

Population genetic structure and colonization history of *Bombus terrestris* s.l. (Hymenoptera: Apidae) from the Canary Islands and Madeira

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The bumble bee *Bombus terrestris* L. is a geographically variable species with a wide distribution in Europe, the near East, northern Africa, Mediterranean islands, the Canary Islands and Madeira. Based on morphological and coat colour pattern differences, the bumble bee populations of the Canary Islands and Madeira are currently treated as separate species, *B. canariensis* and *B. maderensis*, respectively. To analyse the phylogeographical associations of these bees with continental *B. terrestris*, one population each from four islands of the Canaries and one population from Madeira were studied. Genetic variability was assessed at nine microsatellite loci and a fragment of the mitochondrial gene cytochrome b. Genetic differentiation among islands, and between islands and the continent, was extensive. A NJ-tree based on microsatellites strongly supported the distinctness of the Canary Island populations, whereas the Madeira sample was genetically more similar to the continental populations of *B. terrestris* from Europe. MtDNA sequence data were in good agreement with nuclear markers. They suggest that haplotypes ancestral with respect to *B. lucorum* occur on the Canary Islands, whereas derived haplotypes were found on the European continent. The Madeira population shares the most common haplotype of continental *B. terrestris*. Nuclear and mtDNA data both indicate that bumble bees from the Canaries and Madeira do not share a common colonization history.

Keywords: *Bombus*, microsatellites, mtDNA, phylogeography.

Introduction

The bumble bee *Bombus terrestris* L. (Hymenoptera: Apidae) is the most intensively studied non-*Apis* bee. *Bombus terrestris* s.l. is widely distributed in continental Europe, occurs in North Africa, and has colonized most major Mediterranean and several Atlantic Islands (the Canary Islands and Madeira). Populations from the Canary Islands show a distinct colour pattern in that they lack the yellow bands on the thorax and abdomen that are typical for *B. terrestris* throughout a large part of the European continent (Fig. 1). Therefore, the populations from the Canary Islands were initially described as a colour morph of *B. terrestris* but later considered a

separate species, *B. canariensis* (Erlandsson, 1979). Specimens from Madeira, on the other hand, are very similar, despite some minute morphological differences, to continental *B. terrestris lusitanicus* from the Iberian peninsula (Rasmont *et al.*, 1986) (Fig. 1). Nevertheless, Erlandsson (1979) also described the populations from Madeira as a separate species, *B. maderensis*. Rasmont (1983) also lists *canariensis* and *maderensis* as separate species, although other authors still treat these taxa as subspecies (Hohmann *et al.*, 1993). Here, we will refer to *B. terrestris* s.l. when referring to these several taxa. In Europe, about 800,000 colonies are currently commercially produced every year for crop pollination in the greenhouse industry (R. De Jonghe, pers. comm.). Because they originate from different stocks, commercially bred bumble bees differ genetically from local populations and the

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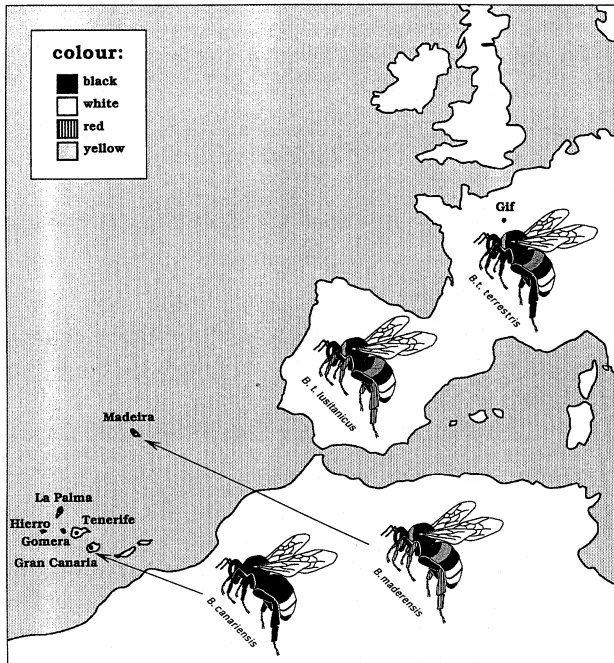


Fig. 1 Map of western Europe and northern Africa indicating sampled populations, taxonomic status and coat colour variation of *Bombus terrestris*, *B. canariensis* and *B. maderensis*. The designation of taxa follows Rasmont (1983).

escaping sexuals may introduce foreign alleles. Therefore, the commercial use of *B. terrestris* s.l. for pollination should be of concern with respect to genetic changes in local populations. However, our understanding of the genetic population structure of *Bombus* spp. is rather limited (Estoup *et al.*, 1996).

A preliminary genetic analysis of a population of *Bombus* from Tenerife (Canary Islands) revealed significantly lower levels of genetic variability at microsatellite loci and a distinct autapomorphic mtDNA haplotype (COII gene) compared to continental populations (Estoup *et al.*, 1996). These preliminary genetic data thus suggested that the Canary Island populations may be derived from the continental ancestral stock of *B. terrestris*, and that these populations went through a bottleneck with reduced effective population size (Estoup *et al.*, 1996). However, as only one population was studied, the robustness of this scenario is low. The aim of this study was to investigate the population genetic structure and colonization history of *B. terrestris* s.l. populations from the Canary Islands and Madeira in relation to the European continent and Mediterranean basin. In particular, the level of nuclear genetic variability within and among populations from the Canary Islands and Madeira, the genetic differentia-

tion among Canary Island populations, and the relationships between these islands and continental populations of *B. terrestris* s.l. were investigated. The results support the special status of the Canary Islands populations but suggest that the colonization history of the Canary Islands has been different from that depicted by Estoup *et al.* (1996). The findings shed new light on the pattern of colonization and present-day population structure of an important temperate-area pollinator.

Materials and methods

Study area and biological material

The Canary Islands and Madeira are located in the Atlantic ocean at a distance of about 1400 km and 900 km, respectively, from the European continent. The Canary Islands consist of seven large islands, five of which (Tenerife, La Gomera, El Hierro, La Palma and Gran Canaria) are inhabited by *Bombus canariensis*. No bumble bees occur on Fuerteventura and Lanzarote, located between the African continent and the other Canary Islands (Hohmann *et al.*, 1993). The distance between islands inhabited by *B. canariensis* ranges between 30 (Tenerife-La Gomera) and 210 km (El Hierro-Gran Canaria). The island ages vary considerably. K-Ar datings (Ancochea *et al.*, 1990; Coello *et al.*, 1992) reveal a general decrease in the age of the islands from east to west. The data suggest that Tenerife formed about 11.6 Ma BP, Gran Canaria 14–16 Ma BP, La Gomera about 10 Ma BP, and El Hierro about 1 Ma BP. None of the Canary Islands seems ever to have been connected to the African continent. The colonization of the Canary Islands by bumble bees must therefore have occurred over open water surfaces. Distances among islands, and between islands and the African continent, were smaller during the ice ages, because the sea level was lower (Hohmann *et al.*, 1993). Madeira, which lies about 630 km off the western coast of Morocco, is of Tertiary volcanic origin and has an estimated age of 60–70 million years.

Bumble bee queens were sampled in April 1995 on Tenerife, La Gomera, El Hierro and Gran Canaria. The sample from Madeira consisted of workers which were collected as described in Estoup *et al.* (1996). The specimens were stored at ambient temperature in pure ethanol. The geographical origins of sampled populations are indicated in Fig. 1. To test for differences in genetic variability and amount of genetic differentiation between conti-

mental and insular populations, some of the microsatellite data from Estoup *et al.* (1996) were added. These came from the following populations: Avelino (Italy), Banyuls (France), Gif-sur-Yvette (France), Lublin (Poland), Madrid (Spain), Nea-Moudania (Greece), Pleven (Bulgaria) and Tours (France) from the European continent, and the Mediterranean island populations from Cyprus (Greece), Samos (Greece), Elba (Italy), Corsica (France) and Sardinia (Italy). A population of *B. lucorum* from Bern (Switzerland) was used as an outgroup in our analyses (Estoup *et al.*, 1996).

DNA isolation, PCR and sequencing

DNA extractions were performed from the head of the specimens as described in Garnery *et al.* (1991) after soaking in sterile water for 10 min to remove any excess of ethanol. The genotypes were assessed at nine microsatellite loci: B10, B11, B96, B100, B116, B118, B124, B126 and B132. All primer sequences are given in Estoup *et al.* (1995, 1996). Multiplex PCRs were run for combinations of the loci B10-B11-B96, B124-B126 and B100-B118. PCR reactions were performed in a final volume of 10 μ L. The reaction mixtures contained 5–10 ng of total DNA, 400 nM of each primer, 75 μ M of each nucleotide dGTP, dCTP, dTTP, 6 μ M dATP, 1.36×10^4 Bq alpha-³³P-dATP, 1.0 or 1.2 mM MgCl₂, 20 μ g/mL Bovine Serum Albumin (BSA), 1 \times reaction buffer and 0.4 units of Taq Polymerase. PCR conditions were as in Estoup *et al.* (1996). 0.4 vol. of Formamide/EDTA/XC/BPB gel-loading buffer (Sambrook *et al.*, 1989) was added to the PCR products prior to heat denaturation. Samples were run on 6% denaturing polyacrylamide sequencing gels for 3 h at constant voltage (2500 V) and temperature (45°C). Fixed and dried gels were exposed for 24 h.

For the study of mitochondrial DNA sequence variation, a fragment of the mitochondrial cytochrome b gene was amplified by PCR. PCR and sequencing reaction conditions were carried out as described in Pirounakis (1995). PCR fragments were produced using the mtDNA primers CB1 (coordinates 11400–11425 as in Crozier & Crozier, 1993) and CB2 (coordinates 11859–11884 as in Crozier & Crozier, 1993). In a first step, double-stranded PCR products were generated, an aliquot of which was used as template in a second PCR reaction with only one of both primers to produce a single-stranded PCR product. Ss-PCR products were sequenced using the Sequenase version 2.0 kit (USB). Sequence information was collected from 21 *B. terrestris* s.l., six *B. canariensis*, three *B. maderensis*

and from one *B. lucorum* as the outgroup to root the tree.

Data analysis

Allele frequencies, observed heterozygosities and unbiased estimates of expected heterozygosities (Nei, 1978) were calculated for each population separately, using the BIOSYS-1 package (version 1.7) (Swofford & Selander, 1989). Departures from Hardy-Weinberg equilibrium (HWE) were assessed with the software package GENEPOP (version 1.2) (Raymond & Rousset, 1995). *F*-statistics were computed with FSTAT (Goudet, 1996) and *F*-values were tested for their statistical significance using permutation tests (Goudet, 1996). The appropriate permutation units depend on the *F*-value under consideration. For *F*_{ST} we permuted genotypes among populations, for *F*_{IS}, alleles were permuted among individuals within populations, and for *F*_{IT}, alleles were permuted among populations. Confidence intervals for *F*-values were obtained by jackknifing over populations. We calculated a global *F*_{ST} over the Canary Islands and Madeira, and exclusively for the Canary Islands, to estimate the effective number of migrants (*N*_m) between Madeira and the Canary Islands, as well as among the Canary Islands themselves, using the private alleles method according to Barton & Slatkin (1985). This estimator is most appropriate when levels of gene flow are low (Barton & Slatkin, 1985). In addition, pairwise multilocus *F*_{ST}-values and estimates of *N*_m based on these *F*_{ST}-values, were calculated between pairs of populations of *B. canariensis* and *B. maderensis* using FSTAT.

Comparisons of genetic variability between the Canary Islands and Madeira, Mediterranean Islands and the continent were calculated for six microsatellite loci used in this study and also by Estoup *et al.* (1996): B11, B96, B100, B116, B118 and B132. Because the number of alleles depends on the sample size, the sample size was adjusted to 40 alleles per population, using Ewens' formula (Ewens, 1972). One-tailed Mann-Whitney *U*-tests were used to test for differences in *H*_{exp}. Where multiple significance tests were performed, *P*-values were adjusted using the sequential Bonferroni procedure to control for Type I error (Rice, 1989).

A Neighbour-Joining (NJ) tree (Saitou & Nei, 1987) relating the island and continental populations, was constructed based on microsatellite data using the chord-distance (Cavalli-Sforza & Edwards, 1967). To assess the stability of the tree nodes, we ran 2000 bootstrap replications (Hedges, 1992). All

calculations were carried out using programs provided by J.-M. Cornuet (personal communication).

Analyses of phylogenetic relationships among *B. terrestris* s.l. mtDNA haplotypes were carried out with *B. lucorum* as an outgroup taxon. A maximum likelihood (ML) analysis was carried out with the program DNAML in the PHYLIP package (Felsenstein, 1993) to assess phylogenetic relationships among haplotypes. The optimal transition/transversion ratio was determined in repeated runs by maximizing the log likelihood value. To construct ML trees, an extensive search for the best tree was run, using global rearrangement. A maximum parsimony analysis (MP) was performed with the software package PAUP 3.1.1 (Swofford, 1993). Searches for all most parsimonious trees were performed with the exhaustive search algorithm assuming equal weights for transitions and transversions. The presence of phylogenetic signal in the sequence matrices was tested using the g_1 statistic test based on the skewness of tree-length distributions (Hillis & Huelsenbeck, 1992). The g_1 value was obtained from the exhaustive search with PAUP.

Results

All nine loci revealed genetic polymorphisms within or among the Canary Islands and Madeira populations (Table 1). Genetic variation was higher in Madeira than in the Canary Island populations (Table 2). Samples from Tenerife and Gran Canaria revealed a similar mean number of alleles per locus (2.3 and 2.5, respectively) and the same number of polymorphic loci (five each). At La Gomera and El Hierro, only three loci were polymorphic. Locus B124 was fixed for allele 50 on El Hierro, but polymorphic on La Gomera, whereas locus B126 was monomorphic on La Gomera and polymorphic on El Hierro (Table 1). Two loci (B10 and B100) showed significant deviations from HWE ($P < 0.001$ for B10, and $P = 0.033$ for B100), but only the deviation at locus B10 remained significant after sequential Bonferroni adjustment of P for the number of loci tested. Strong deviations from HWE at this particular locus were found in the samples from Tenerife and La Gomera, and are responsible for the positive F_{IS} -values in these samples (Table 2). The only population where an excess of heterozygotes was observed, was Gran Canaria ($F_{IS} = -0.06$).

To test whether populations from the Canary Islands and Madeira display reduced genetic variability, the mean number of alleles and expected

Table 1 Allele frequencies, observed heterozygosity (H_{obs}), and unbiased, expected heterozygosity under Hardy–Weinberg equilibrium (H_{exp}), by locus and by locality in populations of *Bombus canariensis* and *B. maderensis*. The sample size is indicated in parentheses for each locality and locus

Allele	El		La	Gran	
	Tenerife	Hierro	Gomera	Canaria	Madeira
Locus B10					
(n)	(59)	(30)	(22)	(18)	(20)
142	—	—	—	—	0.150
144	0.144	0.117	—	—	—
146	0.085	—	—	—	—
148	0.297	0.133	0.136	0.111	0.075
150	0.390	0.583	0.455	0.111	—
152	0.042	0.033	0.364	0.306	—
154	0.017	—	0.045	0.278	—
156	0.025	0.133	—	0.139	—
158	—	—	—	0.056	—
162	—	—	—	—	0.450
164	—	—	—	—	0.300
166	—	—	—	—	0.025
$H_{(obs)}$	0.576	0.600	0.318	0.889	0.500
$H_{(exp)}$	0.736	0.620	0.655	0.805	0.696
Locus B11					
(n)	(62)	(30)	(23)	(20)	(20)
158	—	—	—	—	0.475
166	—	—	—	—	0.225
168	—	—	—	—	0.100
172	—	—	—	—	0.200
174	1.000	1.000	1.000	1.000	—
$H_{(obs)}$	0.000	0.000	0.000	0.000	0.500
$H_{(exp)}$	0.000	0.000	0.000	0.000	0.691
Locus B96					
(n)	(62)	(30)	(23)	(20)	(20)
238	—	—	—	—	1.000
242	1.000	1.000	1.000	1.000	—
$H_{(obs)}$	0.000	0.000	0.000	0.000	0.000
$H_{(exp)}$	0.000	0.000	0.000	0.000	0.000
Locus B100					
(n)	(62)	(30)	(23)	(20)	(19)
146	—	—	—	—	0.026
152	—	—	—	—	0.105
154	1.000	1.000	1.000	1.000	0.526
156	—	—	—	—	0.132
158	—	—	—	—	0.079
162	—	—	—	—	0.053
170	—	—	—	—	0.079
$H_{(obs)}$	0.000	0.000	0.000	0.000	0.579
$H_{(exp)}$	0.000	0.000	0.000	0.000	0.697
Locus B116					
(n)	(55)	(30)	(23)	(20)	(20)
172	0.900	1.000	1.000	1.000	0.725
174	0.100	—	—	—	0.275
$H_{(obs)}$	0.164	0.000	0.000	0.000	0.450
$H_{(exp)}$	0.182	0.000	0.000	0.000	0.409

Table 1 Continued

Allele	El		La	Gran	Madeira
	Tenerife	Hierro	Gomera	Canaria	
Locus B118					
(n)	(21)	(30)	(23)	(20)	(20)
209	0.738	1.000	1.000	0.725	—
211	0.190	—	—	0.275	0.575
217	0.071	—	—	—	0.350
219	—	—	—	—	0.075
$H_{(obs)}$	0.429	0.000	0.000	0.550	0.650
$H_{(exp)}$	0.424	0.000	0.000	0.409	0.555
Locus B124					
(n)	(32)	(30)	(21)	(18)	(18)
48	—	—	0.167	—	—
50	0.859	1.000	0.690	0.389	—
52	0.141	—	0.024	0.028	0.556
54	—	—	0.119	0.444	—
56	—	—	—	0.139	0.444
$H_{(obs)}$	0.219	0.000	0.524	0.722	0.333
$H_{(exp)}$	0.246	0.000	0.492	0.649	0.508
Locus B126					
(n)	(32)	(30)	(23)	(13)	(20)
48	—	—	—	—	0.025
50	1.000	0.367	1.000	0.346	0.625
52	—	—	—	—	0.225
54	—	—	—	0.654	—
56	—	0.617	—	—	—
58	—	0.017	—	—	—
60	—	—	—	—	0.125
$H_{(obs)}$	0.000	0.467	0.000	0.385	0.600
$H_{(exp)}$	0.000	0.493	0.000	0.471	0.556
Locus B132					
(n)	(47)	(30)	(23)	(19)	(15)
159	—	—	—	—	0.233
161	—	—	—	—	0.133
163	—	—	—	—	0.433
165	—	—	—	—	0.200
167	0.011	—	—	—	—
169	—	—	—	0.237	—
171	0.574	0.150	0.717	0.237	—
173	0.096	—	0.130	0.105	—
177	0.053	—	0.130	0.395	—
179	0.255	0.850	—	0.026	—
181	0.011	—	0.022	—	—
$H_{(obs)}$	0.511	0.233	0.435	0.684	0.867
$H_{(exp)}$	0.599	0.259	0.461	0.740	0.724

multilocus heterozygosities were estimated from the six loci used in both this study and Estoup *et al.* (1996) (see Methods for list of localities). Genetic variability of the Canary Island populations ($\bar{H}_{exp} = 0.23 \pm 0.08$) was low compared to the continental populations of *B. terrestris* ($\bar{H}_{exp} = 0.62 \pm 0.04$), or those of Madeira ($\bar{H}_{exp} = 0.54 \pm 0.08$; one-tailed

Mann–Whitney; $P < 0.008$ for both comparisons). Similarly, the mean number of alleles for Canary Island populations ($x = 2.1 \pm 0.4$) was lower than that found on Madeira ($x = 3.6 \pm 0.6$) or on the continent ($x = 6.8 \pm 0.3$; $P < 0.05$ for both comparisons).

The Mediterranean islands investigated by Estoup *et al.* (1996) provide an interesting comparison. For those, the average number of alleles per locus (one-sided Mann–Whitney; $P = 0.006$), but not heterozygosity ($P = 0.064$), was significantly reduced relative to continental populations. But even with respect to the Mediterranean Islands, the Canary Islands populations harboured less variability ($P = 0.008$ for expected heterozygosity and mean number of alleles). Bonferroni adjustment did not change the significance of these comparisons.

Populations of *B. terrestris* s.l. from the Canary Islands and Madeira were strongly differentiated from each other, as indicated by a highly significant overall F_{ST} -value (average $F_{ST} = 0.411$, $P < 0.001$). The corresponding average estimate of gene flow, based on the private alleles method, was thus low ($Nm = 0.15$). Multilocus F_{ST} estimates among the four populations from the Canary Islands also revealed strong genetic differentiation ($F_{ST} = 0.281$, $P < 0.001$) among islands, with correspondingly low gene flow ($Nm = 0.18$). Multilocus pairwise F_{ST} estimates between pairs of islands (Canary Islands and Madeira) revealed a relatively weak differentiation between Tenerife and La Gomera ($F_{ST} = 0.092$), as compared to other pairs of island populations ($F_{ST} \geq 0.274$) (Table 3). As expected, when compared to continental populations, the Canary Islands and Madeira showed highly significant levels of genetic differentiation. Pairwise F_{ST} estimates between Gif, a French continental population analysed by Estoup *et al.* (1996) that we took here as a reference, and Tenerife or Madeira was $F_{ST} = 0.426$, and $F_{ST} = 0.107$, respectively ($P < 0.001$ for both comparisons).

The results of the analysis of variability and differentiation are corroborated by the NJ-tree based on populations (Fig. 2). The Canary Islands represent a clearly distant group from continental *B. terrestris*, *B. maderensis* and *B. lucorum* (Fig. 2). The bootstrap values on loci or individuals reached 100%, confirming the differentiation of these populations. Another well supported cluster is the populations from the Tyrrhenian Islands (Elba, Corsica and Sardinia; Fig. 2), as in Estoup *et al.* (1996). On the other hand, Madeira surprisingly clusters with the continental and Mediterranean populations.

Cytochrome b sequences revealed intraspecific mtDNA sequence variation in samples of *B. terrestris* s.l. Six different haplotypes with clear distribution

Table 2 A summary of genetic information for each population of *Bombus canariensis* and *B. maderensis*. Values in parentheses are standard errors

Island	N_{\dagger}	n_{\ddagger}	Polymorphic (%)§	\bar{H}_{obs}	\bar{H}_{exp}	F_{IS}
Tenerife	48.0 (5.3)	2.3 (0.76)	55.6	0.211 (0.079)	0.243 (0.094)	0.157*
El Hierro	30.0 (0.0)	1.7 (0.46)	33.3	0.144 (0.079)	0.152 (0.082)	0.054
La Gomera	22.7 (0.2)	2.0 (0.50)	33.3	0.142 (0.073)	0.179 (0.091)	0.212*
Gran Canaria	18.7 (0.8)	2.5 (0.65)	55.6	0.359 (0.122)	0.341 (0.115)	-0.066
Madeira	19.1 (0.6)	3.6 (0.60)	88.9	0.498 (0.079)	0.537 (0.076)	0.083*

†Mean sample size (number of individuals) per locus.

‡Mean number of alleles per locus.

§Percentage polymorphic among all loci.

* $P < 0.05$.

Table 3 Multilocus pairwise F_{ST} -values between pairs of populations of *Bombus canariensis* and *B. maderensis* are indicated above the diagonal. Pairwise estimates of Nm , as estimated from the pairwise F_{ST} -values, are given below the diagonal

	Tenerife	El Hierro	La Gomera	Gran Canaria	Madeira
(1) Tenerife		0.295***	0.092**	0.275***	0.537***
(2) El Hierro	0.599		0.417***	0.405***	0.621***
(3) La Gomera	2.473	0.350		0.274***	0.557***
(4) Gran Canaria	0.660	0.367	0.662		0.457***
(5) Madeira	0.216	0.153	0.199	0.297	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

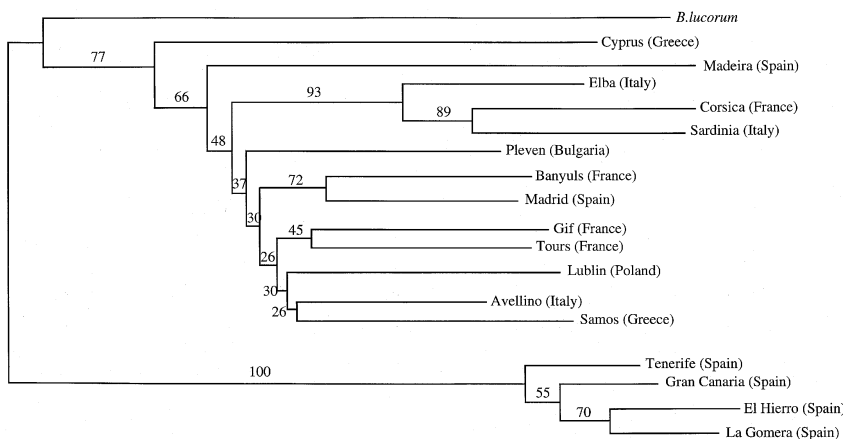


Fig. 2 NJ-tree (unrooted) based on Cavalli-Sforza & Edward's (1967) distance measures, relating continental and insular populations of *Bombus terrestris*, *B. canariensis* and *B. maderensis*. Genetic distances were estimated from six microsatellite loci using a program provided by J.-M. Cornuet (personal communication). Small figures are bootstrap values, computed over 2000 replications.

pattern were observed (Table 4). Haplotype A was found in the five continental populations surveyed. Haplotype B was found only once in Nea-Moudania (mainland Greece). Haplotype C was observed in two queens analysed from Great Britain, suggesting that these populations of *B. terrestris* s.l. are distinct from those of European continent. Two haplotypes could be found on the Mediterranean islands: the continental haplotype A on Mallorca and on the three eastern Mediterranean islands (Samos, Cyprus and Crete), and haplotype D which was exclusively found on the Tyrrhenian islands. The Canary Islands harboured two endemic haplotypes, E and F. All bees from Madeira had the most common continental haplotype A.

Results of the ML and MP analyses of mtDNA haplotypes were in good agreement. The tree obtained from the ML analysis (TI:TV ratio = 2.5; ln likelihood = 505.45) was identical with one of the 10 shortest trees (tree length = 21, consistency index = 0.905) obtained in the MP analysis (Fig. 3a). Other shortest trees differed in the placement of haplotypes A-D or placed the Canary Island haplotypes (E, F) as a sister group to the continental haplotypes (A-D; Fig. 3b). The g_1 -value ($g_1 =$

Table 4 Distribution of the six mtDNA haplotypes detected in continental and island populations of *Bombus terrestris*, *B. canariensis* and *B. maderensis*. The number of individuals sequenced (n) and the mtDNA haplotype found (A...F) are given for each sampling locality

Sampling localities	n	Haplotype
<i>Europe</i>		
Avellino (Italy)	2	A
Gif-sur-Yvette (France)	1	A
Nea-Moudania (Greece)	2	A,B
Madrid (Spain)	1	A
Pleven (Bulgaria)	1	A
Silwood (Britain)	2	C
<i>Mediterranean islands</i>		
Corsica (France)	1	D
Elba (Italy)	3	D
Sardinia (Italy)	2	D
Mallorca (Spain)	2	A
Samos (Greece)	1	A
Kreta (Greece)	2	A
Cyprus (Greece)	1	A
<i>Canary Islands and Madeira</i>		
Tenerife (Spain)	2	E,F
La Gomera (Spain)	1	F
El Hierro (Spain)	2	F
Gran Canaria (Spain)	1	F
Madeira (Spain)	3	A

-0.644 , $P < 0.05$) indicates a significantly skewed tree-length distribution, suggesting that the dataset contains a phylogenetic signal (Hillis & Huelsenbeck, 1992). All phylogenetic analyses indicated that the most primitive haplotypes are those from the Canary Islands (E and F), followed by haplotype D from the Tyrrhenian Islands. The continental and remaining Mediterranean islands, as well as Madeira, had derived haplotypes (A, B and C).

Discussion

The results of this study show that Canary Island populations are characterized by low variability in both allele numbers and heterozygosity, compared to the mainland populations of *B. terrestris*. At the

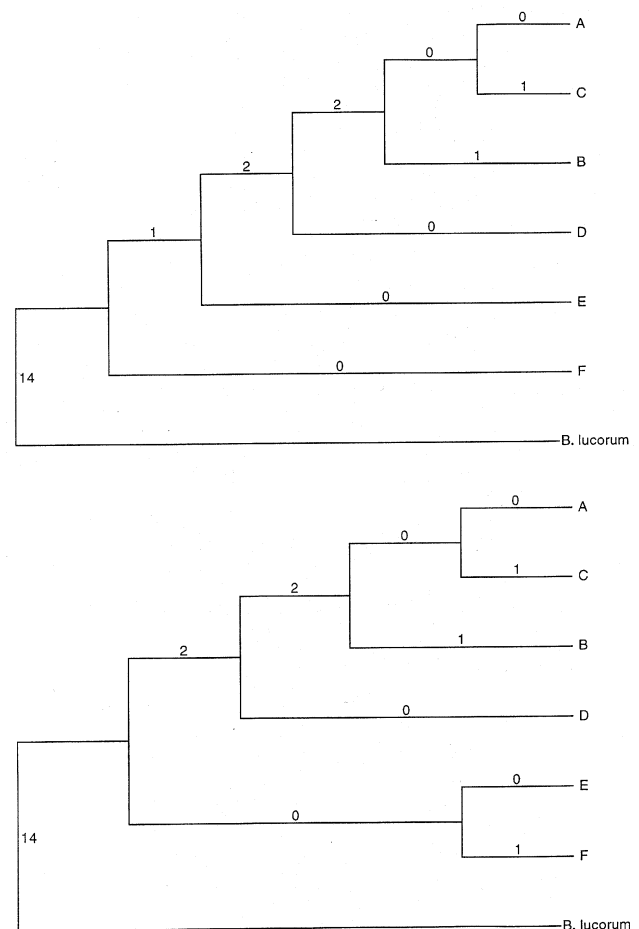


Fig. 3 Two of 10 most parsimonious trees of *Bombus terrestris* s.l. mtDNA haplotypes: (a) MP identical with the tree obtained in a ML analysis with haplotypes E and F from the Canary Islands ancestral to European haplotypes (A-D); (b) haplotypes E and F form the sister group to the European haplotypes (A-D).

same time, the mtDNA haplotypes are diverged from those of the continent. These findings are in good agreement with the preliminary study of Estoup *et al.* (1996). As mentioned by these authors, such low variability could be explained with a founder event by a population derived from the European continental stock. Similarly, it could reflect repeated bottlenecks of a resident population that had immigrated from the continent, coupled with small effective size over extended periods of time (Nei *et al.*, 1975). This would explain the large genetic distance of the Canary Islands from the other populations, as estimated from microsatellite data, because bottlenecks can inflate measures of genetic distance (Chakraborty & Nei, 1977). However, when the mtDNA-sequence data are considered (Fig. 3), the pattern is more suggestive of an ancient, primary colonization of the islands and a long history of the resident population in the archipelago.

Two mtDNA haplotypes, E and F, are endemic to the Canary Islands, whereas haplotypes A, B and C are found on the European continent, most Mediterranean islands and the British Islands, respectively. The Canary Island haplotypes, and to some degree those of the Tyrrhenian Islands, are ancestral with respect to all others if the closely related taxon *B. lucorum* is taken as an outgroup (Fig. 3). *Bombus terrestris* and *B. lucorum* are closely related, as suggested by a phylogenetic analysis of subgenus *Bombus* s.str., based on mtDNA sequence variation (Widmer *et al.*, unpublished results).

Given the close proximity to the African continent, it is most likely that *B. canariensis* originated from northern African *B. terrestris* s.l. To what extent the islands of Fuerteventura and Lanzarote, located between the African continent and Gran Canaria, and which today are not inhabited by bumble bees (Hohmann *et al.*, 1993), acted as stepping-stones in the colonization process cannot be answered. Unfortunately, samples from northern Africa (ssp. *africanus*) were not available for analysis and we tacitly assumed throughout this study that the African populations are not differentiated from continental European populations at the mtDNA and microsatellite level. However, it now becomes clear that molecular data from these bees are urgently needed to understand the divergence of the Canary Island populations in particular, as well as the colonization process in the Mediterranean basin and continental Europe.

The phylogeographical analysis of mtDNA haplotypes (Avice, 1994) supports the close relationship between populations from some Mediterranean

islands (Cyprus, Crete, Mallorca, Samos) and the European continent, as they all share haplotype A (Table 4). The Tyrrhenian Islands (Elba, Corsica and Sardinia), on the other hand, form a monophyletic group in the NJ-tree, based on nuclear genetic variation (Fig. 2), which is supported by the exclusive occurrence of haplotype D and high bootstrap values. The colonization history inferred for Canary Island populations based on sequence variation of the cytochrome b gene is not in agreement with the conclusions drawn by Estoup *et al.* (1996) based on sequence information of the gene COII (247 bp). They found a single substitution between haplotype I from the European continent and the haplotypes from the Tyrrhenian Islands and Tenerife (*B. canariensis*). The insular haplotypes were autapomorphic, which indicated that island haplotypes could be derived from an ancestral European continental stock (Estoup *et al.*, 1996). In agreement with the view advocated here, an old age of Canary Island populations has also been inferred in other cases, for example, for *Drosophila subobscura*, where the abundance of endemic nucleomorphs and their great divergence are likely to be the result of the very old age of these *Drosophila* populations rather than a consequence of drift and founder effects (Afonso *et al.*, 1990). Furthermore, the relationships among the insular populations of *B. canariensis* are in partial agreement with other animal taxa that have a long history on the archipelago. Phylogenetic studies on darkling beetles of the genus *Pimelia* (Juan *et al.*, 1995), also found strong evidence for a colonization route from east (Fuerteventura, Tenerife) to west (La Gomera, El Hierro). A slightly different dispersal scenario has been invoked for the lizard genus *Gallotia* (Thorpe *et al.*, 1994). Molecular data suggest that the western species, *G. galloti*, arose in Tenerife and dispersed westward in two independent pathways: north to La Palma and south to La Gomera and El Hierro (Thorpe *et al.*, 1994). In contrast to the cases of darkling beetles and lizards, where most islands harbour endemic subspecies or species (Thorpe *et al.*, 1994; Juan *et al.*, 1995), differentiation of bumble bee populations has apparently not led to speciation among the different Canary Island populations, despite significant genetic differentiation. These differences may be the result of different dispersal capabilities which determine inter-island gene flow. *Bombus* queens have indeed been observed to migrate long distances, sometimes also over open water (Mikkola, 1984). Whether the estimated levels of gene flow among populations of *B. canariensis* are sufficient to prevent speciation or whether the separation of the

insular gene pools is too recent for speciation to occur is not known.

In contrast, the case of Madeira seems to be different. Variability at microsatellite loci in this population was similar to Mediterranean island populations and larger than on the Canary Islands. This observation, and the occurrence of the continental and Mediterranean mtDNA haplotype A on Madeira is indicative of a different evolutionary history. The independent origin of the Madeira and Canary Island populations is also evidenced by the NJ-trees based on microsatellite markers (Fig. 2). Populations and individuals from Madeira are clearly more closely related to *B. terrestris* from the European continent and Mediterranean islands than to *B. canariensis*. Interestingly, also in the case of *Drosophila subobscura*, allozyme, chromosomal and mtDNA polymorphisms suggest that the Madeira populations are more related to those of the continent than to those of the Canary Islands (Larruga *et al.*, 1983; Afonso *et al.*, 1990). However, in contrast to *B. terrestris* s.l., populations of *D. subobscura* from Madeira carry several distinct mtDNA haplotypes (Afonso *et al.*, 1990).

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