extension at 72 °C. The annealing temperature started at 65 °C and was decreased by 1 °C per cycle. This was followed by 15 cycles of 30 s at 95 °C, 30 s at 45 °C and 30 s at 72 °C. Reactions ended with a final extension at 72 °C for 7 min. The PCR products were visualized using an Applied Biosystems ABI Prism 3100 Genetic Analyzer. Allele sizes were scored using Applied Biosystems GeneMapper v3.7 software and reference to an internal size standard (Applied Biosystems GeneScan 500 ROX).

To quantify microsatellite genotyping errors, 439 workers were genotyped a second time, at one or more loci. In total, 1629 worker genotypes were re-genotyped. Repeats consisted of a new PCR from the original extraction, using one or more of the eight loci (except B11, at which only workers from Orkney were retyped because B11 was monomorphic at all other sites). The per-allele rates of both dropout error (*ab* changes to *aa* or *bb* or vice versa) and non-dropout error (other forms of mismatch between original and repeat genotypes, e.g. *ab* changes to *ac*) were estimated as the number of mismatched alleles divided by the number of re-typed alleles (Broquet & Petit 2004; Hoffman & Amos 2005).

### *Partitioning of genotypes*

Population-genetic analyses were conducted only on worker genotypes. Because workers within sites included individuals that were likely to have been sisters and hence to have exhibited non-independent genotypes (Thomas & Hill 2000), workers were first partitioned into sisterhoods using the sibship reconstruction software COLONY 1.3 (see below). Within sites, partitioning of the data with COLONY 1.3 was repeated ten times. This was because each run of the analysis did not always return the same sibship configuration, although the variation in number of sibships returned was very small (1–2 sisterhoods; Table 1). From each partitioned dataset a worker was chosen at random from each sisterhood. This procedure generated ten datasets, each consisting of non-sister workers, per site. For population-genetic analyses, partitioned datasets were pooled, as appropriate, across sites within islands or island groups, or across years, to increase sample sizes. Pooling within islands was

justified because, on islands with more than one sampling site (South Uist and Mainland, Orkney), sites were at least 2.7 km apart, rendering sites unlikely to share workers from the same colonies (see Results). Pooling across years (i.e. of different sites within a group of sites) was justified because, in the single site that was sampled over consecutive years (Milton on South Uist), there was no significant genetic differentiation across years (see Results). However, the Milton 2005 sample was omitted from other analyses to avoid duplicating lineages present in the Milton 2004 sample. Datasets were pooled as in the following example: for an island with sites A and B, site A having ten partitioned datasets $A_1$, $A_2$, $A_3$, …, $A_{10}$ and site B having ten partitioned datasets $B_1$, $B_2$, $B_3$, …, $B_{10}$, then $A_1$ was pooled with $B_1$, $A_2$ with $B_2$, and so on up to $A_{10}$ and $B_{10}$, to create 10 pooled datasets for the island. Location-specific genetic parameters (e.g. inbreeding coefficients) were then calculated as means across ten (pooled) datasets, and comparisons between (pooled) datasets were likewise calculated as means of ten comparisons (hence the degrees of freedom always reflected the numbers of separate sisterhoods). In the analyses of genetic differentiation, sample sizes within partitioned datasets were equalized to the number of sisterhoods in the site with the lowest number of sisterhoods, which was necessary to achieve balanced sample sizes in FSTAT (Goudet *et al.* 1996). Regarding the use of partitioned datasets in general, note that because there were relatively few workers per colony represented in each site, results from the above analyses and results from analyses using the entire dataset were quantitatively and qualitatively similar.

### *Inbreeding, linkage disequilibrium, genetic diversity and bottlenecking*

We used FSTAT 2.9.3.2 (Goudet 1995) to test for Hardy–Weinberg equilibrium of individual loci and linkage disequilibrium between loci and to calculate expected heterozygosity ($H_E$), allelic richness and inbreeding coefficients ($F_{IS}$). Bonferroni-adjusted $P$ values for these tests as given by FSTAT 2.9.3.2 are reported as appropriate. FSTAT 2.9.3.2 was also used to test whether inbreeding coefficients differed significantly from zero. As another means of detecting possible inbreeding, the genotypes of all males were inspected for heterozygosity, since heterozygosity would indicate male diploidy. The worker data were also tested for recent demographic bottlenecks using BOTTLENECK 1.2.02 (Cornuet & Luikart 1996). The heterozygosity expected at each locus was estimated for each island under three different models of microsatellite evolution (with 1 000 iterations), the Infinite Allele Model (IAM), Two-Phase Model (TPM) and Single-step Mutation Model (SMM). Under the TPM, the frequency of single-step mutations was set at 95% and the variance among multiple steps at 12, as recommended by Piry *et al.* (1999).

### *Genetic differentiation between years, sites and island groups*

FSTAT 2.9.3.2 was used to test for genetic differentiation ($F_{ST}$) between years at the same site, between different sites within an island group and between islands of different island groups. Genetic differentiation between years was tested by comparing the Milton 2004 and Milton 2005 samples (Table 1). Because this comparison showed no genetic differentiation across years (see Results), other analyses included samples regardless of the year of collection. Genetic differentiation between sites within an island group was tested using data from the seven sites sampled for workers in the Outer Hebrides (on North Uist, Benbecula and South Uist; Table 1). In this dataset, the relationship between genetic differentiation between sites and the geographical distance between them (isolation by distance) was investigated by plotting pairwise $F_{ST}$ against geographical distance and statistically testing this relationship using a Mantel test with 10 000 permutations (implemented in FSTAT 2.9.3.2). Genetic differentiation between islands of different island groups was analysed by using data from South Uist (Stoneybridge sample), Coll, Tiree and the Orkney Islands (Brodgar and The Loons samples pooled). To facilitate comparisons with other studies, we also calculated the standardized genetic differentiation measure $G'_{ST}$ following the method of Meirmans (2006).

### *Number of colonies represented within sites*

To estimate the number of colonies represented by workers sampled at each site, the sibship reconstruction software COLONY 1.3 (Chapman *et al.* 2003; Wang 2004), which incorporates an algorithm developed by Thomas & Hill (2000), was used to partition workers within sites into sisterhoods. The software was run using the genotypic error rates (dropout and non-dropout) calculated from retyping individuals (see Results). We assumed that all workers within a colony were offspring of one, singly-mated queen, i.e. were full sisters. These assumptions were justified because single-queening (monogyny) is the rule in temperate bumblebees (Goulson 2003) and because, to date, single mating has been found in all bumblebee subgenera investigated except *Pyrobombus* and *Psithyrus* (Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000; Payne *et al.* 2003; Takahashi *et al.* 2008b). In addition, consistent with single queen mating, the seven workers from the nest comprising the Howmore sample (Table 1) proved to share multilocus

genotypes compatible with full sisterhood. As described above, because each run of COLONY 1.3 within a site did not always generate the same sibship configuration, COLONY 1.3 was run ten times within sites. The number of colonies within sites was then estimated as the mean number of full sisterhoods present, including sisterhoods represented by single workers. (As also described above, each run provided a dataset for the calculation of population genetic parameters.) The estimated number of colonies was a minimum number, because some colonies may have been represented at sites by foraging workers but not sampled. However, the number of unsampled colonies was not estimated because the relatively low number of colonies detected per site precluded a reliable estimation of the size of the missing class (Chapman *et al.* 2003; Darvill *et al.* 2004). Given monogyny and single queen mating, minimum effective population size ($N_e$) was then calculated as 1.5 × number of colonies, since, from Crozier (1979), $N_e$ in social haplodiploids = $(4.5Nmn)/(1 + 2m)$, where $N$ = number of colonies, $m$ = mating frequency and $n$ = number of queens per colony (see also: Ellis *et al.* 2006; Kraus *et al.* 2009).

### *Direct estimates of foraging distance*

During the period of sampling from the Milton 2005 transect (Table 1), some of the 167 workers that were paint-marked as part of the sampling procedure were resighted. The distance travelled by a resighted worker was calculated as the mean of the distance between its resighting location and (a) the nearest and (b) the furthest of the set of positions at which all workers sharing the focal worker's paint-mark (which was specific to one of the six sampling points) were sampled. In addition, 11 workers from the Howmore nest were paint-marked at the nest entrance and the distance between the nest and the location of any resightings of these workers was recorded.

### *Genetic estimates of foraging distance and nest density*

Building on previous methods (Darvill *et al.* 2004; Knight *et al.* 2005), we developed a new technique for estimating foraging distance and nest density from genetic data, which we applied to the Milton 2005 dataset (transect dataset, Table 1). In brief, our method adjusted the proportion of worker pairs classified as sisters to account for false positive and false negative classifications (Step 1). This was to allow the true proportion of sister-pairs to be calculated. We then fitted a parametric model (half-normal) to our dataset to describe the relationship between the proportion of pairs of workers that were sisters and the exact distance between the sampling locations of pair members (as established with GPS), which we term the separation distance (Step 2). Next we performed simulations to determine the effect on this relationship of varying the distribution of workers' foraging distances and nest density (Step 3). Lastly, we compared the simulations with the data to estimate foraging distance and nest density (Step 4). In detail, the steps of the procedure were as follows:

1. Workers were allocated into pairs of putative (full) sisters using KINSHIP 1.2 (Goodnight & Queller 1999). We used KINSHIP 1.2 for this purpose because, unlike COLONY 1.3, it returns pairwise relatednesses associated with a pre-selected Type 1 error rate (rate of false positives, i.e. of classifying females as sisters when they are not). We assumed three false positive error rates, $P < 0.05$, $P < 0.01$ and $P < 0.001$ (where, for example, $P < 0.05$ means that up to 5% of declared sister-pairs are pairs of non-sisters). These error rates, and the rates of the corresponding Type II errors (rate of false negatives, i.e. of classifying genuine sisters as non-sisters), were assigned within KINSHIP 1.2 using 2 000 000 simulations. We defined $p_{sis}'$ as the observed probability that any pair of sampled workers would apparently be sisters rather than non-sisters (according to the KINSHIP 1.2 classification). If $r^+$ = the false positive rate, $r^-$ = the false negative rate and $p_{sis}$ = the true proportion of sister-pairs, we took $p_{sis}'$ to be given by:

$$p_{sis}' = p_{sis}(1 - r^-) + (1 - p_{sis})r^+, \quad (1)$$

from which

$$p_{sis} = (p_{sis}' - r^+)/(1 - r^+ - r^-). \quad (2)$$

2. To model the relationship between $p_{sis}$ and separation distance, we took data for all possible pairings of sampled workers, sorted the pairs into bins of separation distance and then obtained the proportion of pairs ($p_{sis}'$) that were assigned as sisters at each of the three assumed false positive rates. The $p_{sis}'$ values were then transformed to $p_{sis}$ using eqn 2. The results suggested that a negative sigmoid relationship would give a reasonable fit regardless of the false positive rate assumed (Fig. 2). We therefore assumed $p_{sis}$ to be a half-normal function of the separation distance, $d$:

$$p_{sis} = a\exp(-d^2/(2q^2)), \quad (3)$$

where $a$ and $q$ are constants. Larger values of $q$ would imply a slower rate of decrease in $p_{sis}$ with increasing distance. It is possible that other functions would describe this relationship equally well. However, we selected a half-normal function because it has just two parameters, only one of which ($q$) determines the rate of decline of $p_{sis}$ with distance. Other functions with

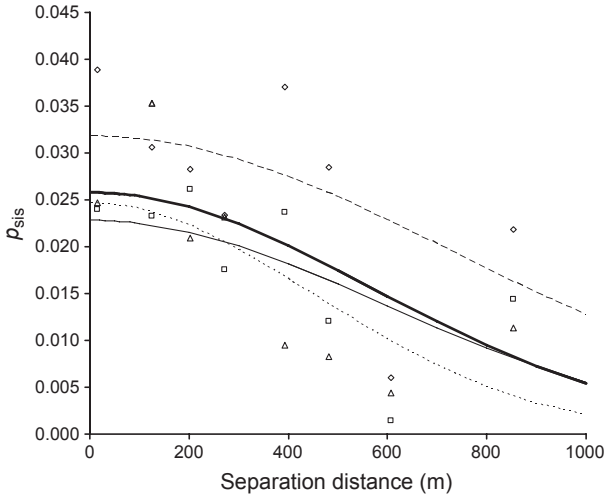


**Fig. 2** Probability that two *Bombus distinguendus* workers are sisters ($p_{sis}$) as a function of their separation distance. Data from the Milton 2005 dataset. Each symbol represents a value of $p_{sis}$ estimated using eqn 2 for a bin of separation distance selected to include at least 1600 pairings of workers. Curves represent half-normal models fitted to data using eqn 4 for three false positive rates ($r^+$) separately or for all rates combined. Dashed line and diamond symbols, $r^+ = 0.05$; thin solid line and square symbols, $r^+ = 0.01$; dotted line and triangular symbols, $r^+ = 0.001$; thick solid line, all rates combined.

this property either predicted negative proportions at large separation distances (linear relationship) or failed to describe the data well (exponential relationship; Fig. 2). Furthermore, simulations described below revealed that assuming a half-normal relationship between the density of foraging workers and distance from the nest, which made estimating nest density tractable, yielded a half-normal relationship between $p_{sis}$ and separation distance. This meant our choice of a half-normal function for the latter relationship was desirable for consistency.

Substituting eqn 3 into eqn 1 yielded:

$$p_{sis}{}' = (a\exp(-d^2/(2q^2)))(1 - r^-) + (1 - (a\exp(-d^2/(2q^2))))r^+. \quad (4)$$

We calculated the binomial log-likelihood of the data under this model and then obtained maximum-likelihood values of $a$ and $q$ using the NONLIN module of SYSTAT 5.03. We fitted this relationship to the sister-pair assignments made by assuming each of the three false positive error rates above and their associated false negative rates. We also fitted the model to the assignments made by combining all three false positive rates by maximizing the sum of their log-likelihoods.

3. To determine how nest density affects the relationship between $p_{sis}$ and separation distance, we performed simulations of sampling workers in a landscape. We simulated a random distribution of nests in a $10 \times 10$ km landscape at a range of different nest densities (1000 replicates per each density value). We assumed that the average number of workers from a given nest, captured at a sampling location at distance $x$ from the nest, was given by a negative sigmoid form approximated by the half-normal expression, $S\exp(-x^2/(2Q^2))$, where $S$ is the expected number of workers captured from a nest when the sampling location is immediately adjacent to it, $x$ is the distance between the nest and the sampling location and $Q$ is a constant. We then calculated the number of workers, from each nest in the landscape, that would be captured at each of seven sampling points distributed every 250 m along a 1.5 km linear transect in the centre of the landscape. We tested various transect lengths and point spacings and found that they substantially affected the simulation outputs only if transect length was much less than, or point spacing much greater than, the average foraging distance (unpublished). For every pairing of sampling points (including each point paired with itself), we calculated the number of possible pairings of different workers and the number of these that were sister-pairs. These numbers were accumulated for each separation distance and the number of sister-pairs as a proportion of all pairings of different workers (i.e. $p_{sis}$) at that separation distance was obtained. Results were averaged over 1000 simulations. Choosing different values of $S$, fixed for a given simulation, did not affect the estimates of $p_{sis}$, and neither did using values of $S$ that varied among colonies within a simulation, obtained by drawing values at random from a normal distribution. Hence, we used $S = 50$ throughout.

4a. We fitted the model defined in eqn 3 relating $p_{sis}$ to separation distance to the simulated data. Plots of simulated $p_{sis}$ against separation distance showed a relationship closely resembling the half-normal. The fitted values of $q$ from the model in eqn 3 were approximately 1.5 times the input value of $Q$ used to generate the simulated data, though the precise ratio varied with the nest density used in the simulation. Analysis of the simulated data also showed a clear relationship between the nest density used in the simulation and the fitted value of $a$ from the model of the simulated data defined by eqn 3. It was found that the nest density input to the model (in nests $km^{-2}$) was closely linearly related to $1/(aq^2)$ across a wide range of simulated nest densities, with the quantity $1/(aq^2)$ derived from the simulations averaging 6.103 times more than the input density. Geometrical considerations (S. T. Buckland, personal communication) predict nest density to be given by $1/(2\pi aq^2)$. Since 6.103 is close to $2\pi$ (6.283), we concluded that nest density could be derived by estimating $a$ and

$q$ from the empirical data via fitting the model in eqn 4 and then calculating nest density as $1/(2\pi aq^2)$.

4b. We next estimated the value of $Q$, which describes the distribution of foraging workers in relation to distance from the nest. Because the precise ratio of $Q$ to $q$ in simulations varied according to nest density, we estimated $Q$ iteratively. We ran simulations with a range of assumed values of nest density and $Q$ until we obtained values of $a$ and $q$ by fitting eqn 3 to the model output that matched those from the data. We took the input value of $Q$ from the final iteration of this procedure to estimate $Q$. The amount of worker foraging activity that occurs in a given distance band around the nest is the product of the average density of worker activity and the area of the band. Hence, the amount of worker activity at a given distance $x$ is proportional to $x\exp(-x^2/2Q^2)$. This function implies that the highest amount of activity occurs at distance $Q$ from the nest (Fig. 3). Therefore, $Q$ estimates the mode of workers' foraging distance. For each estimate of $Q$, we also calculated the expected amount of foraging activity in each of the set of 1 m-wide annuli placed at an increasing distance from the nest at their centre. We then summed these values to give the cumulative proportion of foraging activity contained within a circle with given radius centred on the nest. From this, we obtained the foraging distance within which 95% of the foraging activity of a nest occurs.

## Results

### *Analysis of error rates*

Of 1629 genotypes (from 439 workers) that were retyped, 692 (from 342 workers) were heterozygotes in at least one of the two typing runs. Of these, 11 differed on repeat typing in a manner indicating that either the original or the repeat genotype contained a dropout error, yielding a mean rate of dropout error of $11/(2 \times 692) = 0.8\%$ errors per allele. Twenty-two alleles in the dataset of 1629 genotypes differed in other ways in repeat typing, yielding a mean rate of non-dropout error of $22/(2 \times 1629) = 0.7\%$ errors per allele. These error rates were employed in sibship reconstruction using COLONY 1.3 (see Materials and methods), but, because they were very low, we conclude that genotyping error would not have systematically biased our other analyses.

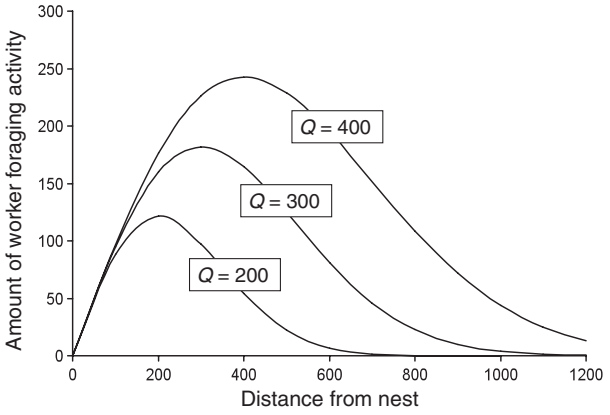


**Fig. 3** Amount of worker foraging activity as a function of distance from the nest ($x$) and $Q$, where amount of worker foraging activity is given by $x\exp(-x^2/2Q^2)$. Maximum activity occurs when $x = Q$. See Materials and methods for further details.

### *Inbreeding, linkage disequilibrium, genetic diversity and bottlenecking*

No locus within any island exhibited evidence for deviation from Hardy–Weinberg equilibrium (all $P > 0.05$, versus Bonferroni-adjusted critical $P = 0.001$). Nor was there any evidence for significant linkage disequilibrium between loci either within islands (all $P > 0.05$, Bonferroni-adjusted critical $P = 0.0003$) or across all islands combined (all $P > 0.07$, Bonferroni-adjusted critical $P = 0.002$). Expected heterozygosity and allelic richness were low overall (Table 2), and varied little in the islands of the Inner and Outer Hebrides, while being slightly higher in the Orkney Islands (Table 2). Inbreeding coefficients were not significantly different from zero within any island (Table 2). Ninety-six of the 97 males sampled (Table 1) were successfully typed, and of these all had genotypes consistent with haploidy.

None of the six islands sampled exhibited evidence for demographic bottlenecking under any of the models

**Table 2** Data on genetic diversity and inbreeding from genotyping of *Bombus distinguendus* workers. Samples within each island were pooled across sites and years (see Materials and methods). Islands represented by the following sites/samples: Balranald, Borve, Garrynamonie, Milton 2004, Ormiclate, Stilligarry, Stoneybridge, Red Rock, The Reef, Brodgar and The Loons (Table 1); hence, mean $N = 27$ colonies (sisterhoods) per island (range, 9–83). None of the inbreeding coefficients differed significantly from zero (all $P > 0.09$)

| Island | Expected heterozygosity ($H_E \pm$ SE) | Allelic richness ($A \pm$ SE) | Inbreeding coefficient ($F_{IS} \pm$ SE) |
|---|---|---|---|
| North Uist | 0.398 ± 0.090 | 2.6 ± 0.4 | −0.184 ± 0.093 |
| Benbecula | 0.384 ± 0.113 | 2.5 ± 0.5 | 0.105 ± 0.097 |
| South Uist | 0.381 ± 0.086 | 2.6 ± 0.4 | 0.052 ± 0.020 |
| Coll | 0.361 ± 0.082 | 2.2 ± 0.2 | 0.060 ± 0.059 |
| Tiree | 0.381 ± 0.085 | 2.5 ± 0.3 | 0.019 ± 0.032 |
| Orkney | 0.439 ± 0.068 | 3.1 ± 0.4 | −0.042 ± 0.057 |
| Mean (range) | 0.391 (0.361–0.439) | 2.6 (2.2–3.1) | 0.002 (−0.184 –0.105) |

**Table 3** Population-genetic structuring in *Bombus distinguendus* between islands of different island groups. Island groups represented by Stoneybridge, Red Rock, The Reef, Brodgar and The Loons (Table 1); $N = 15$ colonies (sisterhoods) per island group. Above diagonal: pairwise $F_{ST}$ values (mean ± SE); below diagonal: pairwise $G'_{ST}$ values (mean ± SE)

| | South Uist | Coll | Tiree | Orkney |
|---|---|---|---|---|
| South Uist | – | 0.101 ± 0.005* | 0.058 ± 0.004* | 0.088 ± 0.006* |
| Coll | 0.160 ± 0.008 | – | 0.006 ± 0.002 | 0.060 ± 0.003* |
| Tiree | 0.092 ± 0.007 | 0.009 ± 0.004 | – | 0.055 ± 0.003* |
| Orkney | 0.149 ± 0.010 | 0.100 ± 0.004 | 0.093 ± 0.005 | – |

* $P < 0.01$ (Bonferroni-adjusted).

for microsatellite mutation (all $P > 0.05$), except for Tiree, for which there was evidence for significant bottlenecking under the IAM model ($P = 0.04$) but not under the TPM ($P = 0.40$) or SMM ($P = 0.45$) models. Overall, this suggested that demographic bottlenecking was largely absent.

### *Genetic differentiation between years, sites and island groups*

The comparison of the Milton 2004 and Milton 2005 samples showed that there was no significant genetic differentiation between them (pairwise $F_{ST} = -0.012$, $P = 0.614$, $N = 14$–$15$ colonies per sample). There was also no significant genetic differentiation between the different sites sampled within the islands of the Outer Hebrides (global $F_{ST}$ (95% confidence limits) = -0.009 (-0.024–0.010), $P = 0.692$, $N = 9$–$10$ colonies per sample). Correspondingly, there was no significant isolation by distance within the Outer Hebrides (Mantel test: $r^2 = 12.2\%$, $P = 0.124$). At a larger scale, there was significant genetic differentiation between all pairs of islands or island groups included, except for Coll and Tiree (Table 3). We did not formally test for isolation by distance at this scale because of the small number of pairwise comparisons available (Table 3).

### *Number of colonies represented within sites*

The minimum number of colonies (full sisterhoods) detected by COLONY 1.3 as being represented within 12 sites (all sites from which workers were sampled excluding the Howmore nest sample) ranged from seven to 42 (Table 1). These values yielded a mean per site effective population size of 25.4 (Table 1). The calculated estimates are minimum effective population sizes because there was no correction for unsampled colonies, and there was a clear dependence, for the sample sizes achieved, of the number of colonies returned by COLONY 1.3 on the number of workers sampled (Spearman's rho = 0.98, $n = 12$, $P < 0.001$; Table 1).

### *Direct estimates of foraging distance*

Six of 167 workers marked on the Milton 2005 transect were resighted, at distances (mean of minimum and maximum distances) 186 m, 197 m, 221 m, 231 m, 399 m and 770 m, respectively, from where they were marked. One of eleven workers marked at the Howmore nest was resighted, at a distance 193 m from the nest.

### *Genetic estimates of foraging distance and nest density*

Analysis of the worker genotypes from the Milton 2005 dataset yielded different estimates of the parameters $a$ and $q$ in eqns 3 and 4 according to which false positive rate was assumed in KINSHIP 1.2 (Table 4). From these fitted values, the quantity $Q$ in the half-normal distribution was determined to be 308–510 m, depending on the false positive rate assumed, with a value of 391m for the analysis based upon pooled data for all three

**Table 4** Estimates of foraging distance and nest density of *Bombus distinguendus* derived from worker genotype data in the Milton 2005 dataset using the model described in Materials and methods

| | Assumed false positive rate $r^+$ | | | |
|---|---|---|---|---|
| | 0.05 | 0.01 | 0.001 | All rates combined |
| $r^-$ | 0.1268 | 0.3607 | 0.6781 | – |
| $a$ | 0.0318 | 0.0228 | 0.0247 | 0.0258 |
| $q$ | 738 | 593 | 449 | 566 |
| $Q$ | 510 | 407 | 308 | 391 |
| 95% radius | 1246 | 994 | 752 | 955 |
| Nest density | 9.2 | 19.9 | 32.0 | 19.3 |

Estimates are shown for three different assumed values of the false positive rate $r^+$ and for all three rates considered together. $r^-$, false negative rate; $a$ and $q$, estimated parameters from model; $Q$, quantity (in metres) describing the half-normal relationship between the density of foraging workers and distance from the nest; 95% radius, radius around the nest (in metres) expected to contain 95% of the foraging activity of the colony; nest density, estimated nest density (nests km$^{-2}$).

false positive rates (Table 4). Our best estimate for the modal foraging distance of workers was therefore 391 m. The radius of a circle that contains 95% of the foraging activity of a colony was estimated at 752–1246 m, depending on the false positive rate assumed, with a value of 955 m for the estimate based upon all three false positive rates (Table 4). The analysis also yielded estimates for nest density of 9.2–32.0 nests $km^{-2}$, depending on the false positive rate assumed. The analysis based upon pooled data for all three false positive rates gave an estimate of 19.3 nests $km^{-2}$ (Table 4).

## Discussion

### *Population genetics of scarce or declining bumblebee species*

We found that populations of the scarce and declining bumblebee, *Bombus distinguendus*, in Scotland, UK, were characterized by low levels of genetic diversity (low expected heterozygosity and allelic richness), by lack of conclusive evidence for inbreeding (but see below), by absence of substantial demographic bottlenecking, and by population substructuring (genetic differentiation between sites) at large scales (between island groups, *c.* 100+ km) but not small scales (within island groups, 10s of km). The minimum effective population size at our sampling sites was *c.* 25 on average.

These results show that *B. distinguendus* shares several genetic traits found in the handful of other scarce or declining bumblebee species whose conservation genetics have been investigated (Table 5). Although the relevant studies need comparing with caution because of different methods and sample sizes, it is evident that these species share low levels of genetic diversity and small effective population sizes (Table 5). One or more of these species also exhibits or exhibit above-zero frequencies of diploid males, small-scale population substructuring even among mainland populations (e.g. Lozier & Cameron 2009), and demographic bottlenecking (Table 5). By contrast, population-genetic analyses of widespread and common *Bombus* species typically reveal populations with high levels of genetic diversity, panmixis, absence of diploid males and large effective population sizes (Estoup *et al.* 1996; Widmer & Schmid-Hempel 1999; Chapman *et al.* 2003; Shao *et al.* 2004), with population substructuring and bottlenecking being confined to island populations (Estoup *et al.* 1996; Widmer *et al.* 1998; Shao *et al.* 2004). An exception is a set of non-island populations of *B. pascuorum* in Germany, which exhibited inbreeding and small-scale population substructuring (Herrmann *et al.* 2007). In scarce or declining bumblebee species, it is possible that low population sizes generate inbreeding even if mating is

**Table 5** Data (means and ranges, unless otherwise stated) from genetic studies of scarce or declining bumblebee (*Bombus*) species

| Trait | *B. distinguendus* | *B. florilegus* | *B. muscorum* | *B. pensylvanicus* | *B. sylvarum* |
|---|---|---|---|---|---|
| Expected heterozygosity ($H_E$)* | 0.391 (0.361–0.439) | 0.51 (0.27–0.69) | 0.443 ± 0.014† | 0.584 (0.578–0.591) | 0.39 ± 0.02‡ |
| Allelic richness (*A*) | 2.6 (2.2–3.1) | No data | 3.22 ± 0.06† | 4.82 (4.74–4.93) | 3.12 ± 0.10‡ |
| Inbreeding coefficient ($F_{IS}$) | 0.002 (-0.184–0.105) | No data | 0.01 ± 0.01‡ | No data | No data |
| Diploid males (DM) | 0 of 96 males | In 4 of 14 nests | 2 of 41 males | No data | 1 of 39 males |
| Population substructuring | Large scale | No data | Both scales | Small scale | Large scale |
| Demographic bottlenecking (DB) | 1 of 6 islands | No data | 10 of 16 islands | No data | 1 of 5 sites |
| Effective population size ($N_e$), *N* sites | 25.4 (12.0–63.0)§, 12 | No data | No data | 17.0 (15.0–19.5), 3 | 55.2 (39.0–72.0), 7 |
| Reference; notes | Present study | Takahashi *et al.* (2008a) | Darvill *et al.* (2006)¶; Hebridean data only ($H_E$, *A*, DM, DB) | Lozier & Cameron (2009); 2008 data only (all) | Ellis *et al.* (2006); UK data only ($H_E$, *A*, DB, $N_e$) |

*Observed heterozygosity in *B. florilegus*.
†SD.
‡SE.
§Minimum estimate, as described in Materials and methods.
¶See also expanded dataset of Darvill *et al.* (2010).

indiscriminate (because a comparatively high proportion of potential mates are relatives if population size is small). This could account for inbreeding coefficients of zero (since $F_{IS}$ is zero whenever mating is random; Keller & Waller 2002) and low genetic diversity in these species (Table 5). Hence, in *B. distinguendus*, inbreeding may be present due to small population size, with our failure to detect diploid males having arisen through sampling error. This would be consistent with *B. distinguendus* having the lowest mean level of allelic richness yet found in scarce or declining bumblebees (Table 5). However, apart from low allelic richness and small effective population sizes (although recall our estimates were minima), we found no other evidence of inbreeding. Our overall conclusion from comparison of our findings with previous studies of bumblebees (Table 5) is that rarity and population decline in this group are accompanied by some or all of loss of genetic diversity, increased frequency of diploid males, increased population substructuring and reduced effective population size. This is in line with theoretical expectation and also matches conclusions from other taxa (see Introduction).

### *Foraging distance and nest density*

Our method of estimating foraging distance and nest densities from the genetic data extended previous methods but could be improved by, for example, explicitly modelling variation in foraging distance and forage density over both space and time. In addition, the effects of altering the number of loci sampled and the level of per-locus polymorphism on these estimates remain to be fully explored. However, note that our estimates of foraging distance empirically reflected the average of temporal variations in foraging distance occurring over the sampling period (*c.* 1 month of sampling of Milton 2005 workers; Table 1). Our genetic estimate of the modal foraging distance of *B. distinguendus* workers on coastal grassland was 391 m, with 95% of foraging activity occurring within 955 m of the nest. Direct observation also demonstrated that marked, foraging workers may fly many hundreds of metres. Comparison with other estimates of worker foraging distances in bumblebees, both direct and indirect (Table 6), shows that the estimated foraging distance of *B. distinguendus* lies within the range reported for other bumblebees, with a suggestion that it may be higher than average. This perhaps stems from the open nature of the coastal habitat in which UK populations of *B. distinguendus* largely occur. Our genetic estimate of *B. distinguendus* nest density was *c.* 19 nests $km^{-2}$. Of 14 genetic density estimates in five bumblebee species taken from the literature and the present study (Table 7), this value is the fourth lowest. Note, however, that density estimates from some populations of common species (e.g. *B. pascuorum*, *B. terrestris*) are even lower and that some previous methods returned varying densities depending on the foraging distance employed in calculating density (Table 7). Nonetheless, low density in *B. distinguendus* is consistent with the species appearing never to have been abundant even in its former UK range and with its still being one of the scarcest bumblebees in its remaining Scottish strongholds. For example, *B. distinguendus* workers represented 2.7% and 3.7% of all worker bumblebee records on Coll and South Uist, respectively (Charman 2007).

### *Implications for conservation*

*B. distinguendus* populations in the UK appear to rely on the continued presence of flower-rich, unimproved

**Table 6** Estimated foraging distances of worker bumblebees (*Bombus*). See also Zurbuchen *et al.* (2010)

| Species | Foraging distance (m) | | Method | Reference |
|---|---|---|---|---|
| | Average* | Maximum | | |
| *B. distinguendus* | 391 | 955[†] | Genetic markers | Present study |
| *B. lapidarius* | – | 450 | Genetic markers | Knight *et al.* (2005) |
| | 260 | 1,500 | Direct (marked workers) | Walther-Hellwig & Frankl (2000) |
| *B. muscorum* | 55 | 125 | Direct (marked workers) | Walther-Hellwig & Frankl (2000) |
| *B. pascuorum* | – | 449 | Genetic markers | Knight *et al.* (2005) |
| *B. pratorum* | – | 674 | Genetic markers | Knight *et al.* (2005) |
| *B. terrestris* | 275 | 631 | Direct (radar tracking) | Osborne *et al.* (1999) |
| | – | 758 | Genetic markers | Knight *et al.* (2005) |
| | 267 | 800 | Direct (marked workers) | Wolf & Moritz (2008) |
| | – | 1,500 | Direct (marked workers) | Osborne *et al.* (2008a) |
| | 663 | 1,750 | Direct (marked workers) | Walther-Hellwig & Frankl (2000) |

*Averages are means except for *B. distinguendus*, where value given is the estimated mode
[†]95% of foraging activity estimated to occur within this distance from the nest.

**Table 7** Nest densities estimated using genetic markers in bumblebees (*Bombus*)

| Species | Density (nests km$^{-2}$) | Landscape type, location | Reference |
|---|---|---|---|
| *B. distinguendus* | 19 | Coastal grassland, South Uist, UK | Present study |
| *B. lapidarius* | 117 | Agricultural, Hertfordshire, UK | Knight *et al.* (2005) |
| *B. pascuorum* | 8 | Agricultural, Lower Saxony, Germany | Herrmann *et al.* (2007) |
| | 35 | Agricultural, Hertfordshire, UK | Knight *et al.* (2009)* † |
| | 68 | Agricultural, Hertfordshire, UK | Knight *et al.* (2005) |
| | 104 | Urban, UK | Chapman *et al.* (2003)‡ |
| | 173 | Agricultural, Hertfordshire, UK | Knight *et al.* (2009)* † |
| | 193 | Agricultural, Hampshire, UK | Darvill *et al.* (2004) |
| *B. pratorum* | 26 | Agricultural, Hertfordshire, UK | Knight *et al.* (2005) |
| *B. terrestris* | 13 | Agricultural, Hampshire, UK | Darvill *et al.* (2004) |
| | 15 | Urban, Germany | Kraus *et al.* (2009)‡§ |
| | 29 | Agricultural, Hertfordshire, UK | Knight *et al.* (2005) |
| | 38 | Urban, Germany | Kraus *et al.* (2009)‡§ |
| | 53 | Urban, UK | Chapman *et al.* (2003)‡ |

*Two estimates differ depending on foraging distance employed in calculating density.
†Same location as, but different year to, study of Knight *et al.* (2005).
‡Calculated for this table from data on estimated numbers of colonies at point samples in cited references combined with foraging distance estimates of Knight *et al.* (2005).
§Estimates from same site in successive years.

grassland that provides floral resources throughout the colony cycle (June to September) and contains, or is close to, suitable sites for nesting, mating and hibernation. Management aimed at maintaining these conditions would therefore be the best way of ensuring the persistence of these populations (Charman 2007; Charman *et al.* 2009). The present study shows that there is no conclusive evidence that these populations are in worse 'genetic health' than populations of other scarce and declining bumblebees (Table 5). However, as discussed above, *B. distinguendus* shares with scarce and declining congeners a number of genetic traits of declining species, and its low genetic variation may testify to inbreeding effects. Another caveat is that our study concentrated on sites known to hold *B. distinguendus* in some numbers, and hence our conclusions may not extend to other, smaller and more peripheral populations. However, the lack of small-scale population substructuring suggests that many *B. distinguendus* populations are genetically connected within metapopulations, with gene flow being maintained at least over tens of kilometres (e.g. between sites within the Outer Hebrides) or across open sea (e.g. between Coll and Tiree). If *B. distinguendus* does exist in a set of metapopulations, this would offset the potential risk created by its subpopulations exhibiting low levels of genetic diversity and low effective population sizes. Correspondingly, any habitat management should be carried out in the knowledge that nests are likely to occur only at low density, and that workers forage on a large scale. This suggests that a few (but not too widely separated) large patches of suitable habitat would meet the requirements of *B. distinguendus* better than a greater number (but equal total area) of smaller patches of suitable habitat. This is because, assuming small patches were isolated within unfavourable habitat, both population sizes of *B. distinguendus* and density of available forage might be critically low within small patches. Finally, any translocations of *B. distinguendus* in future, which would potentially be used to supplement declining subpopulations, would need to be carried out bearing in mind the between-population genetic differentiation at large scales detected by this study.

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