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Landscape effects on extremely fragmented populations of a rare solitary bee, *Colletes floralis*

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Abstract

Globally, there is concern over the decline of bees, an ecologically important group of pollinating insects. Genetic studies provide insights into population structure that are crucial for conservation management but that would be impossible to obtain by conventional ecological methods. Yet conservation genetic studies of bees have primarily focussed on social species rather than the more species-rich solitary bees. Here, we investigate the population structure of *Colletes floralis*, a rare and threatened solitary mining bee, in Ireland and Scotland using nine microsatellite loci. Genetic diversity was surprisingly as high in Scottish (Hebridean island) populations at the extreme northwestern edge of the species range as in mainland Irish populations further south. Extremely high genetic differentiation among populations was detected; multilocus F_{ST} was up to 0.53, and G'_{ST} and D_{est} were even higher (maximum: 0.85 and 1.00, respectively). A pattern of isolation by distance was evident for sites separated by land. Water appears to act as a substantial barrier to gene flow yet sites separated by sea did not exhibit isolation by distance. *C. floralis* populations are extremely isolated and probably not in regional migration-drift equilibrium. GIS-based landscape genetic analysis reveals urban areas as a potential and substantial barrier to gene flow. Our results highlight the need for urgent site-specific management action to halt the decline of this and potentially other rare solitary bees.

Keywords: conservation, dispersal, landscape genetics, management unit, mining bee, population structure

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Introduction

Bees perform a vital ecosystem service, acting as pollinators of both wild flora and numerous crops of commercial value (Kearns *et al.* 1998; Kremen *et al.* 2007). Animal-mediated pollination, the majority of which is carried out by bees, accounts for 35% of global foodstuffs (Klein *et al.* 2007; Kremen *et al.* 2007) and has been shown to increase the size, quality or yield of 70% of the world's most important crops (Ricketts *et al.* 2008). The global economic value of pollination, by both commercial and wild species, is estimated to range between $112 and 200 billion annually (Kremen *et al.* 2007).

Recently, there has been much concern, and documented evidence, regarding the global decline of bees (Williams 1982; Steffan-Dewenter *et al.* 2005; Biesmeijer *et al.* 2006; Fitzpatrick *et al.* 2007; Brown & Paxton 2009; Williams & Osborne 2009; but see Ghazoul 2005). The primary deterministic factors driving bee population decline are considered to be habitat degradation and fragmentation, wrought in large part by agricultural intensification and other forms of land use (Ellis *et al.*

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2006; Greenleaf & Kremen 2006a; Klein *et al.* 2007; Kremen *et al.* 2007; Knight *et al.* 2009). Other major factors impacting biodiversity, such as invasive species, parasites, disease and climate change (Memmott *et al.* 2007; Tylianakis *et al.* 2008), also negatively affect bee species diversity (Brown & Paxton 2009).

Population genetic analyses have become important tools in the conservation of threatened species. For rare species, molecular tools may be the only possible method for investigating population connectivity and dispersal and for the appropriate designation of management units (MUs) to aid conservation (Frankham *et al.* 2002; Waples & Gaggiotti 2006; Palsboll *et al.* 2007). Genetic diversity, fundamental for long-term survival, is a key parameter for prioritizing areas or populations for urgent management action (Bonin *et al.* 2007; Jost 2008; Zayed 2009). Furthermore, estimates of genetic differentiation and gene flow between populations are essential to develop management plans that maintain population connectivity (Kremen *et al.* 2007). These arguments are especially relevant for insects such as bees that are often small yet fast flying and difficult to mark and monitor directly. Yet despite the fact that over 90% of the 19 500 currently described bee species are solitary (Michener 2007), most population genetic studies of bees have focussed on the eusocial bumble bees (Estoup *et al.* 1996; Widmer *et al.* 1998; Widmer & Schmid-Hempel 1999; Darvill *et al.* 2006; Ellis *et al.* 2006; Murray *et al.* 2009; Zayed 2009; but see Paxton *et al.* 1996; Beveridge & Simmons 2006; Zayed & Packer 2007; Exeler *et al.* 2008, 2010) that may differ markedly in terms of gene flow and population connectivity from the usually much smaller and potentially more sedentary solitary bees. In addition, solitary bees also play an important role in the provision of pollination services, either directly (Winfree *et al.* 2007, 2008) or indirectly through behavioural interactions with social bee species (Greenleaf & Kremen 2006b).

Many threatened bee species exist in small, isolated populations and are of increasing conservation concern as they are less likely to be able to adapt to changes in their environment (Ellis *et al.* 2006; Zayed 2009). One such solitary species is *Colletes floralis* (Hymenoptera: Colletidae), currently distributed around coastal areas of Ireland and in the north and west of Scotland, particularly the Western Isles (Inner and Outer Hebrides; see Fig. 1). Up to 90% of the world's Atlantic zone, *C. floralis* is thought to be located in the coastal areas of Ireland and the species is listed as vulnerable in Ireland's red data list of bees (Fitzpatrick *et al.* 2006) and 'rare' in Britain, where it is the subject of a so-called Biodiversity Action Plan (Falk 1991; Hunter 2006). Possible reasons for the decline of *C. floralis* are the loss of herb-rich grassland through agricultural intensification and the abandonment of traditional grazing practices, habitat fragmentation and climate change (Environment & Heritage Service, Northern Ireland 2006). In addition, coastal sand dunes, which are nowadays the principal habitat for *C. floralis* around the British Isles, have declined and as such have been designated a priority habitat in Annex 1 of the European Community habitats and species directive (JNCC 1999).

Landscape genetics is a relatively new field that integrates landscape ecology and population genetics to quantitatively examine the impact of landscape parameters on genetic factors such as gene flow and genetic differentiation (Manel *et al.* 2003). For the conservation of a species, especially those that are rare or exist in isolated populations, it is crucial to examine how ecological barriers affect the ease of movement, and therefore gene flow, of species through a given landscape (Leclerc *et al.* 2008). Integrating landscape genetics with traditional population genetic analyses can therefore greatly enrich conservation genetic analyses. The findings of such studies (for example Spear *et al.* 2005; Lada *et al.* 2008; Leclerc *et al.* 2008; Pérez-Espona *et al.* 2008; Spear & Storfer 2008; Wang *et al.* 2008, 2009) can have important implications for the management and, ultimately, the long-term persistence of a species.

Here, we utilize microsatellite markers to study the population genetic structure of *C. floralis* in Ireland and Scotland. We had the following two main aims: (i) to investigate population differentiation and (ii) to determine the impact of landscape on the population genetic structure of this threatened insect by integrating geographic information systems (GIS)-based landscape analysis with our population genetic data. Specifically, we examined whether genetic differentiation reflected isolation by distance (IBD) or rather the role of particular landscape barriers, of which we examined six: agricultural land; beaches, dunes and sand; natural grassland; semi-natural vegetation; urban land; and woodland. This information will help in the designation of specific MUs and reserve design for the appropriate conservation management of this rare bee species by determining (i) the level of genetic differentiation/gene flow between populations (ii) whether certain land classes are more permeable to gene flow for *C. floralis* and (iii) the scale at which management should be applied.

Materials and methods

Study system

Colletes floralis is a medium-sized (females: 9–12 mm body length) univoltine bee nowadays associated with flower-rich sand dune systems in Britain and Ireland (Falk 1991). *C. floralis* is a sub-boreo-alpine species that

is found in limited numbers in alpine areas such as the Pyrenees, the Alps and further east into central Asia, and in coastal Baltic areas (Kuhlmann *et al.* 2007). However, up to 90% of the world's Atlantic zone population is believed to be located in the coastal areas of Ireland and in the north and west of Scotland, particularly the Western Isles (Fitzpatrick *et al.* 2006). Adults fly between May and August, when mating, foraging and nest provisioning are carried out. Males patrol nesting sites in search of receptive females, while females are thought to mate soon after emergence and then set about constructing a fossorial nest, as is typical for solitary bee species (Paxton 2005). Nests are found in sandy soil and often in close proximity, forming aggregations (containing from 2 to 100 000 nests) that may be separated from the next aggregation by kilometres of unsuitable habitat. Females construct subterranean brood cells at the end of their tunnels, provision them with pollen and regurgitated nectar or honey (Sommeijer *et al.* 2009) and finally seal them once an egg has been laid in an individual cell.

Sampling regime

C. floralis was collected from 12 localities around the coast of Ireland and Scotland (Fig. 1; Table 1) from the only known sites with adequate bee abundance to allow sufficient sampling. Given that nests are dispersed and difficult to locate at any one site, we could not estimate the absolute number of nests (i.e. reproducing females) per site or population size. However, bees were collected from at maximum a 500 m stretch of coastline.

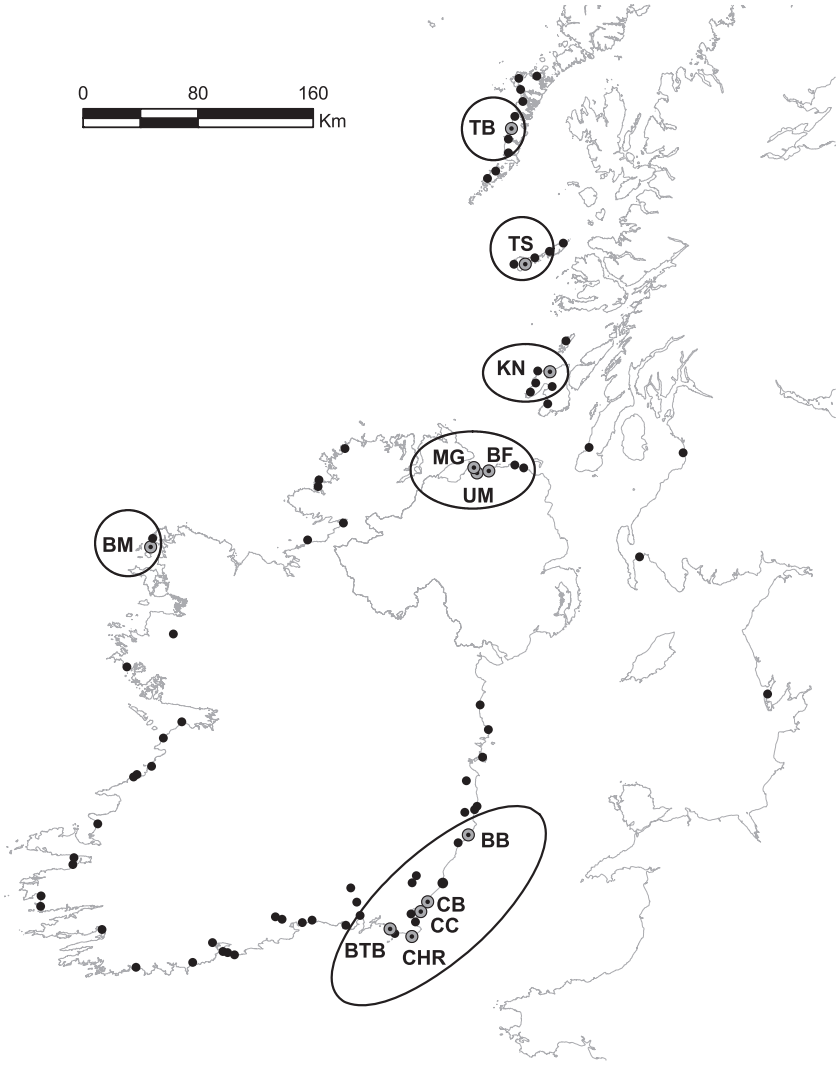

Fig. 1 Historical distribution of *Colletes floralis* in Britain and Ireland, with the 12 sample sites highlighted in grey. Population acronyms correspond to the site codes specified in Table 1. The six groupings shown above correspond to Structure results.

Table 1 Sample sites, date, sample sizes and diversity measures at nine microsatellite loci

Site code	Location	Coordinates	Date	Sample size	H_o	H_e	A_r
BB	Brittas Bay, Wicklow, Ireland	52°53′21″N 06°03′23″W	04–11/07/2005	29♀	0.53	0.51	2.92
BF	Bushfoot Strand, Antrim, Northern Ireland	55°13′11″N 6°31′56″W	28/06/2009	60♂	n.a.	0.26	2.07
BM	Belmullet, Mayo, Ireland	54°09′23″N 10°05′39″W	18/07/2005	30♀	0.57	0.57	4.09
BTB	Ballyteige Burrow, Wexford, Ireland	52°11′47″N 06°37′46″W	31/07–01/08/2007	15♀	0.51	0.54	2.78
CB	Curracloe Beach, Wexford, Ireland	52°23′01″N 06°21′49″W	13/07/2005	36♀	0.52	0.52	3.17
CC	The Raven at Curracloe, Wexford, Ireland	52°20′51″N 06°21′43″W	31/07/2007	24♀	0.49	0.50	3.08
CHR	Chour, Wexford, Ireland	52°10′54″N 06°23′40″W	01/08/2007	28♀	0.52	0.58	3.23
KN	Killinallan, Islay, Inner Hebrides, Scotland	55°51′55″N 06°19′01″W	08–21/07/2008	29♀	0.47	0.57	4.20
MG	Magilligan, Londonderry, Northern Ireland	55°10′17″N 6°54′16″W	30/06/2009	59♂	n.a.	0.24	2.10
TB	Tobha Mor, South Uist, Outer Hebrides, Scotland	57°18′14″N 07°23′37″W	12/07/2007	60♂	n.a.	0.51	3.22
TS	Crossapol, Tiree, Inner Hebrides, Scotland	56°29′31″N 06°52′05″W	02/07/2005	69♂	n.a.	0.58	3.67
UM	Umbra, Londonderry, Northern Ireland	55°09′50″N 06°51′38″W	12–24/07/2008	25♀	0.34	0.35	3.38

H_o, observed heterozygosity; H_e, expected heterozygosity (with Levene's correction); A_r, allelic richness (based on a minimum sample size of the equivalent of 15 diploid individuals); n.a., not applicable.

A resurvey during 2008 and 2009 of historical coastal and inland sites (Fig. 1) found that the species was locally extinct at over half of historically occupied sites (Davis, unpublished), including all historical inland sites. Adults were caught using an insect net and, when possible, non-lethal tarsal samples of the mid-leg were taken, which was obligatory at some sites because of protective legislation (Holehouse *et al.* 2003). Samples were placed in 99% ethanol and stored at 4°C until DNA extraction. In general, 30 (diploid) females were sampled at each site. However, at some sites, 60 (haploid) males were sampled, which was necessary because of the rare nature of the species. This compromised subsequent analyses of inbreeding and bottlenecking at those sites where males were sampled. Sample sizes were reduced at one locality, Ballyteige Burrow (Table 1), as bees were at very low abundance, probably because of small population size; across sites, the mean sample size is equivalent to 28 diploids.

DNA extraction and amplification

DNA was extracted from thoracic tissue or tarsal samples using a high-salt protocol (Paxton *et al.* 1996). Extracts were amplified in 10 μL reaction volumes using nine primers specifically designed for the species: cf1, cf2, cf10, cf11, cf12, cf13, cf14, cf16 and cf17, and reactions were carried out as described in Murray *et al.* (2009 in Molecular Ecology Resources Primer Development Consortium *et al.* 2009; available from http://tomato.biol.trinity.edu/). Products were resolved on 6% polyacrylamide sequencing gels (8 M urea), visualized by autoradiography and scored independently by ESD (all sites), TEM (four sites) and RJP (the remaining eight sites). Repeated amplification of *c.* 5% of the individuals gave identical results at all loci, suggesting that rates of allele mis-calling for all loci were extremely low.

Genetic diversity

Microsatellite data were checked for null alleles, large allele dropout and scoring errors using MicroChecker version 2.2.3 (Van Oosterhout *et al.* 2004). Tests for departure from Hardy–Weinberg equilibrium and genotypic linkage disequilibrium were carried out using GENEPOP web version 3.4 (Raymond & Rousset 1995), applying a sequential Bonferroni correction to minimize type 1 errors. For each population, the observed heterozygosity (H_o: diploid data set only), expected heterozygosity (H_e) and allelic richness (A_r) corrected for equal

sample size were calculated using microsatellite analyser (MSA) version 4.05, which accepts both haploid and diploid individuals in the same dataset (Dieringer & Schlötterer 2003). These measures of genetic diversity were compared across populations using ANCOVA, controlling for the differences in the variability of each locus by using mean locus H_o as a covariate (Petit *et al.* 2005). The inbreeding coefficient, F_{IS}, across and within populations was calculated with FSTAT 2.9.3.2 (Goudet 1995).

Population structure

Levels of genic differentiation (allelic frequency differences) were assessed by an exact test using GENEPOP and the *F*-statistic estimator F_{ST} (Weir & Cockerham 1984) using MSA. R_{ST} was calculated using SPAGeDi version 1.2 (Hardy & Vekemans 2002) and compared with F_{ST} values. However, no consistent difference between F_{ST} and R_{ST} was found and therefore analyses were restricted to F_{ST}.

There is concern over the use of F_{ST} and its analogues because of the frequent underestimation or nondetection of genetic differentiation at highly variable loci (Hedrick 2005; Meirmans 2006; Jost 2008). This problem can be overcome by using Hedrick's (2005) standardized measure of genetic differentiation, G'_{ST}, that takes into account the locus-specific levels of genetic variation within each population and rescales differentiation to between 0 and 1. Another measure is Jost's (2008) actual differentiation, D_{est}, that overcomes the same problem by calculating genetic differentiation independently of variation among subpopulations in allele frequencies. Rather, D_{est} is based on the proportion of alleles that are unique to a subpopulation; Jost (2008) suggests that it replaces conventional measures of genetic differentiation. G'_{ST} was calculated for population pairs using MSA, and correlated with D_{est}, estimated using SPADE (Chao & Shen 2003), which also corrects for bias caused by small sample sizes (Jost 2008).

Population structure was also examined using Bayesian model–based clustering, as implemented in Structure version 2.2 (Pritchard *et al.* 2000). This approach assigns individuals to one or more groupings based on their genotypes. The number of populations (*K*) was varied between $K = 1$–13, one more than the number of sampling sites, using a burn-in of 10 000 repetitions, followed by 100 000 repetitions. Each value of *K* was run five times to ensure consistency of the results, after which the posterior probability of each *K* value was calculated.

Population bottlenecks

We tested for recent reduction in effective population size as a decrease in allelic richness for a given level of heterozygosity using the Wilcoxon signed rank statistic, as implemented in Bottleneck version 1.2.02 (Cornuet & Luikart 1996) and with 10 000 iterations. Three mutational models were examined, the infinite alleles model (IAM), the stepwise mutational model (SMM) and the two-phase mutational model (TPM; with the variation in multistep changes set at 10%). As we did not have a reliable means of estimating the number of bees at a site, it was not possible to relate bottleneck statistics with current population size.

Isolation by distance

Tests of IBD were carried out using Genepop. Rousset's (1997) distance $F_{ST}/(1 - F_{ST})$ was plotted against geographic distance and tested for significance using a Mantel test with 10 000 randomizations. Two separate measures of geographic separation were used: (i) the logarithm of the Euclidean (straight line) geographic distance, accounting for dispersal in two dimensions and (ii) the coastal distance between sites, accounting for dispersal in one dimension. This latter measure was investigated to account for the apparent coastal restriction of suitable habitat for the species. Coastal distances were estimated from Google Earth 5.0 (Google Corporation 2009) using a 'broken-stick' method, which uses a series of straight lines fitted to the coastline to estimate distance. As there is no information available on solitary bee dispersal distances, three different sizes of break of sea (e.g. for inlets) were allowed along the coastal route. Darvill *et al.* (2006) found that *Bombus muscorum* populations in the Western Isles showed marked population structuring for islands more than 10 km apart. This was used as an upper limit as *C. floralis* is smaller and therefore less likely to be able to disperse as far as *B. muscorum* over sea. A lower limit of 0.4 km was set using the mean foraging distance of similar sized solitary bees (Gathmann & Tscharntke 2002), as dispersal distances are potentially at least as large as foraging distances. The third value of 5.2 km was calculated as the mean of the first two values (Table S1, Supporting information). When calculating coastal distances, an inlet <10, 5.2 or 0.4 km in width for the three estimates was considered traversable for the species. Conversely, a larger inlet was followed inland until its width became traversable. However, where this was not possible (e.g. between Ireland and the Western Isles), the shortest possible sea distance was used (% distance over sea given in Table S1, Supporting information).

In addition, the effect of open ocean on genetic differentiation $[F_{ST}/(1 - F_{ST})]$ between population pairs was examined using a partial Mantel test, carried out with ZT (Bonnet & Van de Peer 2002), controlling for

geographic (Euclidean) distance and using 10 000 randomizations. In this case, population pairs were divided into two groups: those separated at some point by >10 km of sea (i.e. among the Western Isles, between Irish and Western Isles locations) and those separated only by land (i.e. within Ireland).

Landscape genetics

The effect of landscape parameters on population structure was investigated by combining GIS-based techniques with genetic analysis of population differentiation for the nine populations in Ireland following the methodology of Pérez-Espona *et al.* (2008). ArcGIS® 9.3 (ESRI) was used to compute landscape-scale habitat parameters from the Corine Land Cover Map 2000 (CLC2000) of Ireland. Environmental parameters were chosen intuitively based on landscape characteristics that were likely to influence *C. floralis* dispersal. Six land classes were investigated: agricultural land; beaches, dunes and sand; natural grassland; semi-natural vegetation; urban land; and woodland. A raster dataset was created of 250 m^2 grids representing a single land class (if more than one was contained within a single square, the one that constituted the largest proportion of the square was used). This resolution was used as a trade-off between fine resolution and computing time, as over 1.3 million pixels were contained within the resulting raster dataset of Ireland.

The cost of travelling through each land class was then investigated by assigning a range of fixed values to every cell on the raster grid representing the difficulty of travelling through a land class. Only one land class was investigated at a time (by assigning costs of <1 or >1), while every other land class was assigned a basic cost of 1. A cost of <1 indicates that the land class facilitates gene flow, while a cost >1 suggests that the land class acts as a barrier to gene flow. As there is a lack of information on how landscape features affect *C. floralis* dispersal, we investigated six arbitrary values, as approximations of cost values, for each land class: 0.001, 0.01, 0.1, 10, 100 and 1000. The ArcGIS® 9.3 spatial analyst function, cost-weighted distance, was then used to calculate the minimum travel costs (along the least-cost pathway) between every population pair for every arbitrary cost value for each land class.

The relationship between cost-weighted distance between every population pair and pairwise population differentiation, estimated as F_{ST}, was investigated using linear regression. The best fit cost value for each land class was determined from the r^2 values, whereby the highest r^2 value indicates the cost value that accounts for most of the variation in the pairwise population differentiation values and maximizes the relationship between genetic differentiation and the land class of interest. To ensure that the r^2 values were not biased because of sampling error, a jackknife procedure was also conducted following the methodology of Pérez-Espona *et al.* (2008). All r^2 values were recalculated, omitting one of the nine Irish populations in turn. Pseudovalues, which are estimates of the bias encountered because of interpopulation differences, were calculated and used to perform *t*-tests to investigate whether the original r^2 values produced were unbiased (indicated by significant *t* values) and therefore representative of the entire study area (Pérez-Espona *et al.* 2008).

Results

Genetic diversity

In total, 216 *Colletes floralis* females and 248 *C. floralis* males from 12 populations were genotyped at nine microsatellite loci. One female did not amplify at all loci, while one male and one female did not amplify at two loci each, giving an estimated rate of nonamplification of 0.12%. Loci were not in significant linkage disequilibrium for all pairwise comparisons between loci and across populations ($P > 0.05$).

Expected heterozygosity (H_e) ranged from 0.24 to 0.58 (Table 1) and differed subtly between populations (ANCOVA: $F = 5.138$; d.f. = 11; $P < 0.001$). Expected heterozygosity was extremely low at the three sites in Northern Ireland, even though at one of these sites, Umbra, allelic richness was high (Table 1). Surprisingly, the Scottish island and other Irish mainland sites exhibited similar levels of heterozygosity (Table 1). Allelic richness (A_r) ranged between 2.07 and 4.20 and also varied significantly between populations (ANCOVA of log (A_r): $F = 4.546$; d.f. = 11; $P < 0.001$); Scottish island populations exhibited high A_r relative to Irish mainland sites. These patterns were consistent across loci (Table S2, Supporting information).

Global F_{IS} was found to be subtly but significantly different from zero ($F_{IS} = 0.045 \pm 0.031$; 95% confidence intervals). Generally, population F_{IS} values were not found to differ significantly from zero and populations were not out of Hardy–Weinberg equilibrium (Table S3, Supporting information), with the exception of the Killinallan site on Islay, where significant deviation (lack of heterozygotes) was seen at five of the nine loci and an excess of heterozygotes at one of the nine loci (Table S3, Supporting information).

Population structure

Genic differentiation (allelic frequency differences) for each population pair across all loci was found to be

highly significant ($P < 0.001$), apart from the two closest population pairs in SE Ireland: Ballyteige Burrow and Chour ($P = 0.40$) and Curracloe Beach and The Raven ($P = 0.14$). These population pairs were located relatively close to one another: 16.2 and 4 km, respectively (Fig. 1; Table 2).

Considerable genetic differentiation was found between other population pairs, with extremely large values of F_{ST} and G'_{ST} (global $F_{ST} = 0.3398$, $P < 0.0001$; global $G'_{ST} = 0.6608$, $P = 0.0001$; see Table 2). Only between two population pairs (Ballyteige Burrow and Chour; Curracloe Beach and The Raven) was F_{ST} not significantly different from zero (Table 2), mirroring the results of the exact tests for genic differentiation. Other populations located relatively close together on the SE coast of Ireland were generally found to have significant but low pairwise F_{ST} values ($F_{ST} < 0.07$ for each population pair). However, for every other population pair, population differentiation was extremely high ($F_{ST} = 0.14$–0.53; Table 2). All three Northern Irish sites were found to be exceptionally highly differentiated from every other population. Within these three Northern Irish sites, F_{ST} values ranged from 0.17 to 0.26, while F_{ST} values ranged between 0.37 and 0.53 for all combinations of one of the three Northern Irish sites to one of the other nine sites.

Genetic differentiation was found to be substantially larger when the standardized measure (G'_{ST}) was utilized (Table 3). Pairwise G'_{ST} varied from 0 to 0.85, with highest values being found in pairwise comparisons including populations in Northern Ireland. G'_{ST} was also found to be slightly higher compared to F_{ST} within the populations located in the southeast of Ireland ($G'_{ST} = 0.05$–0.17).

Jost's (2008) D_{est} was the highest of the three estimators of genetic differentiation (Table 3). D_{est} was found to range between 0.02 and 1.00. Bushfoot Strand and Magilligan, both in Northern Ireland, showed the highest D_{est}. However, unlike for other estimators, Umbra in Northern Ireland was no longer found to have one of the highest levels of differentiation. Belmullet, for example, showed higher D_{est} than Umbra (Table 3). As with the other measures, D_{est} was low in the SE Irish populations ($D_{est} = 0.03$–0.10).

There was a highly significant linear relationship between both G'_{ST} and F_{ST} ($r = 0.920$; $P < 0.001$), and between D_{est} and F_{ST} ($r = 0.869$; $P < 0.001$). This indicates that all three measures give similar relative estimates of population differentiation; hence, we use F_{ST} in subsequent analyses.

Using model-based clustering, Structure assigned individuals to six distinct groupings ($P > 0.88$; Fig. S1, Supporting information; Table S4, Supporting information). These are Ballyteige Burrow, Chour, The Raven, Curracloe Beach, and Brittas Bay; Magilligan, Umbra and Bushfoot Strand; Belmullet; Killinallan; Tiree; and Tobha Mòr (Fig. 1). Bayesian clustering structured populations similarly to that produced using measures of genetic differentiation. The only difference is that Structure reduced both the three Northern Irish populations and also the five populations in SE Ireland to one group apiece, whereas F_{ST} suggests that each of the 12 population should be placed in a separate group apart from those two population pairs with nonsignificant differentiation, namely Ballyteige Burrow and Chour, and Curracloe Beach and The Raven.

Population bottlenecks

Bottleneck suggested that most populations may have experienced recent bottlenecks (Table S5, Supporting information). Nearly every population showed

Table 2 Pairwise F_{ST} values below diagonal, Euclidean geographic separation (km) above diagonal. All pairwise F_{ST} values significant ($P < 0.0006$) except those highlighted by an asterisk (based on 10 000 iterations)

	BB	BF	BM	BTB	CB	CC	CHR	KN	MG	TB	TS	UM
BB	–	261.0	302.6	86.4	60.0	63.7	82.0	331.8	259.8	498.8	404.4	258.7
BF	0.484	–	258.1	336.2	315.5	319.5	337.9	73.06	24.3	237.9	143.1	21.8
BM	0.339	0.449	–	318.1	317.5	320.2	330.9	308.1	234.5	389.3	330.9	236.8
BTB	0.046	0.518	0.342	–	27.6	24.8	16.2	409.0	331.2	570.7	478.4	330.7
CB	0.045	0.452	0.308	0.049	–	4.0	22.6	387.6	312.0	551.7	458.5	311.3
CC	0.032	0.510	0.343	0.062	0.005*	–	18.6	391.7	316.0	555.7	462.5	315.3
CHR	0.041	0.462	0.296	−0.003*	0.053	0.070	–	410.1	334.1	573.8	480.8	333.4
KN	0.269	0.496	0.228	0.256	0.229	0.242	0.231	–	85.6	172.9	77.4	86.0
MG	0.503	0.197	0.479	0.524	0.471	0.528	0.480	0.515	–	239.1	146.9	2.9
TB	0.378	0.506	0.234	0.370	0.341	0.367	0.328	0.241	0.530	–	95.9	240.5
TS	0.313	0.462	0.288	0.296	0.294	0.306	0.275	0.252	0.486	0.142	–	147.8
UM	0.393	0.262	0.404	0.409	0.386	0.430	0.374	0.422	0.170	0.455	0.418	–

Table 3 Pairwise G'_{ST} values below diagonal; pairwise D_{est} differentiation values above diagonal

	BB	BF	BM	BTB	CB	CC	CHR	KN	MG	TB	TS	UM
BB	–	1.000	0.615	0.060	0.058	0.048	0.050	0.455	0.631	0.659	0.532	0.470
BF	0.716	–	0.996	0.973	1.000	0.965	0.996	0.965	0.797	0.970	0.992	0.867
BM	0.694	0.707	–	0.672	0.571	0.623	0.582	0.375	0.680	0.413	0.592	0.601
BTB	0.085	0.568	0.695	–	0.063	0.081	0.026	0.493	0.640	0.678	0.542	0.495
CB	0.100	0.721	0.617	0.098	–	0.016	0.078	0.423	0.616	0.589	0.509	0.509
CC	0.079	0.712	0.704	0.114	0.049	–	0.103	0.432	0.667	0.615	0.520	0.541
CHR	0.097	0.721	0.652	0.084	0.099	0.167	–	0.465	0.670	0.628	0.516	0.512
KN	0.534	0.793	0.486	0.519	0.442	0.486	0.500	–	0.708	0.359	0.481	0.597
MG	0.739	0.323	0.755	0.556	0.748	0.733	0.745	0.819	–	0.722	0.718	0.140
TB	0.744	0.785	0.473	0.687	0.668	0.709	0.687	0.489	0.820	–	0.247	0.671
TS	0.663	0.811	0.640	0.605	0.619	0.649	0.614	0.556	0.849	0.321	–	0.674
UM	0.644	0.303	0.729	0.548	0.673	0.682	0.667	0.761	0.179	0.790	0.813	–

significant evidence of a recent bottleneck under the IAM, a third of the populations under the TPM, while three populations were significant under the SMM. Umbra and Magilligan were the only populations not to exhibit significant bottlenecking under the IAM, TPM or SMM. Lack of significant bottlenecking in Magilligan and Umbra is likely to be caused by the extremely low heterozygosity within those populations, while allele richness remained relatively high, possibly because of recent founder events.

Isolation by distance

Across the entire dataset, there was a significant relationship between genetic [$F_{ST}/(1 - F_{ST})$] and geographic [log (Euclidean)] distance ($r^2 = 0.230$, $P = 0.037$). Coastal distance, allowing for three different minimum breaks of sea distances (0.4 km: $r^2 = 0.053$, $P = 0.118$; 5.2 km: $r^2 = 0.065$, $P = 0.097$; 10 km: $r^2 = 0.063$, $P = 0.101$), was not found to have a strong IBD relationship.

The effect of sea on genetic differentiation while controlling for Euclidean distance was not significant (partial Mantel test, $r = -0.16$; $P = 0.33$); whereas the relationship between Euclidean distance and genetic differentiation while controlling for sea was found to be highly significant ($r = 0.50$; $P = 0.0001$). The difference between over-land versus over-sea differentiation can be highlighted by dividing the data set into two groups of population pairs: those separated at some point by sea (>10 km) and those separated only by land (Fig. 2). A strong positive correlation ($r^2 = 0.771$, $P < 0.001$) is found between genetic differentiation and distance (Euclidean) for those population pairs that were only divided by land, while there is a lack of IBD ($r^2 = 0.110$, $P = 0.073$) when sea (>10 km) separated populations. F_{ST} is generally greater than 0.25 across sea for distances of separation from 77 to 574 km, and there is much scatter in pairwise values around the line of best fit.

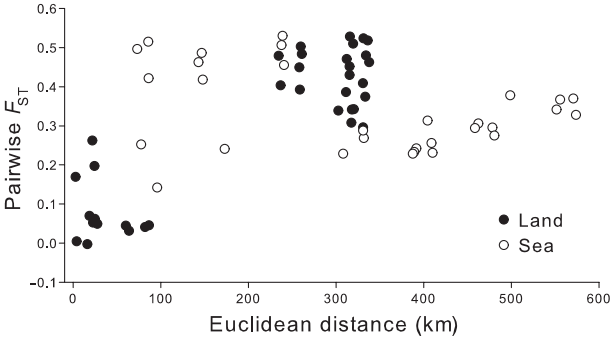

Fig. 2 Isolation by distance relationship for pairs of populations. Pairs divided at some point by sea in open circles, those divided only by land in filled circles.

Landscape genetics

All r^2 values obtained from the regression of pairwise genetic differentiation upon cost-weighted distance are significantly different from zero ($P < 0.015$; Table 4), except for a single cost of a single land class (agriculture costed at 0.001; $P = 0.16$). Agricultural land and beaches, dunes and sand were identified as possible land classes that facilitated gene flow as the least-cost value was found to be <1 (0.1 for both classes). In contrast, natural grassland, semi-natural vegetation, urban areas and woodland were all associated with cost values >1, and therefore, they potentially act as barriers to gene flow. There was no single best fit cost value for natural grassland; rather, all the cost values >1 resulted in the same r^2 value through the regression analysis. This is caused by the fact that natural grassland does not account for a large proportion of the grid squares in Ireland (only 1.7%) and, when these squares increase in cost, the least-cost pathway does not go through them and the cost-weighted distance value remains the same. The urban land class was found to have the highest

Table 4 Values of r^2 obtained from the regression between cost-weighted distance between every population pair and pairwise population differentiation, estimated using F_{ST}. The best fit cost value for each land class is highlighted by values in bold

	Cost values					
Land class	0.001	0.01	0.1	10	100	1000
Agricultural land	0.0565	0.1679	**0.6487**	0.6359	0.4428	0.3944
Beaches, dunes & sand	0.7794	0.7795	**0.7805**	0.7728	0.7387	0.3262
Natural grassland	0.6740	0.6763	0.6974	**0.7728**	**0.7728**	**0.7728**
Semi-natural vegetation	0.5419	0.5580	0.6523	**0.7504**	0.7501	0.7501
Urban	0.6881	0.6899	0.7060	0.7749	0.7885	**0.8300**
Woodland	0.7478	0.7478	0.7509	**0.7721**	0.7558	0.2479

best fit cost value (1000) and also explained the greatest amount of the variation in genetic differentiation (83.0%). Among those classes that appear to facilitate gene flow, beaches, dunes and sand explained the largest amount of genetic differentiation (78.1%). For comparison, the regression between genetic differentiation and Euclidean distance (equivalent to a cost value of 1 for every land class) produced a marginally lower $r^2 = 0.771$ for populations in Ireland.

In general, the jackknife procedure confirmed that results of the regression were not biased by outlier populations and could be considered representative of the study area (Fig. 3). Eight of the 36 *t*-tests of land class cost values were found to be nonsignificant but these did not include any cost values that were considered to be the best fit cost value for that land class (Table S6, Supporting information).

Discussion

Populations of the threatened solitary bee *C. floralis* exhibit extremely high genetic differentiation, and island populations in particular appear to be effectively isolated. This has general implications for solitary bee conservation and specifically suggests that management of this species needs to be site-focused and site-specific.

Population differentiation

A remarkable result of this study was the extremely high levels of population differentiation (global $F_{ST} = 0.34$; global $G_{ST} = 0.66$), which are considerably higher than those observed for temperate eusocial and solitary bee species across similar geographic distances (Zayed *et al.* 2005; Darvill *et al.* 2006; Ellis *et al.* 2006; Zayed *et al.* 2007; Zayed & Packer 2007; Exeler *et al.* 2008). As expected, G'_{ST} was found to be greater than F_{ST}, while D_{est} was larger again. All three estimators were closely correlated. The two measures that were independent of locus variability, G'_{ST} and D_{est}, disagreed slightly with respect to those populations with the highest levels of differentiation yet they both gave valuable, independent information. G'_{ST} provides useful insights into differentiation, gene flow and priority populations for conservation, while D_{est} highlights populations with unique alleles. G'_{ST} suggests the Northern Irish sites as priority populations for conservation given that they are extremely differentiated. D_{est} on the other hand also draws attention to Belmullet, because this population contains a greater number of unique alleles. The presence of unique alleles is an important feature for conservation consideration given that small, isolated populations frequently suffer loss of genetic variation and rare alleles through genetic drift and inbreeding (Allendorf & Luikart 2006).

Structure provided a conceptually alternative outlook on population structure. It differed slightly from F_{ST}, G'_{ST} and D_{est} with respect to Northern Ireland, grouping all three sites together, and with respect to the finer-scale structure of the SE Irish populations. The low levels of differentiation among SE Irish populations, as deduced from F_{ST}, suggested the five populations could be successfully managed as three MU: Ballyteige Burrow and Chour; Curracloe Beach and The Raven; and finally Brittas Bay. Alternatively, Structure suggested that these populations could be conserved as a single MU by grouping all five populations together. All measures of differentiation suggest that all populations outside of Northern Ireland and the SE of Ireland should be considered independent MUs.

While genetic diversity did not vary considerably between the 12 populations sampled, an interesting feature was that genetic diversity remained as high in northern populations in the Western Isles of Scotland, located at the extreme northwestern edge of the species range, as in the mainland Irish populations further south. In the Western Isles populations (TS, TB and KN), A_r and H_e were found to be similar to, or even higher than, Irish populations. That the genetic diversity found in these northern populations was high

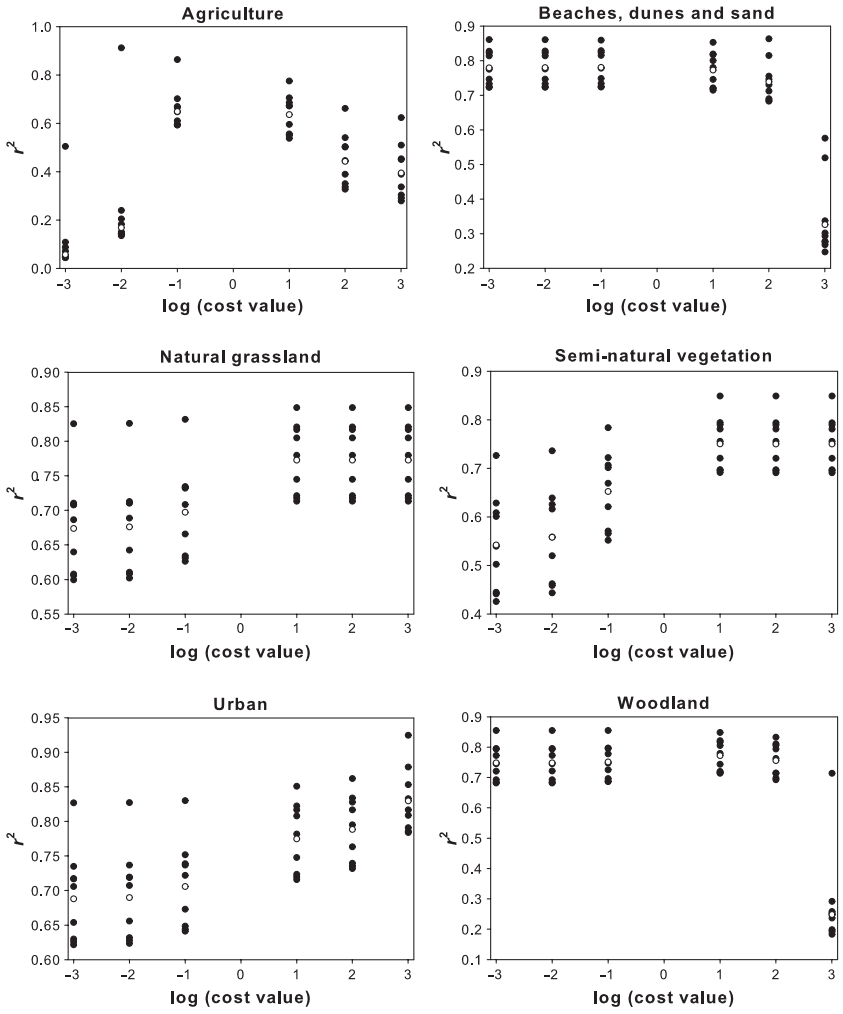

Fig. 3 Graphs showing the jackknife procedure of regression between genetic differentiation (F_{ST}) and cost-weighted distance values, which have been log-transformed for ease of illustration.

relative to more southern populations is unlike most other European bee species (Ellis *et al.* 2006) and other temperate biodiversity (Hewitt 2000; Provan & Bennett 2008) that have higher levels of genetic diversity in the southern regions of their range.

Landscape genetics

That *C. floralis* exhibits a significant IBD relationship across Ireland suggests that gene flow between populations separated by land occurs via the stepping stone model. It is probable that minimal gene flow occurs between distant populations >90 km, which will be more likely to become genetically distinct from each other through drift. This reinforces the view that populations separated by greater distances should be treated as distinct for conservation management purposes, as they may respond differently to environmental and ecological stresses.

Sea appears to act as a substantial barrier to gene flow for *C. floralis*. The absence of an IBD relationship for those population pairs separated by more than 10 km of sea and their generally high and variable levels of differentiation (F_{ST} = 0.14–0.46) suggest that gene flow is low over water. Increased levels of differentiation (F_{ST} > 0.25) across longer stretches of sea (77–574 km) suggest that sea may play a more important role in acting as a barrier to gene flow over both medium and long distances (Fig. 2). Indeed, island populations may not be in migration-drift equilibrium, and regional differentiation may be more determined by drift than gene flow (Hutchison & Templeton 1999). Migration-drift equilibrium may also not pertain to populations in Northern Ireland; although separated by <25 km, these three populations also exhibited high and variable genetic differentiation.

The presence of a significant IBD relationship for *C. floralis* with Euclidean distance and not with coastal

distance is perhaps surprising given the current coastal distribution of the species in Britain and Ireland. This could be explained in two ways. First, dispersal may not be carried out via a coastal route, and is perhaps, as suggested by Euclidean distance, mainly inland across direct, straight-line flight distances. However, given the ecology of the species, this may seem unlikely. Second, and perhaps more feasibly, dispersal may be carried out by a mixture of coastal and inland movement. The multiple areas of unsuitable habitat (e.g. cliffs or forested areas) or urban areas (e.g. coastal towns or ports) situated along the coastline may force the species to bypass these areas that could then act as a barrier to gene flow, while other suitable coastal areas may be traversed with ease. Passive dispersal, involving prevailing westerly winds in Ireland and Scotland, could also potentially account for a small proportion of dispersal in an eastern direction.

A caveat of this correlational approach is that F_{ST} measures historical population connectivity, whereas our CORINE LAND COVER MAP reflected land use in 2000. Nevertheless, the results of our GIS-based landscape analysis show that a large proportion of the variation in genetic differentiation can be accounted for when landscape parameters are included (up to 83.0%). In addition, landscape genetics can reveal insights into how a species disperses, which may be different to expectation (Pérez-Espona *et al.* 2008; Spear & Storfer 2008; Wang *et al.* 2009). For *C. floralis,* beaches, dunes and sand and agricultural land were identified as possible land classes that facilitate gene flow. That coastal sandy areas facilitate dispersal is perhaps not surprising as the natural habitat of *C. floralis* would be expected to be easily traversable. Agricultural land in Ireland is composed of arable land, complex cultivation patterns and pastures. While it might be expected that agriculture would not have a beneficial effect on gene flow, its positive association with inferred dispersal may be explained by the composition of agricultural land in Ireland past and present. These areas, which account for 67.6% of the 1.3 million pixels of the raster grid of Ireland, are not dominated by complex cultivation or intensified practices, but by areas designated as pastures (accounting for 74.9% of all agriculture) which have the potential to contain abundant floral resources and therefore increase the ease of passage for the species. In addition, land currently classified as agricultural may formerly have been far more porous to *C. floralis*, reflecting its low best fit cost value to genetic differentiation.

Natural grassland, semi-natural vegetation, urban areas and woodland all had cost values that were larger than one and therefore could potentially act as barriers to gene flow. The most important finding of this analysis was the substantial anthropogenic effect on gene flow through urban areas, which had the highest best fit cost value, explained the greatest amount of the variation in genetic differentiation and potentially acts as the greatest barrier to gene flow. While sizeable urbanized areas were not always found near the more isolated coastal sites, such as Belmullet, it is predicted that the proportion of the Irish human population living in urban areas will increase by 3.9% to 94% (United Nations 2010) over the next 40 years, undoubtedly necessitating extensive urban development. This could result in significant detrimental effects on the ability of *C. floralis* to disperse successfully between sites and therefore on the persistence of the species in Ireland.

Population bottlenecks

Most *C. floralis* populations may have experienced recent bottlenecks. Three of the five southeast Irish populations exhibited a significant probability of recent population bottlenecks under all three mutational models, while the majority of the west coast of Ireland and Western Isle populations exhibited bottlenecking under one or more mutation models. Given that overall gene flow is low, especially where sea is found between population pairs, the limited evidence for bottlenecking in western Ireland and Western Isles populations could be a consequence of better quality habitats or reduced anthropogenic disturbance because of the relatively isolated localities of these sites (Goulson *et al.* 2006; Fitzpatrick *et al.* 2007; Berry 2009).

The three sites in Northern Ireland are enigmatic populations. All exhibited extremely low heterozygosity; Magilligan and Bushfoot Strand were monomorphic at four loci (cf10, 12, 13 and 16), while Umbra was monomorphic at one locus (cf13). Yet Umbra exhibited high allelic diversity; it had the highest A_r of all sampled populations at loci cf1 and cf17. In addition, there was no evidence of bottlenecking at either Magilligan or Umbra. One explanation is that these populations may have been recently re-established. With only a small number of foundresses from multiple sites, the outcome would be a noticeable change in allele frequency (high F_{ST}) and an unpredictable change in genetic variation that depended upon the sources of the founders (Allendorf & Luikart 2006).

Management implications

This study has important implications for the management and conservation of *C. floralis* and potentially many other solitary bees. First, the extremely high levels of genetic differentiation suggest that populations are largely isolated, even over short distances, such that population demography will be determined by local

factors rather than immigration (Waples & Gaggiotti 2006). A second implication is with regard to the Northern Irish populations, Magilligan, Umbra and Bushfoot Strand. The low genetic diversity (extremely low H_e) of these sites suggests that these Northern Irish populations could be extremely small and even more isolated than elsewhere, or are possibly recently re-founded from neighbouring localities and forming a metapopulation. An additional factor that may highlight the need for site-specific management is that populations that are genetically distinct from one another may begin to respond differently to environmental or climatic pressures. The risk to survival may be intensified if gene flow is further reduced because of environmental barriers such as increased urbanization and erosion of suitable habitat.

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This study is part of ESD's PhD thesis on the conservation biology and genetics of *Colletes floralis* in the United Kingdom and Ireland. RJP's main interests cover insect conservation genetics, social evolution in the Hymenoptera, bee diseases and pollination. TEM's interests span conservation genetics and invasion biology. MJFB's research centres on the evolutionary ecology of host–parasite systems and the conservation of insects, specifically bees. UF focuses on biodiversity conservation and is currently employed by the National Biodiversity Data Centre in Ireland.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Coastal geographic separation (km) above diagonal allowing for a 5.2-km break of sea (e.g. for inlets); percentage of this distance that was over sea below diagonal

Table S2 Observed heterozygosity (Ho)*, expected heterozygosity (He) and allele richness (Ar) per locus and per population

Table S3 *F*IS values per locus and per population, and significance of deviation from Hardy–Weinberg equilibrium by an exact test (as implemented in GENEPOP)

Table S3 *F*IS values per locus and per population, and significance of deviation from Hardy–Weinberg equilibrium by an exact test (as implemented in GENEPOP)

Table S4 The mean likelihood of each model for $K = 1$–13 established over five independent repetitions in STRUCTURE. The most likely value occurs at $K = 6$

Table S5 One-tailed *P*-values of the Wilcoxon test for heterozygosity excess under three mutational models (IAM: infinite alleles model; TPM: two-phase mutational model; SMM: stepwise mutational model)

Table S6 Results of the *t*-tests using pseudovalues (estimates of the bias encountered because of interpopulation differences) from the jackknife procedure

Fig. S1 Plot showing the assignments of each individual to each cluster; the most likely number of clusters is $K = 6$.

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