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Fine-scale genetic structure of two carabid species with contrasted levels of habitat specialization

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Abstract

Using microsatellite markers, we compared the genetic structure of populations of two carabid species, one described as a generalist (commonly found in forest and in open habitats) and the other known as a forest specialist. Both species were sampled in the same forest plots, which were separated from each other by either open or forested areas. At the local scale considered (13.6 km separating the most distant plots), genetic differentiation was substantial for both species studied, but populations of the forest specialist *Carabus punctatoauratus* appeared to be more spatially structured than those of *C. nemoralis*. Isolation by distance analyses showed that nonforested areas are partial barriers to gene flow for both species studied, although more clearly for the forest specialist. Between and within forests, dispersal capacity of the generalist *C. nemoralis* was shown to be higher than that of the specialist *C. punctatoauratus*.

Keywords: carabid, Coleoptera, dispersal, forest landscape, gene flow, microsatellite

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Introduction

Genetic differentiation between populations is supposed to be high in fragmented habitats, especially due to restricted or absent migration between fragments. In continuous habitats where no obvious fragmentation prevents individual movements, genetic differentiation can also be present (Ehrich & Stenseth 2001). Local genetic drift associated with preferential dispersion between neighbouring areas may create genetic structure, a process called 'isolation by distance' (Wright 1943). The scale at which isolation by distance occurs depends on the dispersal behaviour of the species considered. Populations of long-range dispersive species can be weakly differentiated in continuous habitats, with no genetic isolation by distance (Ehrich & Stenseth 2001).

Dispersal rates of animals may vary according to habitat types. The nature of the landscape separating populations can thus have a different influence on the degree of their genetic differentiation (Nève *et al*. 1996; King 1997; Keyghobadi *et al*. 1999; Monaghan *et al*. 2002). No natural habitat is truly continuous. Although the effect on genetic differentiation of discrete structures that may act as barriers (e.g. valleys, ridges, rivers) has been abundantly demonstrated, subtle differences in habitats that may affect gene flow have been little studied (Keyghobadi *et al*. 1999). Species often have patchy geographical distributions (Newman & Squire 2001) and even in apparently continuous landscapes such as forests, populations of microhabitat specialist species could be restricted to local areas. The potential for the species to migrate through less favourable areas may act on the level of genetic differentiation between populations.

There has been considerable recent interest among conservation biologists in the identification of robust indicators to assess ecosystem responses to environmental changes (Lindenmayer *et al*. 2000; Lawton & Gaston 2001). In the case of forest ecosystems, carabids have been extensively used as indicators of changes generated by management (Lenski 1982; Parry & Rodger 1986; Niemelä *et al*. 1994; Butterfield *et al*. 1995; Greenberg & Thomas 1995; Beaudry *et al*. 1997) or fragmentation (Niemelä *et al*. 1993; Desender *et al*. 1999; Lövei & Cartellieri 2000; Heliölä *et al*. 2001; Koivula *et al*. 2002). Spatial distribution of carabids is often related to microhabitat features such as soil moisture (Niemelä *et al*. 1992), litter depth (Michaels & McQuillan 1995) and soil organic matter (Jukes *et al*. 2001). Most

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ecological studies based on carabid communities show a decline of forest specialist species when management intensity (Niemelä *et al*. 1994) or forest fragment isolation (Desender *et al*. 1999; Koivula *et al*. 2002) increase, or when forest fragment size decreases (Desender *et al*. 1999; Koivula *et al*. 2002). Generalist species found in different forest types and open areas were shown to be less sensitive than forest specialists to habitat changes (Niemelä 1997; Gliwicz & Glowacka 2000; Koivula *et al*. 2002). This intuitively implies higher dispersal capabilities across nonforested areas for generalists than for forest specialist species. Although forest insect specialists have often been suggested to be bad dispersers (Komonen *et al*. 2000; Koivula *et al*. 2002), tests of this hypothesis based on comparative assessment of migration levels are lacking.

One indirect method to estimate gene flow in natural populations is to use gene frequency data (Slatkin 1987). Comparative studies reporting genetic structure of populations of (at least) two species living in the same area with contrasted ecology are rare, and to our knowledge are restricted to mammal species (Matocq *et al*. 2000; Ehrich *et al*. 2001) or species living in aquatic ecosystems (Monaghan *et al*. 2002). Concerning carabids, several studies based on allozyme markers have shown that populations of specialist species can be strongly structured (Assmann 1990; Terlutter 1990; Assmann *et al*. 1994; Niehues *et al*. 1996; Assmann & Weber 1997). In this paper, we present a comparative study of the genetic structure of two carabid species living in the same area, and differing in their habitat requirements. *Carabus nemoralis* is abundant and widespread in Western Europe (Penev & Turin 1994). Although described as a forest species (Valemberg 1995; Jukes *et al*. 2001), it can also be found in relatively open fields (Lövei & Sunderland 1996). *Carabus punctatoauratus* [often considered as a synonym of *C. auronitens* (Assmann & Weber 1997)] is endemic to the Pyrénées and known to be a forest specialist (Assmann 1990). Both species are brachypterous (i.e. short-winged, therefore flightless), and share globally the same functional role as predators of slugs and earthworms in the forest ecosystem (Thiele 1977). Both are spring breeders, mainly laying eggs in spring or summer and with larval development in summer. Tenerals emerge in summer and autumn and overwinter in shelter sites (i.e. stumps, dead trunks, or directly in the soil) prior to reproducing the following spring.

For these two taxonomically-related species, we chose to work at a small spatial scale to minimize effects of differing habitat types or landscape history. Recent development of genetic markers such as microsatellites has made it feasible to study genetic variation at a local scale (Avise 1994). The questions addressed in this paper are: (i) is habitat specialization reflected in local patterns of genetic differentiation; (ii) are dispersal capabilities across nonforested areas higher for the forest generalist species than for the specialist; (iii) do either of these species qualify as an appropriate genetic model for studies dealing with the impact of forest fragmentation?

Materials and methods

Study area and sampling

The Plateau de Sault (42°52′ N, 2°53′ W) is characterized by the presence of large and continuous forests, which have been in existence for at least the past three centuries according to historical records. The total area of the plateau de Sault is around 650 km^2, and forests cover more than half of this total area (*c*. 380 km^2). In the forested area of the Plateau de Sault, we defined 26 sampling plots distant from each other by at least 450 m and at most 13.6 km. Pairs of plots were separated by various distances in terms of forested/open areas (Fig. 1). For example, Be.Q was equally distant from Ba02 and So29 (about 3.7 km), but was separated by about 1.4 km of open area from Ba02, and only by a continuous forest from So29.

Carabids were collected in each plot (between 14 and 30 individuals per plot) using pitfall traps. Traps were checked every week from May to the end of September 2000. Each individual was stored separately in alcohol before genetic analysis. From the 26 plots, a total of 586 *C. nemoralis*, and 559 *C. punctatoauratus* were collected. Three plots for *C. nemoralis* (Bc06, Cl15 and Pa18S) and one plot for *C. punctatoauratus* (Cl16) were not included in the analyses because of low numbers of captured individuals (less than 10 per plot).

Laboratory methods

DNA was extracted from one part of the leg of each individual using a cetyltrimethyl ammonium bromide (CTAB) protocol adapted from Doyle (1991).

Quantification of genetic variation in *C. nemoralis* was performed using 10 microsatellite loci cloned from *C. nemoralis* DNA [Cn 1/180, Cn 5/1, Cn 7/56, Cn 11/70, Cn 12/99, Cn 3/C, Cn 4/44, Cn 11/152, Cn 8/27 and Cn 3/145 (Brouat *et al*. 2002)]. For *C. punctatoauratus*, we used five microsatellite loci cloned from *C. punctatoauratus* DNA [Cp 2/19, Cp 1/121, Cp 1/92, Cp 2/125 and Cp 6/13 (Garnier *et al*. 2002)], and three other microsatellite loci developed on related species of *Carabus* [Cs 1/134 and Cs 2/10 on *C. splendens*, Csol 9170 on *C. solieri* (Garnier *et al*. 2002)]. Cross-species amplifications with primers developed on *C. nemoralis* or *C. punctatoauratus* did not give a reliable result for either of the two species (i.e. no PCR product, or band pattern of poor quality).

The polymerase chain reaction (PCR) amplifications and electrophoresis of the fragments on polyacrylamide gels were carried out as described in Brouat *et al*. (2002) for *C. nemoralis*, and in Garnier *et al*. (2002) for *C. punctatoauratus*.

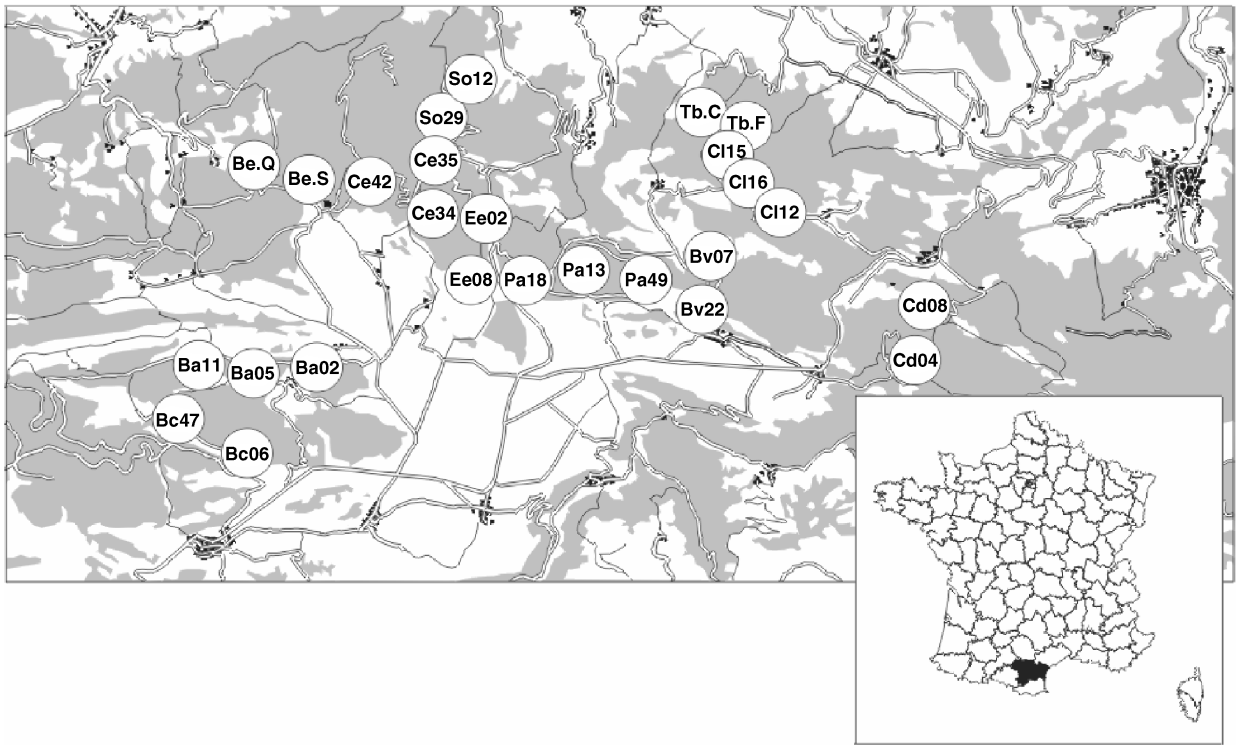

Fig. 1 Location of the study area and position of trapping plots. Open areas are represented in white and forests in grey.

Population structure analysis

Mean number of alleles per locus, observed (H_O) and expected (H_E) heterozygosities (Nei 1987) were calculated over all loci at each sampling location using the program POP100GENE 1.1.02 (http://www.ensam.inra.fr/URLB). Tests of conformation to Hardy–Weinberg equilibrium (HWE) and genotypic linkage disequilibrium were performed using Markov chain methods in GENEPOP 3.2 (Raymond & Rousset 1995). Markov chain methods in GENEPOP were also used to test for genotypic divergence among populations for all loci and population pairs. The extent of population differentiation was then quantified by computing the Weir & Cockerham's (1984) estimator of F_{ST} (θ) using GENEPOP 3.2. Ninety-five percent confidence intervals (CI) for means *F*-statistics were generated by jackknifing across loci (Weir 1990) using GENETIX 4.01 (Belkhir *et al*. 2001).

The procedure described in Kinnison *et al*. (2002) was employed to correct for multiple contrasts in tests of HWE and genotypic divergence. For each locus and each plot, we estimated the likelihood (*L*) of obtaining as many (or more) significant tests as actually obtained by chance using the binomial likelihood function (Chapman *et al*. 1999)

$$L = \sum_{i=r}^{n} (n!/(r!(n-r)!))(1-\alpha)^{n-i}(\infty)^{i}$$

where n is the total number of tests and r is the number of significant tests at a given α level of significance. When *L* was < 0.05, the null hypothesis of such a result by chance was rejected and the overarching hypothesis was considered supported.

Cavalli-Sforza & Edwards's (1967) chord distance (D_{CE}) was used to construct a population phenogram using Saitou & Nei's (1987) neighbour-joining algorithm. D_{CE} was chosen because it leads to a higher probability of depicting correct tree topology than other distances tested by Takezaki & Nei (1996). Confidence in tree topology was assessed by bootstrapping over loci (2000 iterations) using a personal program (J.-M. Cornuet, personal communication).

Under a model of isolation by distance, genetic distance between populations is expected to increase with geographical distance. Isolation by distance was analysed by regressing pairwise estimates of $F_{ST}/(1-F_{ST})$ against ln-distance between plots (Rousset 1997). Mantel tests were performed to test the correlation between matrices of genetic differentiation and geographical distance between sampled populations using GENEPOP 3.2. (10 000 permutations) (Raymond & Rousset 1995). Ninety five percent confidence intervals for slopes of the relationships between genetic differentiation and total geographical distance were performed using an adapted (Leblois *et al*. 2003)

nonparametric ABC bootstrap procedure from DiCiccio & Efron (1996). In this procedure, we considered genotypic data at each locus as independent replicates of the genealogical process. The ABC bootstrap procedure, which generates approximated bootstrap confidence intervals without real resampling, is useful for estimations with high computation time needs (Leblois *et al.* 2003).

Additionally, the spatial pattern of genetic variation was investigated using spatial autocorrelation analyses (Hardy & Vekemans 1999), which have the advantage over Mantel testing of providing results on the shape of the spatial relationship (Stow *et al.* 2001). Moran's *I* statistics for diploid multilocus genotypes were computed for 10 geographical distance classes using the program SPAGEDI 1.0 (O. Hardy and X. Vekemans; available at ohardy@ulb.ac.be). The significance of each Moran's *I* was calculated using a randomization procedure (10 000 permutations). Pairs of individuals belonging to the same plot were excluded from the analysis.

To discriminate the effects of geographical distance and forest discontinuities on genetic structure of both species, we also performed Mantel test analyses considering two other types of geographical distance: (i) distance through open habitats; and (ii) distance through forested areas. For these tests, we did not use the logarithm transformation for distances, as some of the pairwise distances through open habitats were null, plots being separated by only forested areas. Distances were measured using topographic maps (1 : 25 000 scale), the distance through forest and the distance through open habitat necessarily summing to the total distance.

Results

Intrapopulation genetic diversity

Basic statistics summarizing genetic diversity observed in each plot for the two *Carabus* species are presented in Table 1. Although all microsatellite loci were polymorphic in all local samples, genetic variability differed considerably among loci. The number of alleles per locus ranged from four to 23 for *C. nemoralis* (mean number of alleles per locus = 12.2 ± 6.01) and 25 for *C. punctatoauratus* (mean number of alleles per locus = 15.4 ± 7.23). Average values for numbers of alleles and heterozygosities were similar overall between sampled plots for each species. However, mean number of alleles and observed and expected heterozygosities were higher for the forest specialist *C. punctatoauratus* than for the forest generalist *C. nemoralis* (Mann–Whitney *U*-tests, $P < 0.0001$).

All loci for each of the two species were at HWE (Table 1). Only one plot deviated from HWE for each species (Table 1). The Be.S plot for *C. nemoralis* and the Cl15 plot for *C. punctatoauratus* exhibited significant heterozygote deficiencies ($P < 0.05$ in all cases). No linkage disequilibrium between loci occurred at any locality. Hence, independence among loci was assumed in the subsequent analyses.

Population differentiation

Microsatellites revealed significant genotypic differentiation among populations both in *C. nemoralis* ($0.002 < P < 0.05$ for each locus) and *C. punctatoauratus* ($P < 0.0001$ for each locus). Counts of significant ($P < 0.05$) pairwise genotypic tests of frequency differences by locus (Table 2) indicated numerous cases in which samples differed by more loci than expected by chance for the two species. The level of differentiation appeared to be higher in *C. punctatoauratus* (mean $F_{ST} = 0.017 \pm 0.002$) than in *C. nemoralis* (mean $F_{ST} = 0.008 \pm 0.001$), the 95% confidence intervals of the F_{ST} estimates being mutually exclusive. Tests for divergence were significant for 32% of pairs of populations with pairwise F_{ST} estimates ranging from 0.0001 to 0.03 for *C. nemoralis* (Table 2a), and for 49% of pairs of populations with pairwise F_{ST} estimates ranging from 0.006 to 0.059 for *C. punctatoauratus* (Table 2b).

For *C. nemoralis*, the population phenogram inferred from D_{CE} distance illustrated the overall lack of population grouping by geographical proximity. The tree was star-like, with all branches of approximately equal length and generally poorly supported (Fig. 2a). However, for *C. punctatoauratus*, the phenogram illustrated how populations clustered together depending on forest discontinuities in the plateau de Sault (Fig. 2b); the southwesternmost plots, Bc47, Bc06, Ba11, Ba05 and Ba02, clustered together in a highly-supported clade. These five plots are grouped in the same forest, separated from other forested areas of the plateau de Sault by open fields (Fig. 1). Similarly, the easternmost populations, Cd08 and Cd04, were found to form a highly-supported clade.

Isolation by distance

No pattern of isolation by distance was apparent for the generalist species *C. nemoralis* when total geographical distance (Fig. 3a; Mantel test, $P = 0.05$) or distance through forest (Fig. 4a; Table 3) were taken into account. However, isolation by distance through open areas was significant (Table 3; Fig. 4a), suggesting that open areas could act as barriers to gene flow for *C. nemoralis*. Slopes of the relationships concerning distance through open areas and distance through forests were significantly different ($F_{1;505} = 45.9$; $P < 0.0001$).

For *C. punctatoauratus*, genetic differentiation was positively correlated with total geographical distance (Fig. 3b; Mantel test, $P = 0.0004$). This significant positive correlation

Table 1 Diversity of allelic variation. N = range of sample size per sample and per locus, n = total number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity. Results of sample by locus and overall locus, and sample tests of conformance to Hardy–Weinberg expectations are provided. (a) *Carabus nemoralis*; (b) *C. punctatoauratus*. *Deviation from Hardy–Weinberg expectations ($P < 0.05$). †Failed likelihood method test by exceeding number of samples or loci expected to violate tests of Hardy–Weinberg equilibrium by chance

(a)

N		Ba02 26–27	Ba05 14–15	Ba11 20	Bc06 0	Bc47 16–17	Be.Q 26–28	Be.S† 27–28	Bv07 18	Bv22 17–18	Cd04 28–29	Cd08 30	Ce34 28–29	Ce35 29–30	Ce42 23–24	Cl12 29–30	Cl15 0	Cl16 15	Ee02 28–29	Ee08 29	Pa13 30	Pa18 0	Pa49 27	So12 28	So29 28–29	Tb.C 27–28	Tb.F 28
Cn1/180	n	4	5	7	—	4	7	6	4	5	6	6	5	5	6	5	—	5	6	6	6	—	6	5	6	7	7
	H_O	0.54	0.64	0.75	—	0.65	0.71	0.57*	0.67	0.78	0.59	0.63	0.55	0.6	0.79	0.5*	—	0.47	0.45	0.62	0.57	—	0.7	0.54	0.72	0.79	0.75
	H_E	0.54	0.70	0.71	—	0.73	0.71	0.73	0.64	0.79	0.7	0.68	0.64	0.69	0.74	0.66	—	0.5	0.51	0.71	0.63	—	0.67	0.72	0.72	0.75	0.76
Cn5/1	n	4	4	5	—	3	4	4	4	5	4	4	5	4	5	3	—	5	5	6	4	—	5	5	5	3	4
	H_O	0.56	0.64	0.60	—	0.24	0.5	0.22	0.28	0.44	0.54	0.47	0.43	0.53	0.58	0.38	—	0.53	0.38	0.66	0.3*	—	0.41	0.46	0.45	0.41	0.36
	H_E	0.52	0.65	0.65	—	0.38	0.61	0.27	0.38	0.38	0.47	0.44	0.42	0.5	0.61	0.37	—	0.49	0.34	0.52	0.55	—	0.55	0.49	0.44	0.43	0.42
Cn7/56	n	7	5	6	—	6	6	7	6	7	6	6	7	8	8	6	—	5	8	6	6	—	6	5	7	6	6
	H_O	0.74	0.80	0.90*	—	0.69	0.71	0.75*	0.78	0.83	0.72	0.73	0.76	0.9	0.78	0.63	—	0.73*	0.86	0.72	0.77	—	0.74	0.86	0.82	0.79	0.79
	H_E	0.73	0.77	0.65	—	0.72	0.75	0.77	0.77	0.81	0.76	0.72	0.77	0.79	0.84	0.75	—	0.78	0.84	0.77	0.73	—	0.75	0.74	0.84	0.75	0.78
Cn11/70	n	6	6	6	—	6	8	8	5	6	5	7	10	8	7	7	—	6	6	6	7	—	7	8	7	8	6
	H_O	0.59	0.67	0.60	—	0.82	0.64*	0.75*	0.72	0.67	0.55	0.67	0.55*	0.77	0.67	0.73	—	0.67	0.68	0.76	0.67	—	0.7	0.71	0.76	0.75	0.75
	H_E	0.61	0.64	0.74	—	0.76	0.78	0.78	0.77	0.7	0.73	0.72	0.77	0.73	0.76	0.75	—	0.75	0.71	0.66	0.75	—	0.67	0.81	0.8	0.78	0.76
Cn12/99	n	8	6	5	—	7	7	6	6	7	6	7	7	7	7	8	—	7	7	6	6	—	7	7	7	7	5
	H_O	0.82	0.6*	0.65	—	0.82	0.81	0.86	0.72	0.83	0.66*	0.67	0.69	0.76	0.65	0.8	—	1	0.83	0.86	0.93	—	0.7	0.79	0.79	0.82	0.5*
	H_E	0.82	0.81	0.78	—	0.77	0.75	0.82	0.79	0.85	0.74	0.68	0.69	0.8	0.73	0.74	—	0.83	0.74	0.83	0.77	—	0.81	0.8	0.79	0.71	0.64
Cn3/C	n	6	6	7	—	6	4	7	8	6	6	6	8	6	6	7	—	5	8	8	8	—	5	6	6	7	8
	H_O	0.63	0.67	0.50	—	0.59	0.57	0.75	0.67	0.67	0.69	0.63	0.69	0.73	0.54	0.57	—	0.73	0.69	0.86	0.8	—	0.7	0.64	0.64	0.68	0.68
	H_E	0.65	0.66	0.54	—	0.6	0.56	0.68	0.77	0.66	0.66	0.69	0.72	0.64	0.57	0.64	—	0.7	0.65	0.79	0.78	—	0.64	0.6	0.63	0.72	0.72
Cn4/44	n	5	5	7	—	3	5	4	4	5	4	6	7	7	5	4	—	4	5	6	5	—	4	5	7	5	4
	H_O	0.59	0.67	0.75	—	0.53	0.68	0.68	0.33*	0.94	0.79	0.67	0.79	0.73	0.58	0.73	—	0.4*	0.64	0.62	0.73	—	0.7	0.64	0.76	0.78	0.64
	H_E	0.69	0.65	0.74	—	0.43	0.64	0.63	0.59	0.77	0.67	0.7	0.74	0.76	0.68	0.71	—	0.7	0.65	0.69	0.64	—	0.58	0.69	0.75	0.71	0.6
Cn11/152	n	10	7	8	—	10	12	11	10	8	12	11	12	11	9	12	—	11	12	11	10	—	11	11	13	11	14
	H_O	0.74	0.73	0.85	—	0.88	0.79*	0.89	0.89	0.61	0.86	0.87	0.9	0.73*	0.78	0.8	—	0.8	0.86	0.76	0.8	—	0.85	0.89	0.79	0.89	0.96
	H_E	0.84	0.81	0.83	—	0.89	0.85	0.86	0.87	0.78	0.87	0.87	0.87	0.86	0.89	0.88	—	0.85	0.87	0.89	0.82	—	0.84	0.86	0.88	0.87	0.91
Cn8/27	n	3	3	3	—	3	3	3	3	2	3	3	4	4	4	4	—	3	2	3	3	—	3	3	3	4	3
	H_O	0.26*	0.13	0.5	—	0.24	0.43	0.46	0.11	0.28	0.35	0.27*	0.38	0.47	0.54	0.33	—	0.73	0.17	0.17	0.27	—	0.37	0.57	0.55	0.36	0.25*
	H_E	0.35	0.19	0.49	—	0.27	0.45	0.47	0.11	0.25	0.38	0.29	0.44	0.48	0.54	0.34	—	0.59	0.27	0.22	0.24	—	0.35	0.46	0.46	0.36	0.38
Cn3/145	n	8	7	6	—	7	9	7	8	7	9	11	10	11	9	8	—	9	11	8	11	—	8	10	9	12	10
	H_O	0.74	0.71	0.7	—	0.82	0.57	0.68	0.72	0.65	0.79	0.7	0.76	0.7*	0.78	0.8	—	0.73	0.72	0.86	0.83	—	0.7	0.64	0.68	0.89	0.79
	H_E	0.69	0.76	0.64	—	0.72	0.61	0.61	0.7	0.67	0.75	0.71	0.78	0.78	0.65	0.77	—	0.82	0.77	0.78	0.78	—	0.71	0.66	0.75	0.81	0.78
Across all loci																											
	n	61	54	60	—	55	65	63	58	58	61	67	75	71	66	64	—	60	70	66	66	—	62	65	70	70	67
	H_O	0.62	0.63	0.68	—	0.63	0.64	0.66	0.59	0.67	0.65	0.63	0.65	0.69	0.67	0.63	—	0.68	0.63	0.69	0.67	—	0.66	0.68	0.7	0.72	0.65
	H_E	0.64	0.66	0.68	—	0.63	0.67	0.66	0.64	0.67	0.67	0.65	0.68	0.7	0.7	0.66	—	0.7	0.64	0.69	0.67	—	0.66	0.68	0.71	0.69	0.67

Table 1 *Continued*
(b)

N		Ba02 22	Ba05 21–23	Ba11 20–22	Bc06 20–23	Bc47 20–23	Be.Q 22	Be.S 21–23	Bv07 21–22	Bv22 22–23	Cd04 20–23	Cd08 20–22	Ce34 20–23	Ce35 13–14	Ce42 21–22	Cl12 18–23	Cl15† 21–23	Cl16 0	Ee02 22–24	Ee08 20–22	Pa13 22–23	Pa18 21–23	Pa49 19–23	So12 21–23	So29 22–23	Tb.C 21–22	Tb.F 20–22
Cp1/121	*n*	4	5	6	4	4	7	6	4	4	4	4	5	5	6	4	4	—	4	6	4	4	4	6	7	4	4
	H_O	0.64	0.78	0.67	0.57	0.75	0.64	0.83	0.82	0.74	0.83	0.65*	0.48	0.77	0.86	0.73	0.52*	—	0.71	0.73	0.65	0.61	0.46*	0.74	0.65	0.64	0.59
	H_E	0.74	0.69	0.65	0.71	0.68	0.76	0.74	0.73	0.74	0.71	0.74	0.68	0.77	0.76	0.69	0.74	—	0.69	0.71	0.67	0.75	0.7	0.74	0.72	0.71	0.68
Cp1/92	*n*	3	2	2	3	3	3	3	3	3	3	3	3	3	3	4	4	—	3	3	4	3	3	3	3	3	3
	H_O	0.5	0.52	0.5	0.7	0.35	0.55	0.65	0.68	0.48	0.61	0.62	0.32*	0.43	0.55	0.91*	0.52	—	0.5	0.41	0.68	0.78	0.52	0.48	0.57	0.64	0.59
	H_E	0.55	0.49	0.49	0.53	0.41	0.61	0.63	0.63	0.55	0.58	0.62	0.59	0.54	0.57	0.64	0.63	—	0.54	0.53	0.59	0.61	0.55	0.42	0.6	0.67	0.66
Cp2/125	*n*	9	12	9	10	7	8	7	8	7	6	8	8	6	7	9	7	—	7	8	7	7	6	8	8	8	7
	H_O	0.77	0.91*	0.75	0.96	0.77	0.82	0.65	0.81	0.83	0.87	0.71	0.77	0.57*	0.82	0.87	0.86	—	0.71	0.73	0.78	0.78	0.68	0.7	0.65	0.86	0.91
	H_E	0.84	0.84	0.84	0.86	0.73	0.78	0.68	0.81	0.78	0.7	0.73	0.76	0.76	0.71	0.85	0.82	—	0.78	0.8	0.74	0.75	0.72	0.72	0.79	0.78	0.78
Cp2/19	*n*	9	9	9	8	8	7	8	11	11	9	9	12	8	10	10	11	—	15	12	8	13	11	11	10	12	9
	H_O	0.73	0.74	0.82	0.76	0.74	0.73	0.82	0.71	0.82	0.85	0.48*	0.81	0.86	0.82	0.74	0.67*	—	0.79	0.86	0.73	0.91	0.87	0.73	0.78	0.86	0.73
	H_E	0.79	0.82	0.85	0.78	0.85	0.81	0.85	0.66	0.79	0.81	0.78	0.86	0.82	0.82	0.76	0.83	—	0.89	0.81	0.84	0.82	0.85	0.7	0.84	0.86	0.83
Cp6/13	*n*	10	11	9	9	13	14	12	15	10	11	11	11	9	11	12	15	—	10	11	8	11	13	12	14	11	11
	H_O	0.91	0.87	0.96	0.87	0.91	0.73	0.7	0.82	0.83	0.87	0.91	0.83	0.79	0.68	1	0.96	—	0.79	0.91	0.78	0.83	1	0.91	1	0.73	0.91
	H_E	0.81	0.85	0.84	0.83	0.87	0.8	0.78	0.93	0.77	0.87	0.86	0.85	0.79	0.71	0.88	0.92	—	0.74	0.84	0.78	0.85	0.9	0.83	0.9	0.84	0.86
Cs1/134	*n*	10	11	9	11	12	10	9	7	7	9	8	7	6	8	8	10	—	9	8	9	8	7	7	7	8	10
	H_O	0.59*	0.87	0.76	0.74	0.86	0.91	0.7	0.91	0.82	0.62	0.73	0.85	0.71	0.67	0.61	0.62*	—	0.82	0.76	0.77	0.68	0.63	0.57*	0.78	0.68	0.59
	H_E	0.82	0.83	0.86	0.86	0.88	0.85	0.81	0.79	0.85	0.81	0.82	0.78	0.67	0.81	0.83	0.86	—	0.78	0.81	0.85	0.78	0.78	0.76	0.74	0.78	0.8
Cs2/10	*n*	6	8	7	5	6	5	6	5	5	5	5	6	6	7	3	7	—	3	4	5	4	4	5	6	4	4
	H_O	0.82	0.76	0.68	0.75	0.65	0.77	0.48*	0.59	0.7	0.91	0.81	0.7	0.64*	0.82	0.61	0.77	—	0.7	0.68	0.7	0.7	0.73	0.65	0.73	0.59	0.73*
	H_E	0.74	0.8	0.74	0.7	0.71	0.61	0.67	0.7	0.76	0.7	0.69	0.66	0.66	0.71	0.57	0.69	—	0.6	0.65	0.72	0.66	0.67	0.67	0.71	0.66	0.65
Csol9/170	*n*	13	14	12	14	15	12	12	12	11	13	11	11	7	10	10	11	—	12	9	13	11	8	11	11	10	10
	H_O	0.81*	0.87	0.91	0.83	0.91	0.91	0.91	0.91	0.78	0.96	0.91	0.76	0.79	0.77	0.73	0.96	—	0.67	0.85	0.91	0.81	0.86	0.91	0.96	0.91	0.8
	H_E	0.87	0.91	0.89	0.89	0.92	0.85	0.83	0.89	0.84	0.91	0.85	0.87	0.77	0.85	0.8	0.84	—	0.77	0.78	0.89	0.84	0.83	0.87	0.86	0.86	0.82
Across all loci																											
	n	64	72	63	64	68	66	63	65	58	60	59	63	50	62	60	69	—	63	61	58	61	56	63	66	60	58
	H_O	0.72	0.79	0.76	0.77	0.74	0.76	0.72	0.78	0.75	0.81	0.73	0.69	0.69	0.75	0.78	0.74	—	0.71	0.74	0.75	0.76	0.72	0.71	0.77	0.74	0.73
	H_E	0.77	0.78	0.77	0.77	0.76	0.76	0.75	0.77	0.76	0.76	0.76	0.76	0.72	0.74	0.75	0.79	—	0.72	0.74	0.76	0.76	0.75	0.71	0.77	0.77	0.76

Table 2 Pairwise F_{ST} values (below the diagonal) calculated for all loci, and counts of significant ($P < 0.05$) genotypic tests of allele frequency differences (above diagonal) between samples. (a) *Carabus nemoralis*, 10 microsatellite loci: by LM method, likelihood of two or more significant tests is 0.08 and likelihood of three or more significant tests is 0.01 (in bold); (b) *C. punctatoauratus*, eight microsatellite loci: by LM method, likelihood of two or more significant tests is 0.06 and likelihood of three or more significant tests is 0.006 (in bold)
(a)

	Ba02	Ba05	Ba11	Bc47	Be.Q	Be.S	Bv07	Bv22	Cd04	Cd08	Ce34	Ce35	Ce42	Cl12	Cl16	Ee02	Ee08	Pa13	Pa49	So12	So29	Tb.C	Tb.F
Ba02		0	2	1	**3**	**3**	1	1	**3**	0	2	1	1	1	1	**3**	2	0	2	1	**3**	**3**	**3**
Ba05	0.003		1	1	0	1	0	**3**	1	2	0	1	**3**	**3**	2	0	2	0	2	0	**4**	0	1
Ba11	0.015	0.007		**3**	1	**3**	**3**	**3**	**4**	**3**	2	**3**	**3**	**3**	**3**	2	**3**	**4**	2	1	**5**	**3**	2
Bc47	0.023	0.001	**0.015**		2	0	0	1	2	2	2	1	1	2	**3**	2	2	1	1	2	**4**	2	1
Be.Q	**0.012**	0.007	0.005	0.016		2	1	**3**	1	2	2	1	2	3	2	**4**	**3**	2	1	0	**3**	**3**	1
Be.S	**0.021**	0.02	**0.011**	0.015	0.008		1	**4**	2	1	1	2	**3**	2	1	2	**3**	**3**	2	0	**4**	2	2
Bv07	0.007	0.004	**0.023**	0.005	0.009	0.008		1	0	0	1	1	**3**	0	1	0	2	0	0	1	**3**	1	0
Bv22	0.01	**0.022**	**0.019**	0.023	**0.021**	**0.015**	0.013		2	2	**3**	1	**3**	0	**3**	**4**	2	**3**	1	**3**	2	2	**3**
Cd04	**0.008**	0.005	**0.012**	0.008	0	0.006	−0.002	0.009		2	1	1	**3**	0	0	2	2	0	2	1	**3**	2	0
Cd08	0.006	0.015	**0.016**	0.023	0.004	0.007	0.004	0.009	0.002		0	1	1	0	**3**	**3**	2	1	1	1	**5**	2	1
Ce34	0.01	0.008	0.007	0.022	0.007	0.005	0.005	**0.013**	0.003	−0.002		0	1	2	1	1	**3**	0	2	2	1	1	**3**
Ce35	0.005	0.013	**0.007**	0.019	0.008	0.011	0.005	0.001	−0.001	0.004	−0.002		2	2	0	1	**3**	0	1	0	1	2	**3**
Ce42	0.012	**0.009**	**0.008**	0.015	**0.000**	**0.014**	**0.015**	**0.014**	**0.002**	0.01	0.01	0.004		**3**	**5**	**5**	**5**	**5**	2	2	**3**	**3**	**3**
Cl12	0.007	**0.007**	**0.013**	0.011	0.007	0.01	0.003	0.006	−0.005	0.001	−0.002	0.001	**0.006**		1	2	**3**	2	2	**3**	**5**	0	0
Cl16	0.007	0.019	**0.018**	**0.032**	0.025	0.012	0.018	**0.026**	0.004	**0.015**	0.001	0.002	**0.021**	0.006		1	**3**	2	0	1	2	1	0
Ee02	**0.015**	0.015	0.016	0.021	**0.015**	0.02	0.005	**0.029**	0.004	**0.013**	0.001	0.008	**0.021**	0.004	0.012		**4**	**4**	1	**4**	**5**	**3**	2
Ee08	0.015	0.009	**0.021**	0.015	**0.017**	**0.016**	0.009	0.01	0.006	0.007	**0.008**	**0.009**	**0.02**	**0.015**	**0.02**	**0.017**		**3**	**4**	**3**	**5**	2	2
Pa13	0.006	0.001	**0.016**	0.013	0.009	**0.017**	−0.01	**0.018**	−0.001	0.006	0.003	0.004	**0.014**	0.004	0.014	**0.01**	**0.008**		1	0	**4**	1	1
Pa49	0.011	0.007	0.009	0.003	0.009	0.013	0.007	0.013	0.002	0.007	0.005	0.003	0.013	0.01	0.013	0.009	**0.006**	0.007		1	**3**	2	1
So12	0.007	0.005	0.004	0.007	−0.005	0.002	0.005	**0.006**	0	0.001	0.002	0	0.003	**0.004**	0.015	**0.017**	**0.01**	0.004	0.004		**3**	**3**	2
So29	**0.019**	**0.026**	**0.018**	**0.028**	**0.011**	**0.018**	**0.012**	0.011	**0.009**	**0.014**	0.003	−0.002	**0.013**	**0.013**	0.019	**0.012**	**0.017**	**0.017**	**0.008**	**0.004**		**4**	**4**
Tb.C	**0.02**	0.008	**0.016**	0.015	**0.012**	0.012	0.007	0.01	−0.004	0.007	−0.005	0.002	**0.012**	−0.004	0.009	0.007	0.009	0.005	0.006	**0.007**	**0.007**		0
Tb.F	**0.019**	0.012	0.012	0.012	0.002	0.006	0.004	**0.012**	−0.005	−0.002	**−0.003**	**0.005**	**0.006**	−0.001	0.013	0.005	0.007	0.007	0.002	0.004	**0.008**	−0.007	

Table 2 *Continued*
(b)

	BA02	BA05	BA11	BC06	BC47	BE.Q	BE.S	BV07	BV22	CD04	CD08	CE34	CE35	CE42	CL12	CL15	EE02	EE08	PA13	PA18	PA49	SO12	SO29	TB.C	Tb.F
BA02		0	1	**3**	1	**5**	**5**	**3**	**4**	**3**	**5**	**3**	**3**	**4**	**4**	**4**	4	4	2	**3**	**5**	**4**	**5**	**4**	2
BA05	0.004		0	2	0	**4**	2	**3**	**3**	**4**	**5**	**3**	**4**	2	**4**	**3**	**4**	**4**	**3**	**4**	**4**	2	2	**3**	**3**
BA11	0.008	−0.004		0	1	**4**	**3**	**4**	**4**	**5**	**4**	**3**	**3**	**4**	**4**	**5**	**6**	**5**	**4**	**4**	**5**	**4**	2	**4**	2
BC06	**0.018**	0.004	0.001		1	**5**	**7**	**4**	**4**	**4**	**5**	**5**	**4**	**6**	**4**	**5**	**5**	2	**4**	5	**5**	**5**	**3**	**5**	**4**
BC47	0.016	0.003	0.002	0.016		**4**	**4**	**3**	**5**	**4**	**4**	**4**	**6**	1	**4**	2	**4**	**4**	**4**	**4**	**4**	**5**	**6**	**3**	2
BE.Q	**0.035**	**0.019**	**0.017**	**0.03**	**0.023**		0	**5**	**5**	**6**	**5**	**3**	**4**	0	**4**	**4**	**5**	**3**	**4**	**5**	**5**	2	2	**3**	**4**
BE.S	**0.023**	0.017	**0.012**	**0.031**	**0.025**	−0.005		2	**5**	**4**	**3**	1	1	0	**3**	**3**	**3**	2	1	**4**	**3**	2	0	1	2
BV07	**0.035**	**0.021**	**0.027**	**0.024**	**0.046**	**0.031**	0.027		1	0	2	0	1	**3**	1	0	1	1	2	0	1	1	2	0	0
BV22	**0.024**	**0.011**	**0.019**	**0.017**	**0.028**	**0.021**	**0.017**	0.006		**3**	1	2	**3**	**4**	2	1	2	2	1	1	1	**4**	**4**	1	2
CD04	**0.029**	**0.02**	**0.023**	**0.027**	**0.041**	**0.032**	**0.024**	0.009	**0.016**		1	1	2	**4**	**4**	2	**4**	**6**	1	2	2	**5**	**5**	1	**4**
CD08	**0.032**	**0.022**	**0.03**	**0.026**	**0.042**	**0.036**	**0.027**	0.017	0.013	0.009		2	**4**	**3**	2	2	**3**	**3**	1	2	**4**	**6**	**4**	2	**3**
CE34	**0.022**	**0.011**	**0.006**	**0.025**	**0.028**	**0.007**	−0.003	0.004	0.005	0.004	0.02		0	0	0	1	1	0	0	0	1	1	0	0	1
CE35	**0.041**	**0.038**	**0.033**	**0.042**	**0.055**	**0.018**	0.011	0.01	**0.018**	0.022	**0.044**	0.005		2	**3**	2	1	1	1	1	1	1	2	1	1
CE42	**0.03**	0.012	**0.015**	**0.025**	0.024	−0.006	−0.004	**0.028**	**0.015**	**0.02**	**0.027**	0.003	0.011		**3**	2	**3**	2	2	2	**3**	1	1	**3**	**4**
CL12	**0.037**	**0.024**	**0.018**	**0.027**	**0.036**	**0.015**	**0.016**	0.007	0.01	**0.022**	0.023	0.003	**0.019**	**0.019**		0	1	1	2	0	1	**3**	2	1	0
CL15	**0.019**	**0.011**	**0.01**	**0.019**	0.016	**0.013**	**0.009**	0.002	0.003	0.016	0.013	0	0.014	0.012	−0.007		1	1	1	0	2	2	1	0	0
EE02	**0.03**	**0.028**	**0.025**	**0.043**	**0.039**	**0.02**	**0.011**	0.018	0.012	**0.027**	**0.029**	0.003	0.011	**0.017**	0.014	0.011		2	1	0	**3**	2	2	1	1
EE08	**0.043**	**0.032**	**0.028**	0.017	**0.053**	**0.022**	0.023	0.004	0.006	**0.029**	**0.034**	0.01	0.002	0.026	0.015	0.016	0.025		0	2	2	2	**3**	1	0
PA13	0.015	**0.011**	**0.013**	**0.021**	**0.021**	**0.02**	0.008	0.012	0.002	0.006	0.017	−0.006	0.018	0.01	0.01	0.004	0.001	0.016		1	0	**3**	**3**	0	1
PA18	**0.03**	**0.023**	**0.024**	**0.028**	**0.038**	**0.019**	**0.013**	−0.003	0.003	0.007	0.026	−0.004	−0.007	0.015	0.005	0.002	0.004	0.006	0.003		0	2	**3**	0	0
PA49	**0.035**	**0.028**	**0.038**	**0.037**	**0.045**	**0.026**	**0.025**	0.01	0.006	0.014	**0.033**	0.006	0.017	**0.027**	0.009	0.009	**0.014**	0.016	0.003	−0.006		2	1	0	1
SO12	**0.059**	0.04	**0.049**	**0.041**	**0.075**	0.023	0.022	0.012	**0.024**	**0.028**	**0.044**	0.013	0.007	0.013	**0.028**	0.032	0.039	0.011	**0.028**	0.012	0.022		2	**3**	**3**
SO29	**0.019**	0.013	**0.015**	**0.031**	0.031	0.01	0.001	0.007	**0.01**	**0.023**	**0.013**	−0.003	0.016	0.014	0.002	0	0.006	**0.019**	**0.006**	**0.009**	0.01	0.022		**3**	1
TB.C	**0.025**	**0.023**	**0.026**	**0.028**	**0.041**	**0.02**	0.011	−0.001	0.003	0.01	0.016	−0.007	0.007	**0.017**	0.013	0.007	0.005	0.004	−0.001	0.001	0.009	**0.019**	**0.007**		0
TB.F	0.014	**0.017**	0.017	**0.025**	0.028	**0.02**	0.008	0.002	0.003	**0.026**	**0.025**	0.001	0.011	**0.021**	0	−0.009	0	0.013	0.001	0	0.005	**0.029**	−0.005	−0.003	

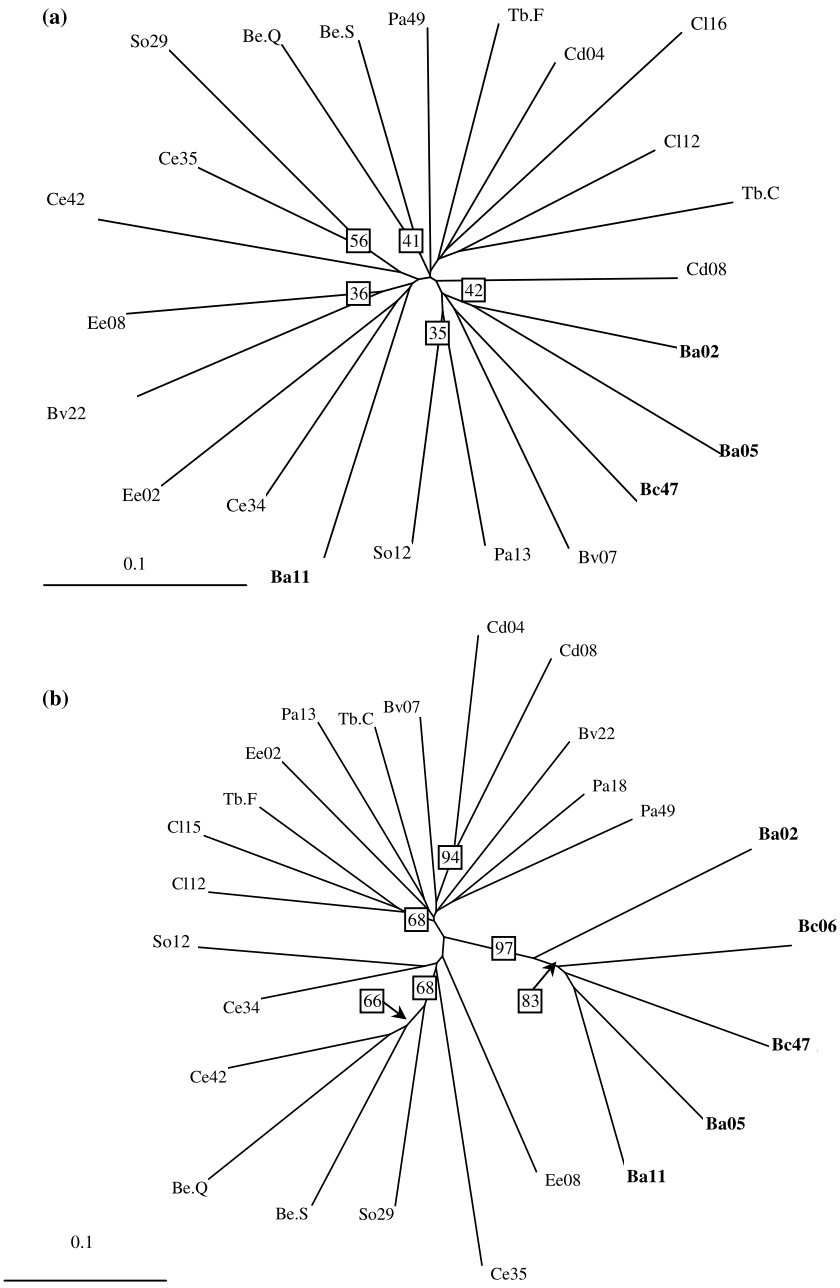

Fig. 2 Neighbour-joining trees based on distances between populations estimated from Cavalli–Sforza and Edwards's chord distances. Values at nodes are bootstrap percentages assessed over loci (2000 iterations); only the highest values are presented. Bold letters correspond to isolated southernmost plots. (a) The forest generalist *Carabus nemoralis*. (b) The forest specialist *C. punctatoauratus*.

Table 3 Isolation by distance (Mantel tests) through forests and through open habitats for *Carabus nemoralis* (forest generalist) and *C. punctatoauratus* (forest specialist). The slope of the relationship between geographical distance and ($F_{ST}/(1 - F_{ST})$) indicates the strength of the isolation

	C. nemoralis			*C. punctatoauratus*		
Distance	Slope	r^2	*P*	Slope	r^2	*P*
Through open habitats	0.001	0.08	**0.004**	0.004	0.27	**0.0000**
Through forests	–0.0001	3E-6	0.54	0.002	0.15	0.0000

remained when isolated southernmost (Ba02, Ba05, Ba11, Bc06, Bc47) and easternmost (Cd04, Cd08) plots were removed from the analysis ($r^2 = 0.15$; slope of the relationship = 0.006; Mantel test: $P < 0.0001$). The correlations between genetic differentiation and distance through open areas or distance through forests were also significant (Table 3; Fig. 4b). The regression coefficient (r^2) was two times higher for distances through open areas than for distances through forests (Table 3), and slopes of the relationships were significantly different ($F_{1;599} = 210.9$; $P < 0.0001$).

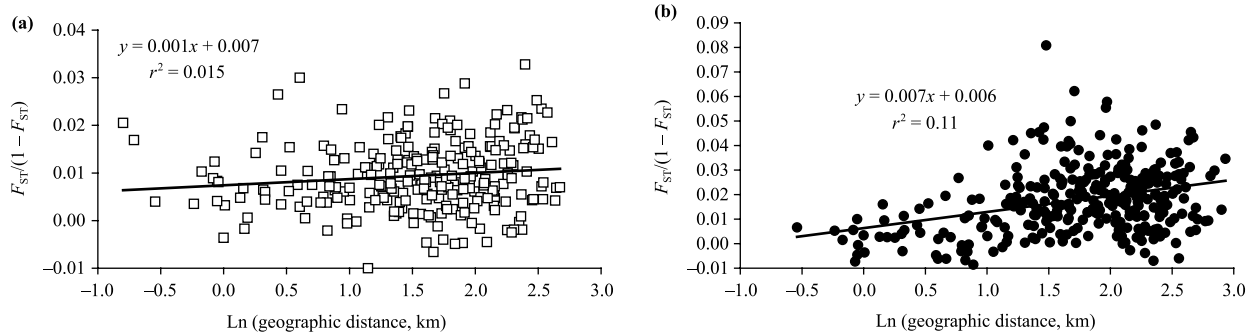

Fig. 3 Relationship between logarithms of geographical distances and genetic dissimilarities [estimated as $F_{ST}/(1-F_{ST})$] for each species. (a) The forest generalist *Carabus nemoralis*. (b) The forest specialist *C. punctatoauratus*.

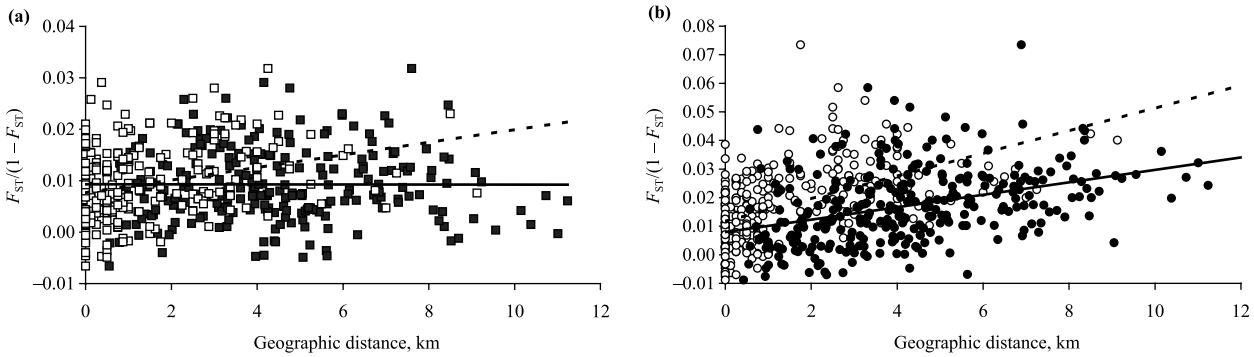

Fig. 4 Comparative effect of open areas and forests on isolation by distance for the two carabid species. Relationships between geographical distances and genetic dissimilarities [estimated as $F_{ST}/(1-F_{ST})$]. White symbols: geographical distance between plots through open areas (dotted line); black symbols: geographical distance between plots through forests (continuous line). (a) The forest generalist *Carabus nemoralis*. (b) The forest specialist *C. punctatoauratus*.

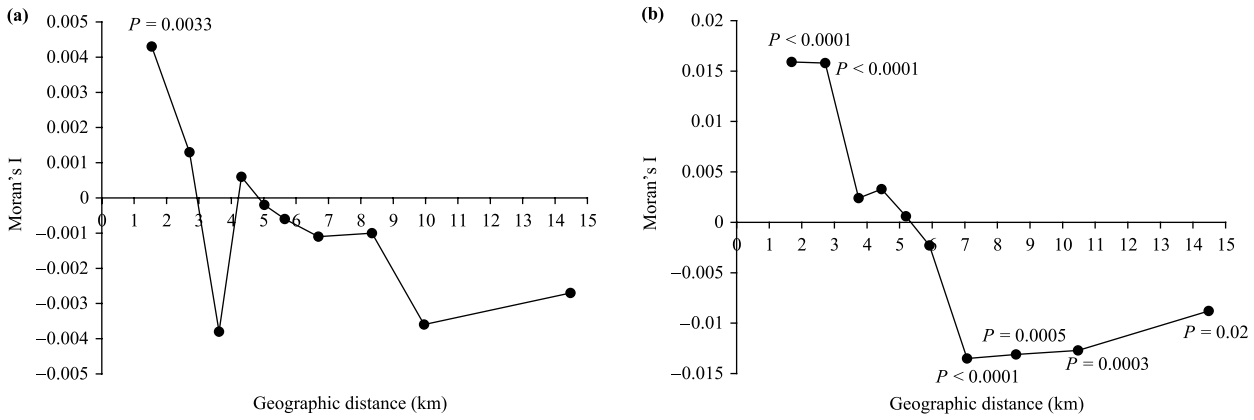

Fig. 5 Spatial autocorrelogram estimated from multilocus microsatellite genotypes. Probabilities are reported for significant Moran's *I*. (a) The forest generalist *Carabus nemoralis*. (b) The forest specialist *C. punctatoauratus*.

For the relationships between genetic differentiation and total geographical distance, ABC bootstrap procedures gave 95% confidence intervals congruent with results of Mantel tests and smartly overlapping for the two species (between 0 and 0.0045 for *C. nemoralis*, and between 0.0042 and 0.0095 for *C. punctatoauratus*).

Spatial autocorrelation

The autocorrelation values were systematically higher for *C. punctatoauratus* than for *C. nemoralis* (Fig. 5). For the forest generalist *C. nemoralis*, there was no clear pattern of variation in spatial autocorrelation indices, as only the first

Moran's *I* was significant (Fig. 5a). For *C. punctatoauratus*, a strictly decreasing pattern was observed (Fig. 5b), indicating isolation by distance. The autocorrelogram indicated a patch size of approximately 5 km for this species (intercept with *x* axis).

Discussion

Genetic diversity

For each species, all sampled populations were similarly polymorphic at all loci. Levels of genetic diversity observed for *C. nemoralis* and *C. punctatoauratus* were relatively high compared with those obtained, also with microsatellite loci, for the alpine carabid species *C. solieri* (Rasplus *et al*. 2001). Allele numbers and heterozygosity values were higher for *C. punctatoauratus* than for *C. nemoralis*, suggesting a higher genetic diversity for the first species. However, the fact that two different sets of microsatellite loci were used for each of the species renders the comparison of polymorphism levels difficult.

For each of the two species, heterozygote deficiencies were found in one plot. Sampling of related individuals can produce heterozygote deficiencies. One hypothesis is that sampled plots could correspond to relatively unfavourable environment for carabids, and be occupied by small resident populations. In accordance with this hypothesis, the plot that was not at HWE for *C. nemoralis* corresponded to a dense fir stand, and C. *nemoralis* is known to occur at lower abundance in such habitat (C. Brouat, unpublished results). The plot Cl15, in which the population of *C. punctatoauratus* was not at HWE, was the most dense of the forested plots. Alternatively, admixture of differentiated gene pools (Wahlund effect) in sampled populations can also produce heterozygote deficiencies. For example, individuals sampled early or late in the activity season could belong to genetically-different cohorts, and considering them together as a population could lead to an observed HW disequilibrium. This hypothesis seems unlikely, as there is no relationship between obtained mean F_{IS} values and the sampling duration for each plot for either of the two species (data not shown).

Genetic differentiation between populations

At the local scale considered, with only 13.6 km separating the most distant populations, genetic differentiation was substantial for both species studied. Even populations in the same forested area distant from each other by only 1 km for *C. nemoralis* (Ce42–BeS) and 2 km for *C. punctatoauratus* (Ce42–Ee02) were detected as genetically distinct.

The overall level of differentiation observed in these *Carabus* species (F_{ST} values ranging from –0.01 to 0.03 for *C. nemoralis*, and from –0.009 to 0.07 for *C. punctatoauratus*) was relatively modest when compared with that reported for the related species *C. solieri* at a similar spatial scale (Rasplus *et al*. 2001: F_{ST} values ranging from 0.09 to 0.12 for populations separated by less than 15 km). The area that we studied was, however, less heterogeneous (in terms of altitude and forest type) than that studied in the Alps for *C. solieri*.

As illustrated by phenograms (Fig. 2) and autocorrelograms (Fig. 5), populations of the forest specialist *C. punctatoauratus* appeared to be more spatially structured than those of *C. nemoralis*. F_{ST} estimates and the number of pairs of genotypically-differentiated populations were higher for *C. punctatoauratus* than for *C. nemoralis*. Theoretical considerations have shown that the level of genetic differentiation between populations is maximized by homozygosity (Hedrick 1999). In our case, F_{ST} for *C. nemoralis* could theoretically attain higher values than for *C. punctatoauratus* as mean homozygosity was found to be higher for *C. nemoralis*. Assuming that 10 microsatellite loci gave sufficient information to estimate genetic differentiation, the lower estimation of F_{ST} for *C. nemoralis* seems thus to reflect a weaker genetic differentiation between populations of this forest generalist species compared with *C. punctatoauratus*.

The geographical pattern of variation observed at microsatellite loci for *C. punctatoauratus* and *C. nemoralis* could have been shaped by isolation by distance processes due to limited dispersal. As they are both brachypterous, long-range dispersal seems unlikely. Higher genetic differentiation between populations of *C. punctatoauratus* may reflect lower levels of gene flow through the Plateau de Sault for this species than for *C. nemoralis*. Radio-tracking experiments have been reported in the literature for both species, giving higher dispersal distances for *C. nemoralis* [mean distance of 50 m per night and per individual (Kennedy 1994)], than for *C. punctatoauratus* [recorded under its synonym *C. auronitens*: mean distance per night and per individual between 0.4 and 4.7 m (Niehues *et al*. 1996)]. However, these experiments were not conducted in similar habitats (arable fields for *C. nemoralis*, forests for *C. auronitens*), rendering the comparison of results impossible. Moreover, observation of individual movements may lead to underestimated dispersal levels as distance detection of tagged individuals is limited (Squire & Newman 2002).

Understanding how genetic differentiation between populations varies with geographical distance can help to determine whether genetic differentiation is primarily due to limited dispersal or to more complex demographic processes (e.g. Leblois *et al*. 2000). At mutation–migration–drift equilibrium, and for species with limited dispersal in space such as those studied here, genetic differentiation is expected to increase with geographical distance (Slatkin 1993; Rousset 1997). However, only one of the two species

that we studied clearly conformed to these theoretical expectations.

For the forest generalist *C. nemoralis*, no relationship was found between genetic differentiation and total geographical distance or distance through forests. The absence of an observable pattern of isolation by distance may suggest that populations of *C. nemoralis* have not yet reached a drift–migration equilibrium, due to recent colonization of the region or to mixing events (Slatkin 1993). Alternatively, gene flow and random dispersal at a range equivalent to the fine geographical scale that we considered could explain the observed lack of isolation by distance and the weakness of spatial autocorrelation for individual genotypes.

Isolation by distance cannot be a valuable hypothesis to explain why some plots appeared to be genetically differentiated from others belonging to the same forest (Table 3). One hypothesis, other than chance, could be that this differentiation is transient and produced by extinction–recolonization dynamics through founder events (McCaughley 1993; Squire & Newman 2002). Alternatively, genetic differentiation between plots and neighbouring ones could be explained by locally unfavourable habitats inhabited by small populations having arisen from few individuals. For example, individuals sampled in plot Ce42, which corresponds to a young and dense fir stand presumably unfavourable for *C. nemoralis* (C. Brouat, unpublished results), were genetically differentiated from individuals of neighbouring plots.

A positive correlation was found between genetic differentiation and distance through open areas for *C. nemoralis*. This result suggests that nonforested areas are partial barriers to gene flow for this species. Although considered as eurytopic by several authors (e.g. Kennedy 1994; Valemberg 1995; Lövei & Sunderland 1996; Luff 1998), our results and others (Wallin 1986; Beaudry *et al*. 1997; Jukes *et al*. 2001) show that *C. nemoralis* could be more dependent on forest than suggested by its eurytopic qualification, at least in some parts of its range.

For the forest specialist *C. punctatoauratus*, genetic differentiation is positively correlated with total geographical distance, distance through open areas and distance through forests. The coefficient of determination (r^2) concerning distance through open areas was almost twice that for distance through forest, suggesting that open areas are strong barriers to gene flow for *C. punctatoauratus*. Dispersal of *C. punctatoauratus* through forests could be limited by subtle differences of microhabitat, such as altitude, canopy cover, humidity, or physical barriers such as roads. The broad habitat requirements of Carabus species are generally known, but there is still no evidence to suggest which environmental variables are responsible for their observed pattern of distribution (Niemelä 1996).

Results of Mantel tests and bootstrap confidence intervals suggested that isolation by distance is more clearly implicated in population genetic differentiation for *C. punctatoauratus* than for *C. nemoralis*. This was confirmed by comparison of regression slopes obtained for each species between genetic differentiation and total geographical distance. Genetic diversity levels such as those obtained for the two species (i.e. H_O between 0.6 and 0.8) are not likely to bias the estimation of slopes in isolation by distance analyses (Leblois *et al*. 2003). The observed difference in the regression slopes between the two species cannot therefore be explained by differences in genetic diversity.

When applied at a small spatial scale, the inverse of the regression slope between genetic differentiation ($F_{ST}/1 - F_{ST}$) and geographical distance provides an estimate of the product of population density (D) by the second moment of the parental axial distance of dispersal (σ^2) (Rousset 1997; Estoup *et al*. 1998), here 61.6 for *C. nemoralis* and 12.1 for *C. punctatoauratus*. Population densities (D) of *C. nemoralis* are clearly lower than those of *C. punctatoauratus* in the Plateau de Sault (C. Brouat, unpublished results). This also supports the idea of higher dispersal capacities for the forest generalist *C. nemoralis* than for the forest specialist *C. punctatoauratus*, both across forests and across open areas.

Implications for conservation

Even at a small spatial scale, *C. nemoralis* and *C. punctatoauratus* seem to be valuable indicators of the forest fragmentation level, as patterns of genetic differentiation for both species were shown to reflect the fragmentation level of the forest habitat. For the forest specialist *C. punctatoauratus*, subtle ecological variables (other than presence/absence of forest between populations) could also explain patterns of genetic differentiation in a single forest. Further studies are necessary to determine whether populations of specialist carabids are good genetic indicators for monitoring the impact of natural and anthropogenic (e.g. management) perturbations in forests.

Common species may serve as model organisms for rare and endangered species (Knutsen *et al*. 2000) for which sampling sizes would be too small for genetic study. *Carabus punctatoauratus* is abundant and widespread in the Pyrénées. However, *C. auronitens* (probably synonym with *C. punctatoauratus*), although widely distributed in northernmost parts of France and Western Europe, is threatened in a large part of its range and is only present in large and old forests (Desender *et al*. 1999). Its decline is partly attributed to forest fragmentation, which continues today (Hansen *et al*. 1991; Desender *et al*. 1999; Heliölä *et al*. 2001) especially where agriculture dominates (Jennersten *et al*. 1997). Our results indicate that forest fragmentation could

lead to a reduction in genetic variation among populations of forest specialist *Carabus* and have an impact on their long-term survival (Templeton *et al*. 1990).

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