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Effect of Habitat Fragmentation on the Genetic Diversity and Structure of Peripheral Populations of Beech in Central Italy

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Abstract

Fragmentation can affect the demographic and genetic structure of populations near the boundary of their biogeographic range. Higher genetic differentiation among populations coupled with lower level of within-population variability is expected as a consequence of reduced population size and isolation. The effects of these 2 factors have been rarely disentangled. Given their high gene flow, anemophilous forest trees should be more affected, in terms of loss of genetic diversity, by small population size rather than geographic isolation alone. We studied the impact of distance from the main range (a measure of isolation) and reduced population size on the within-population and among population components of genetic variability. We assayed 11 isozyme loci in a total of 856 individuals in 27 marginal populations of European beech (*Fagus sylvatica* L.) in Central Italy. Populations were divided into 3 groups with an increasing level of fragmentation. In the most fragmented group, the within-population genetic variability was slightly smaller and the among population differentiation significantly larger than in the other 2 groups. Isolation-by-distance was lost when only pairs of populations involving at least one from the most fragmented group were considered and maintained in the other groups. These results support the role of random genetic drift having a larger impact on the most fragmented group, whereas gene flow seems to balance genetic drift in the 2 less fragmented ones. Given that average distance from the main range is not different between the intermediate and the most fragmented group, but average population size is smaller, we can conclude that gene flow is effective, even at relatively long distances, in balancing the effect of fragmentation if population size is not too small.

Key words: Fagus sylvatica, *gene flow, genetic drift, genetic variability, isolation, isozymes*

In widespread species, population fragmentation is a common feature near the outer boundary of the biogeographic range, where small and relatively isolated peripheral populations are often found (Hampe and Petit 2005). Fragmentation can affect the demographic and genetic structure of these populations (Young et al. 1996; Eckert et al. 2008). In long-lived forest trees, anthropic pressure, as well as changes in climatic or other environmental conditions, can make discontinuities more pronounced on the border of biogeographic ranges (Ledig 1992; Hewitt 1993; Hamrick 2004).

Fragmentation subdivides populations in smaller units and may impose barriers to migration (Ledig 1992; Fahrig 2003). Smaller population size may lead to random loss of genetic variability by genetic drift; whereas isolation, decreasing among population connectivity through dispersal, may cause the prevalence of genetic drift over gene flow and increase differentiation among populations (Ouborg et al. 2006). Erosion of genetic variability within populations, which ranges from loss of rare alleles and lower proportion of expected heterozygotes to complete fixation of alleles, can lead to a higher degree of inbreeding within populations. This may lead to a reduction in fitness and fecundity (Keller and Waller 2002; Reed et al. 2003). The smaller and the more isolated the populations are, the greater the expected effects. In the long term, genetic impoverishment can reduce the species' evolutionary potential to respond to environmental changes and

increase extinction risk (Gilpin and Soulè 1986; Jump and Penuelas 2005; Willi et al. 2006).

Wright's island model postulates that population differentiation depends on both geographic isolation, which in turn depends on distance from the continental population, and population size, which is mainly affected by environmental conditions (Wright 1940). The balance between the impacts of geographic and demographic factors strongly depends on the biological characteristics of the species, and the different effects of these 2 factors have been rarely disentangled (Ouborg et al. 2006). High dispersal via pollen and/or seeds was considered an explanation for the limited impact of fragmentation on genetic diversity and structure in forest tree populations (Hamrick 2004; Piotti 2009). However, it is not clear to what extent gene flow prevails over reduced population size in reducing or delaying fragmentation effects.

Empirical studies of forest species partially reflect this idiosyncrasy (Kramer et al. 2008). No evidence of fragmentation influence on genetic parameters was found in several studies (e.g., Collevatti et al. 2001; Lemes et al. 2003; Muir et al. 2004; Gapare and Aitken 2005). When genetic traces of fragmentation have been found, they spanned from changes in mating system parameters (Aldrich and Hamrick 1998; Fuchs et al. 2003) to the detection of severe genetic bottlenecks in remnant populations (Jump and Penuelas 2006). Significant fragmentation effects were found on the within-population genetic diversity in *Elaeocarpus grandis* (Rossetto et al. 2004), *Abies alba* (Piovani et al. 2010), and *Quercus ilex* (Ortego et al. 2010), on the *among* populations differentiation in *Sorbus aucuparia* (Bacles et al. 2004) and *Pinus sylvestris* (Scalfi et al. 2009), and on both in *Corylus avellana* (Persson et al. 2004), *Acer saccharum* (Baucom et al. 2005), *S. torminalis* (Rasmussen and Kollmann 2008), and *Fagus sylvatica* (Jump and Penuelas 2006).

In our study, we assess the impact of increasing fragmentation levels on genetic variation within and among 27 marginal populations of European beech (*F. sylvatica* L.) in Central Italy. We sampled 3 groups of populations previously classified according to their level of geographical and ecological marginality and thus characterized by different fragmentation levels (low, intermediate, and high). We expected to find an increasing level of genetic differentiation among populations coupled with a decreasing level of within-population variability, with increasing level of fragmentation. Special attention was given to testing the hypothesis that anemophilous forest trees like beech are more sensitive, in terms of loss of genetic diversity, to small population size (induced by suboptimal ecological conditions) rather than to geographic isolation alone.

Materials and Methods

Studied Species

European beech (*F. sylvatica* L.) is a widespread European broadleaved tree species covering 12 million hectares in Europe, from northern Spain to the Ukraine and from Sicily (Italy) to Southern Sweden (Bernetti 1995). In Central Europe, beech is common in forests of low mountain ranges and highlands, whereas in the Mediterranean regions, it is typically found in the mountains, given its preference for cool and wet environments. In Italy, beech is distributed in all mountain areas (except continental internal Alps and Sardinia) from Northern Italy to Sicily; its lower altitudinal limit depends on local humidity (from 300–400 m a.s.l. in wetter areas to 1000 m a.s.l. in drier ones). Beech upper altitudinal limit is about 1300–1600 m a.s.l. in the Alps and the timber line (1600–1800 m a.s.l.) in the Apennines.

Postglacial recolonization history of beech has been previously studied comparing palinological and genetic data by Comps et al. (1990), Leonardi and Menozzi (1995), Comps et al. (2001), and Magri et al. (2006). Beech reached its maximum spread about 1000 years BP in Central Europe (Magri et al. 2006). Model-based simulations showed a northward shift of the southern limit of the distribution of beech because of a loss of suitable habitats in the South of France, Italy, and the Balkan peninsula (Kramer et al. 2010). At present, the species range is shrinking in the South because of climatic change and human land use, and in many Mediterranean regions, several highly fragmented populations can be found (Jump and Penuelas 2006).

In Central Italy, during the last postglacial period (Holocene), beech reached its maximum spread between 8000 and 4000 years BP, and after the subboreal climatic change, it retreated at higher altitude in wetter areas (Chiarugi 1939; Magri 1999). The first evidence of human impact in this area dates back to approximately 2600 year BP (Magri and Sadori 1999). Nowadays, some small relic lowland populations survive in restricted areas with sufficient local summer humidity. In fact, it has been demonstrated that summer drought is the key climatic factor affecting beech growth at lower latitudes (Piovesan et al. 2008).

Sampling

We sampled 27 populations in Central Italy (Tuscany, Lazio, and Umbria regions—Figure 1 and Table 1). About 30 non-adjacent trees randomly selected along a transect were chosen in each population, for a total of 856 individuals. Buds were collected from all trees and were stored at 4 °C until genetic analyses. Populations were classified a priori (i.e., before genetic analysis) in 3 groups (Near-Marginal, Remote-Summit, and Remote-Abyssal) on the basis of their geographical location in relation to the species main continuous range, ecological conditions, and forest physiognomy. This classification was confirmed by a discriminant analysis on latitude, longitude, altitude, and distance from main biogeographic range. Populations were correctly assigned in 100% of cases.

"Near-Marginal populations" are located at the margin of the biogeographic range of European beech for Central Italy and approximately in the "normal" altitude range for this latitude (Figure 1, Table 1). These populations are pure beech stands, with the sporadic presence of individuals of other mountain species. Population size is large (much more than 1000 adults), and geographic isolation is limited.

"Remote-Summit populations" are located on the upper reaches of mountains at "normal" altitude for beech but

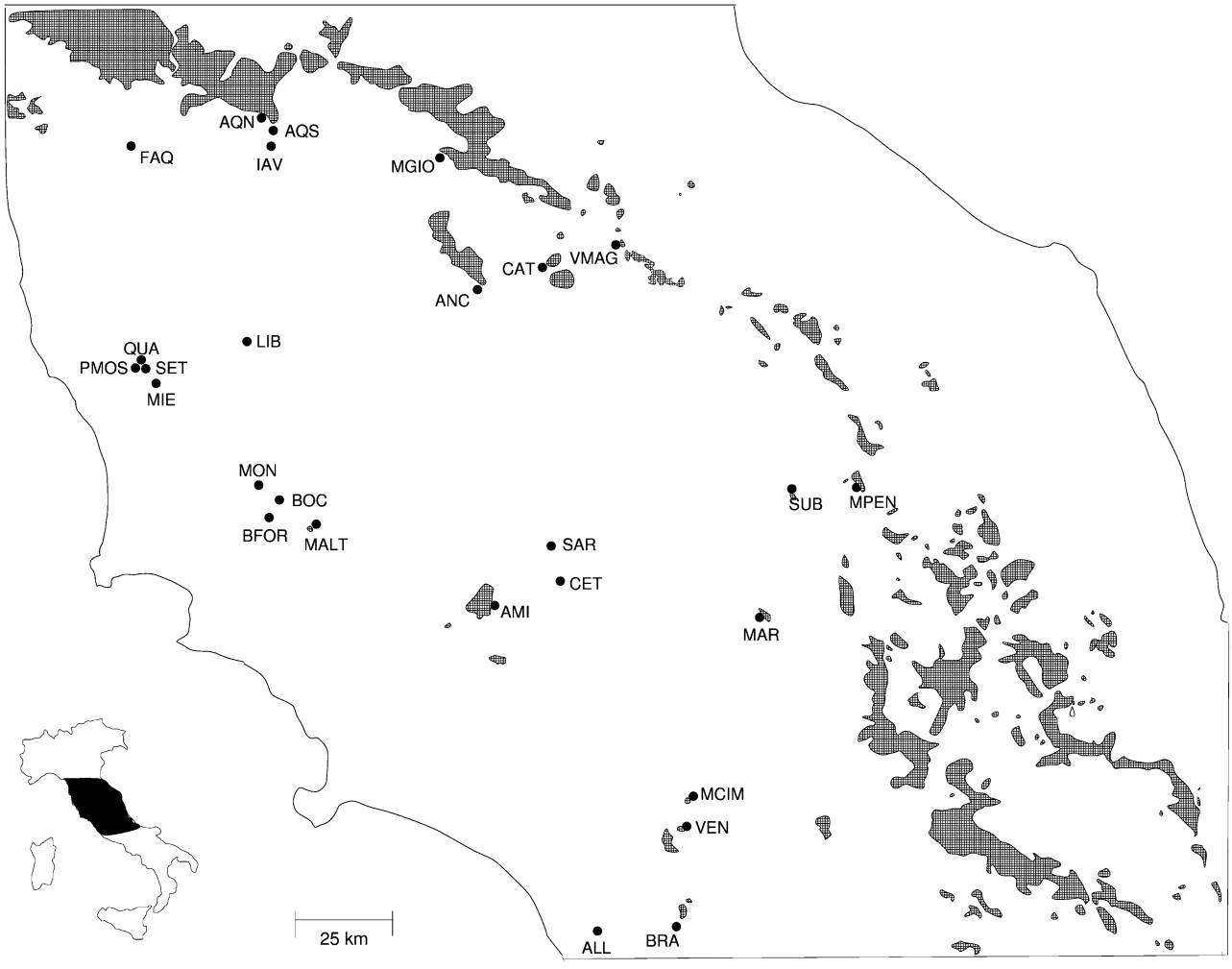

Figure 1. Main distribution range of beech in Central Italy (shaded) and localization of sampling stations.

faraway from its main biographical range (Figure 1, Table 1). A typical example is the Monte Amiata (AMI) population located on the upper slopes of a spent volcano located in a lower altitude area. These populations are generally pure beech stands or, less frequently, mixed with European chestnut (*Castanea sativa*) and Turkey oak (*Q. cerris*). Their population size is generally smaller than Near-Marginal populations (several hundred adult plants). These populations experience a stronger geographic isolation than the Near-Marginal ones.

"Remote-Abyssal populations" are far from the biogeographic range at an altitude of less than 600 m a.s.l. (Figure 1, Table 1), always mixed with several other species typical of Mediterranean or sub-Mediterranean habitats. Beech presence is sporadic with few scattered individuals in intensively managed (coppice) and often degraded semi-deciduous forests. These populations are characterized by small size, and beech trees are usually restricted to small patches with higher humidity, generally at the bottom of small valleys or on north-facing slopes. These small populations, although affected by a geographic isolation equivalent to that of the Remote-Summit group (analysis of variance [ANOVA] on distance from main range: $F_{1,17} = 2.361$, $P = 0.14$), experience suboptimal ecological conditions because of competition by other broadleaved species and longer period of drought stress in summer. These populations are both geographically and ecologically peripheral. For most populations belonging to this group, we sampled all the plants we could find.

Allozyme Analysis

Eleven loci were essayed by means of horizontal starch gel electrophoresis and used to determine the multilocus genotype of each tree: 6PGD-A (E.C. 1.1.1.44), ACO-A and ACO-B (E.C. 4.2.1.3), DIA-A (E.C. 1.6.4.3), GOT-A (E.C. 2.6.1.1), GPI-B (E.C. 5.3.1.9), IDH-A (E.C. 1.1.1.42), MDH-A and MDH-B (E.C. 1.1.1.37), PER-B (E.C. 1.11.1.7), SKDH-A (E.C. 1.1.1.25). Electrophoresis procedures have been reported elsewhere (Leonardi and Menozzi 1995).

Data Analysis

Four multilocus measures of genetic diversity were calculated: allelic richness (*A*), percentage of polymorphic

Table 1 Descriptions of sampled populations

No.	Locality (Province)	Label	Type	Latitude (°)	Longitude (°)	Altitude (m a.s.l.)	Mean sample size
1	Borro Formicciolo (GR)	BFOR	Remote-Abyssal	43.02	11.03	430	28.4
2	Boccheggiano (SI)	BOC		43.05	11.01	450	33.7
3	Bracciano (RM)	BRA		42.10	12.09	490	28.0
4	Libbiano (SI)	LIB		43.29	10.59	330	31.7
5	Miemo (PI)	MIE		43.25	10.40	475	28.9
6	Ponte Moscoso (PI)	PMOS		43.27	10.36	450	18.8
7	Quattrostrade (PI)	QUA		43.28	10.36	500	26.5
8	Settefonti (PI)	SET		43.28	10.36	450	21.6
9	Monte Venere (VT)	VEN		42.21	12.11	550	27.5
10	Allumiere (RM)	ALL	Remote-Summit	42.10	11.49	580	27.3
11	Monte Amiata (SI)	AMI		42.54	11.38	1140	82.2
12	Monte Cetona (SI)	CET		42.56	11.52	1040	22.9
13	Monte Alto (GR)	MALT		43.03	11.08	745	28.2
14	Monte Martano (PG)	MAR		42.45	12.32	1030	35.9
15	Monte Cimino (VT)	MCIM		42.23	12.12	1030	29.1
16	Poggio Montieri (GR)	MON		43.08	11.00	980	27.5
17	Monte Pennino (MC)	MPEN		43.06	12.52	1200	30.3
18	Sarteano (SI)	SAR		43.00	11.49	700	24.7
19	Monte Subasio (PG)	SUB		43.04	12.40	1140	26.6
20	Foro Anciolina (AR)	ANC	Near-Marginal	43.36	11.41	1385	27.4
21	Acquerino Nord (PT)	AQN		44.00	11.01	880	26.9
22	Acquerino Sud (PT)	AQS		44.00	11.01	755	28.6
23	Alpe di Catenaia (AR)	CAT		43.37	11.55	1000	28.5
24	Faggio Aquila (LU)	FAQ		43.56	10.38	1020	29.2
25	Iavello (PO)	IAV		43.58	11.05	525	29.6
26	Monte Giovi (FI)	MGIO		43.55	11.27	900	30.8
27	Viamaggio (FI)	VMAG		43.41	12.47	950	29.5

loci (most frequent allele <99%), mean observed and expected heterozygosity (H_O and H_E, respectively).

Computation of allelic richness was based on the rarefaction method in order to make it independent from sample size (El Mousadik and Petit 1996). Allelic richness is the expected number of different alleles in a sample of n genes, where n (twice the number of sampled individuals) is a reference number often taken to be the smallest sample size in the data set. We chose as reference number 20 and 40. The first is equal to the smallest sample size among our locus-population combinations. The second, despite the fact that is twice the first, still accounts for 97% of our locus-population combinations. Given the 3 levels of hierarchy (populations, groups of population, and total), we estimated allelic richness also for groups of populations following Kalinowski (2004) and using 8 as common minimum reference number for populations. The among populations within groups allelic richness component (A_{SG}) was estimated as in Comps et al. (2001).

In order to test for possible differences among groups of populations in gene diversity measures, we used ANOVA on allelic richness (A_{20} and A_{40}) and generalized linear model (GLM, McCullagh and Nelder 1989) with a binomial error distribution on the other 3 measures. We dropped 9 of 297 combinations with less than 40 alleles in the ANOVA on A_{40}. Because expected and observed heterozygosities are proportions, they were treated as binomial (heterozygous vs. homozygous) observations. A variable with binomial error distribution can be obtained from heterozygosity (and homozygosity) by multiplying it by sample size.

We used the program BOTTLENECK v.1.2.02 (Piry et al. 1999) to test for recent population bottlenecks. A Wilcoxon's sign rank test was used to compare expected heterozygosity from Hardy–Weinberg equilibrium with predicted heterozygosity at mutation–drift equilibrium on the basis of the observed allele number (Piry et al. 1999). The infinite allele model (IAM) (Kimura and Weiss 1964) was chosen because it is considered more reliable for allozyme data (Luikart and Cornuet 1998).

Fixation indexes were estimated according to Weir and Cockerham (1984). F_{SG} (among populations within groups) statistics were also estimated for each population group. Variance of F_{SG} was estimated by bootstrap procedure, as suggested by Weir (1990), resampling over loci. We repeated the procedure 1000 times. To evaluate the statistical significance of differences between F_{SG} values of the studied groups of populations, we followed the method suggested by Van Dongen (1995). Distribution of pairwise differences of F_{SG} values was built, and we checked if the 95% confidence interval included zero.

Principal components analysis (PCA) was performed to obtain a synthetic representation of the genetic structure of analyzed populations. PCA was computed using transformed (arcsin, square root) allele frequencies. For each locus, the lowest allele frequency was excluded from the analysis.

The significance of the relationship between pairwise population genetic and geographic distances was tested by Mantel test (1000 randomizations). Genetic distances were

computed as pairwise linearized F_{ST} values between all pairs of populations (Rousset 1997).

Standard statistic procedures were performed using R statistical package (R Development Core Team 2009).

Results

Within-Population Genetic Variability

Mean expected heterozygosity is 0.19, and the mean observed heterozygosity is 0.17. A general significant homozygote excess was found (overall mean $F_{IS} = 0.07$, $P < 0.05$). Only 6 populations (ALL, AQN, CAT, MCIM, PMOS, and VEN) show a negative mean F_{IS}.

Statistical analysis performed on the 4 parameters describing within-population genetic variability (allelic richness, percentage of polymorphic loci, mean expected and observed heterozygosity) showed that Remote-Abyssal is slightly but significantly less genetically variable than the other 2 groups (Table 2). Given the high difference among loci of the population variability parameters, we decided to remove the variation attributable to the locus effect in ANOVA or GLMs, including the group effect only after the locus effect entered the model. The allelic richness after rarefaction to 20 (A_{20}) was marginally significantly different among groups ($F_{2,284} = 3.01$, $P = 0.051$), but allelic richness after rarefaction to 40 (A_{40}) was significantly different at 5% level ($F_{2,275} = 3.71$, $P < 0.05$). Also expected and observed heterozygosities are significantly different among groups (H_E: deviance = 18.02, degree of freedom [d.f.] = 2, $P < 0.001$; H_O: deviance = 11.20, d.f. = 2, $P < 0.01$). Groups are different at 5% even without removing the locus effects (data not shown). On the contrary, percentage of polymorphic loci is not significantly different among groups (deviance = 2.58, d.f. = 2, $P = 0.28$). Results from a priori orthogonal contrasts show that in Remote-Abyssal A_{40} is significantly lower than in both Remote-Summit and Near-Marginal populations, whereas the heterogeneity among heterozygosities (H_E and H_O) of the 3 groups is due to the differences between the Remote-Abyssal and the Remote-Summit populations only.

Finally, no evidence for recent bottlenecks was found. For all populations, Hardy–Weinberg heterozygosity and expected gene diversity at mutation–drift equilibrium did not differ significantly.

Among Population Genetic Variability

Significant allele frequency heterogeneity was found among populations at all polymorphic loci (except MDH2) (all χ^2 probabilities are less than 0.00002), although differences are modest in absolute terms with no alternate fixations and all populations share the most frequent allele at all loci.

On average, only 4.6% of variability is attributable to the among population component (mean $F_{ST} = 0.046$). *F*-statistics show that 95.4% of variability is due to the

Table 2 Mean allelic richness rarefacted to 20 (A_{20}) and 40 (A_{40}) gene copies, percentage of polymorphic loci (*P*), mean unbiased expected heterozygosity (H_E), mean observed heterozygosity (H_O) for each population

Population	Type	A_{20}	A_{40}	*P*	H_E	H_O
BFOR	Remote-Abyssal	0.60 (0.16)	0.84 (0.20)	36.4	0.12 (0.04)	0.11 (0.04)
BOC		0.66 (0.19)	0.77 (0.22)	54.5	0.15 (0.05)	0.13 (0.04)
BRA		0.95 (0.13)	1.11 (0.15)	81.8	0.21 (0.04)	0.15 (0.04)
LIB		0.66 (0.13)	0.72 (0.14)	63.6	0.17 (0.04)	0.16 (0.05)
MIE		0.86 (0.13)	1.03 (0.16)	72.7	0.22 (0.05)	0.21 (0.05)
PMOS		0.91 (0.16)	1.00 (0.19)	81.8	0.25 (0.06)	0.29 (0.07)
QUA		0.77 (0.16)	0.96 (0.19)	54.5	0.15 (0.04)	0.13 (0.03)
SET		0.47 (0.14)	0.54 (0.16)	45.5	0.12 (0.04)	0.12 (0.05)
VEN		0.60 (0.16)	0.76 (0.21)	45.5	0.14 (0.05)	0.15 (0.05)
ALL	Remote-Summit	0.76 (0.10)	0.93 (0.12)	72.7	0.19 (0.05)	0.19 (0.05)
AMI		0.83 (0.17)	1.01 (0.18)	63.6	0.21 (0.05)	0.18 (0.04)
CET		0.90 (0.18)	1.19 (0.19)	45.5	0.17 (0.05)	0.14 (0.04)
MALT		0.81 (0.18)	0.96 (0.22)	72.7	0.17 (0.04)	0.16 (0.05)
MAR		0.83 (0.20)	0.97 (0.23)	63.6	0.20 (0.06)	0.18 (0.06)
MCIM		0.82 (0.15)	0.93 (0.17)	72.7	0.23 (0.05)	0.23 (0.05)
MON		0.90 (0.12)	0.99 (0.13)	81.8	0.20 (0.03)	0.19 (0.04)
MPEN		0.94 (0.12)	1.11 (0.15)	90.9	0.24 (0.05)	0.19 (0.04)
SAR		0.62 (0.15)	0.70 (0.18)	63.6	0.15 (0.04)	0.15 (0.04)
SUB		0.87 (0.15)	1.03 (0.19)	72.7	0.24 (0.05)	0.22 (0.05)
ANC	Near-Marginal	0.91 (0.14)	1.05 (0.15)	81.8	0.20 (0.04)	0.17 (0.04)
AQN		0.72 (0.13)	1.01 (0.16)	54.5	0.13 (0.04)	0.14 (0.04)
AQS		0.74 (0.16)	0.87 (0.16)	63.6	0.17 (0.05)	0.14 (0.04)
CAT		0.85 (0.10)	1.02 (0.09)	72.7	0.19 (0.04)	0.20 (0.04)
FAQ		0.74 (0.15)	0.98 (0.19)	63.6	0.12 (0.03)	0.11 (0.03)
IAV		0.92 (0.15)	1.12 (0.18)	81.8	0.19 (0.04)	0.19 (0.04)
MGIO		0.88 (0.19)	0.98 (0.19)	63.6	0.21 (0.05)	0.20 (0.05)
VMAG		0.69 (0.19)	0.85 (0.24)	54.5	0.15 (0.05)	0.12 (0.04)

Standard errors are reported in brackets.

within-population component and only a very small fraction of variability (1.4%) is attributable to the among groups component (mean $F_{GT} = 0.014$).

The scatter plot of the first 2 principal component scores does not show a clear separation of groups (Supplementary Figure S1). In particular, Remote-Abyssal populations are obviously more spread out than the other 2 groups. The average distances from the center of the scatter plot are 0.45, 0.35, and 0.26 for the Remote-Abyssal, the Remote-Summit, and the Near-Marginal group, respectively.

The Remote-Abyssal populations seem more differentiated than the other 2 groups. Indeed, Remote-Abyssal populations have an average F_{SG} value that is twice the Near-Marginal ones (Figure 2). This difference was tested using F_{SG} values computed for each group of populations. Variance and confidence interval were estimated by bootstrap. Results are shown in Figure 2. Remote-Abyssal populations have a higher internal degree of differentiation with respect to the Remote-Summit group, and the differentiation of Remote-Summit populations is higher than that of Near-Marginal populations. Only differences between F_{SG} of Remote-Abyssal and Near-Marginal are significant at the 1% level, whereas neither F_{SG} between Remote-Summit and Remote-Abyssal nor that between Remote-Summit and Near-Marginal are significant at 5% level. The among populations within groups component of allelic richness (A_{SG}) estimated according to Kalinowski (2004) and Comps et al. (2001) follows the same pattern as the average F_{SG}. Estimated A_{SG} values are equal to 0.43, 0.40, 0.30, respectively, for the Remote-Abyssal, the Remote-Summit, and the Near-Marginal group.

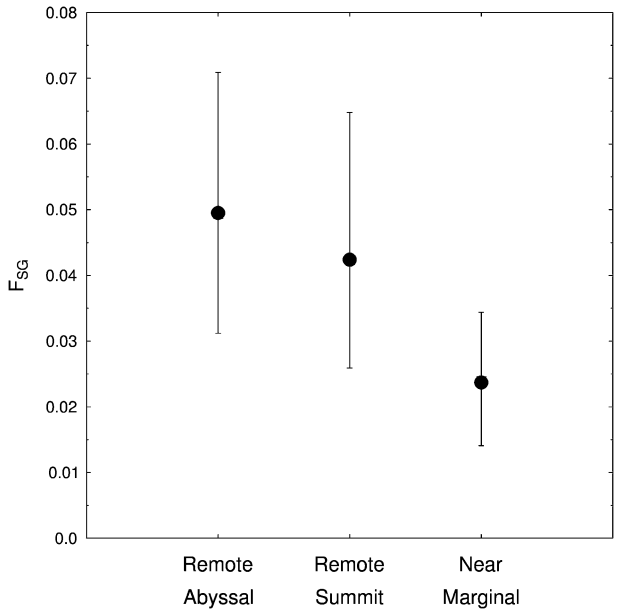

Figure 2. Mean and 95% confidence interval of mean F_{SG} values computed for each population group on 1000 bootstrapping cycles of original data. Every cycle a random sampling of loci is drawn.

If gene flow plays an important role in shaping the among population structure, a positive correlation between geographic and genetic distance is expected. A significant correlation was found between geographic and genetic distance for all pairwise populations (Mantel test $r = 0.21$; $P < 0.01$). A stronger correlation was observed excluding all pairs involving a Remote-Abyssal population ($r = 0.46$; $P < 0.01$) (Figure 3). In fact, no evidence for an isolation-by-distance pattern was found analyzing all pairs involving at least one Remote-Abyssal populations ($r = 0.07$; $P = 0.82$) (Figure 3). Pairwise F_{ST} values are reported in Supplementary Table S1.

Discussion

Our results show a significant effect of habitat fragmentation on European beech. The effect of increasing fragmentation is more evident looking at population differentiation than at genetic diversity. The weak effect on genetic diversity is attributable to the heterogeneity of populations belonging to the same level of fragmentation. In fact, parameters measuring within-population variability are more variable within groups of populations than among groups. This may reflect different evolutionary histories of the populations within a single group, even if present geographical and ecological characteristics are quite similar. Remote-Abyssal populations are affected by both demographic (small population size) and geographic (long distance from main biogeographic range) impacts and are significantly more influenced by fragmentation than Remote-Summit populations that are affected only by geographic distance from the main biogeographic range. Ouborg et al. (2006) pointed out that many studies on fragmentation focused only on the impact of reduced population size and that population size and isolation should be treated separately because different effects are expected from the processes affecting the 2 parameters. Our sampling scheme was aimed at disentangling the effects of population size and isolation on genetic diversity and differentiation.

The different impact of fragmentation on the 3 groups of populations can theoretically be explained by a different balance between genetic drift and gene flow or by the effect of natural selection. The prevailing effect of genetic drift over gene flow is evident in Remote-Abyssal populations, whereas strong gene flow is probably the factor opposing (or delaying) differentiation and loss of genetic variability in Remote-Summit and Near-Marginal populations. Genetic drift theory predicts loss of rare alleles, random fluctuation of allelic frequencies, increased differentiation, and weakening of the isolation-by-distance component of spatial genetic structure among populations (Barrett and Charlesworth 1991; Ellstrand and Elam 1993). Our results regarding Remote-Abyssal populations match all these expectations. For example, the scattered pattern of Remote-Abyssal in the principal components plot is a clear depiction of random allele frequency fluctuations. The differences in F_{SG} and the lack of an isolation-by-distance pattern in the genetic structure confirm this interpretation.

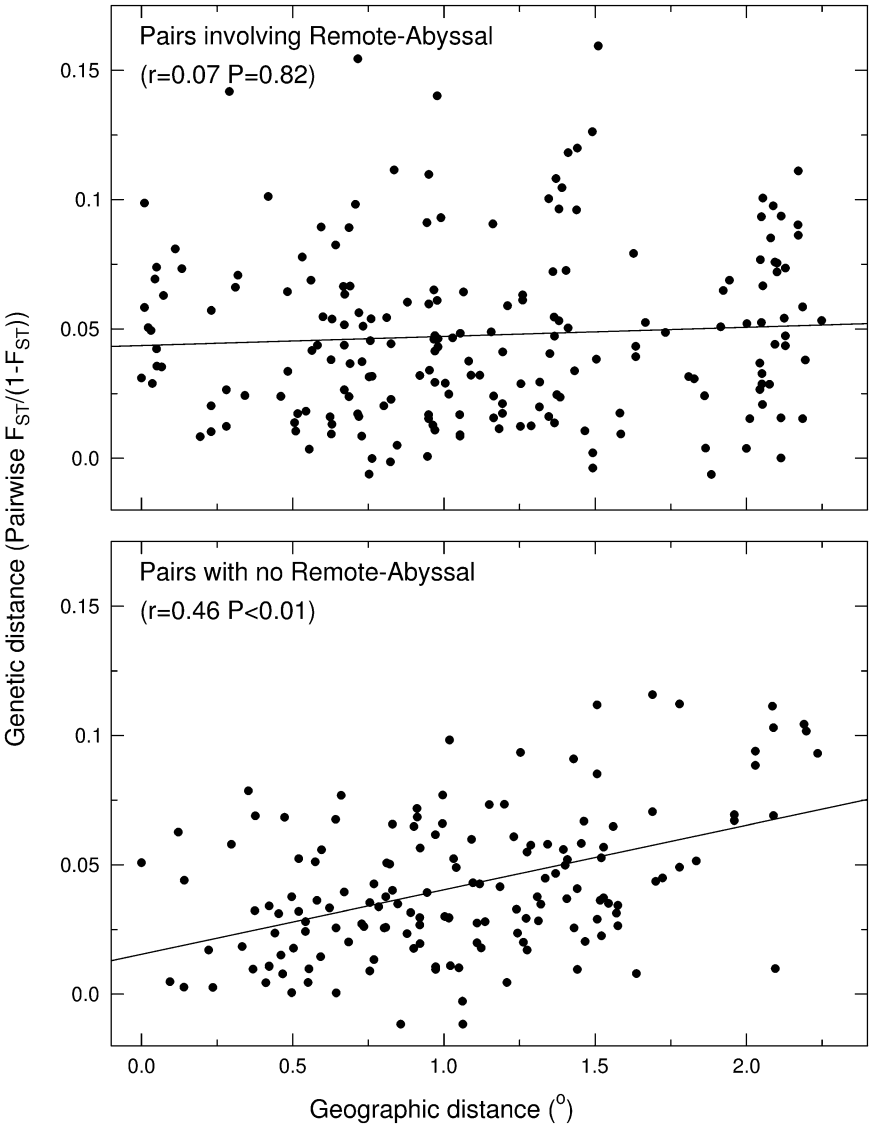

Figure 3. Relationship between pairwise linearized F_{ST} and geographic distance for pairs of populations with a least one Remote-Abyssal (top) and excluding pairs with at least one Remote-Abyssal population (bottom). Pearson's correlation coefficients and *P* values after Mantel test with 1000 random cycles are reported.

Remote-Summit and Near-Marginal populations show a less evident effect of drift. This is probably due to the larger average size of these populations that reduces the effect of drift allowing gene flow to prevail, even if average distance from the main range of Remote-Summit populations is not different from that of the Remote-Abyssal group. Overall, these results support the hypothesis that in anemophilous forest species gene flow is a strong evolutionary force even at relatively long distances and can effectively balance fragmentation if population size is not too small (Robledo-Arnuncio et al. 2005; Craft and Ashley 2010). Gene flow via pollen is usually high (~0.6–0.8) in large continuous stand of beech (Oddou-Muratorio et al. 2010; Piotti et al. 2012), as well as in general in Fagaceae (e.g., Buiteveld et al. 2001; Chybicki and Burczyk 2010). Gene flow investigation in small beech stands, comparable in size to Remote-Abyssal populations in this paper, has never been performed before, therefore it is difficult to predict the extent of genetic connectivity in these populations. However, Piotti et al. (2012) found that in an isolated but large beech population, 50% of pollen came from outside the 2 hectares studied plot, an unusually low estimate for this species. In Fagaceae, gene flow rates below 0.5 have been only found in highly fragmented populations (Hanaoka et al. 2007; Pakkad et al. 2008; Pluess et al. 2009). But little is known about the impact of fragmentation on reproductive patterns of wind-pollinated species because they have not been expected to be as sensitive as insect- and animal-pollinated species (O'Connell et al. 2006). Recently, it has been found that also wind-pollinated tree species can

be vulnerable to fragmentation because wind pollination efficiency may be dependent on spatial structure and density of individuals/populations (Jump and Penuelas 2006; Montoya et al. 2008). Knapp et al. (2001) found that reproduction in blue oak is limited by the availability of local pollen: trees with many pollen-producing neighbors tended to produce larger acorn crops than those that were more isolated. The results of other studies on the relationship between population size and seed production suggested that reproduction in isolated populations of wind-pollinated trees can indeed be limited (e.g., Nilsson and Wastljung 1987).

We cannot exclude the role of natural selection in shaping the genetic structure of the studied populations. The harsh environmental conditions of Remote-Abyssal populations may impose a selection pressure but this is not evident in our data. In only a few cases, evidence of selection was found for isozyme data (Bergmann 1991). Many of our results are difficult to explain as results from a natural selection process. Environmental conditions experienced by all Remote-Abyssal populations are harsh but apparently very similar and homogeneous. Given the homogeneous environmental conditions experienced by Remote-Abyssal populations, disruptive selection determining differentiation of these populations seems unlikely. Furthermore, all our genetic variability parameters are multilocus averages, and principal component scores are linear combination of all allele frequencies. These variables reflect genome-wide patterns, whereas selection is likely to act only upon short portions of genomes.

Decreased genetic variability and increased population differentiation were observed comparing genetic diversity in fragmented versus continuous beech populations in Catalonia (Spain) by Jump and Penuelas (2006). The average differentiation between our Remote-Abyssal populations is indeed higher (F_{SG} = 0.05) than the values observed by Jump and Penuelas (2006) (F_{ST} within the fragmented group = 0.029). This can be explained, beside the use of different genetic markers, by the fact that our populations are geographically distant (range 1–200 km) and have experienced fragmentation for a longer period (about 4000 years) (Magri 1999), whereas populations described in Jump and Penuelas (2006) were distributed on a shorter range (0.5–6 km), and fragmentation was much more recent (about 600 years ago). The range edge location of our Remote-Abyssal populations may in part be responsible for this elevated F_{SG}. Persson et al. (2004) observed that fragmented populations of *C. avellana* in Sweden are genetically impoverished and divergent both from each other and from the rest of the species range. A similar finding was reported for range edge populations of *Cirsium acaule* by Jump et al. (2003).

What are the causes of reduced size of Remote-Abyssal populations? Availability of water in summer is the most limiting factor for beech growth in these sites (Dittmar et al. 2003; Piovesan et al. 2005). At lower elevation, beech actually can survive only in restricted areas with higher humidity (Pignatti 1998). Competition with other woody species (*Quercus* spp., *Carpinus betulus*, *Ostrya carpinifolia*), more adapted to drought than beech (Tognetti et al. 1995), could play an important role in reducing the effective number of beech population. Remote-Summit populations, which are located at higher altitude where climatic conditions are more favorable to beech reducing competition, are indeed "pure" beech forests (Pignatti 1998). Moreover, the intense coppicing practiced by man in lowland populations may have favored other species more efficient in resprouting after cut than beech (Bernetti 1995). Our data cannot help understand the relative role of past climatic changes and human impact in determining genetic consequences of habitat fragmentation in this area. However, according to Magri (1999), the most important reduction of beech presence in this area happened at least 4000 years ago, long before the first evidence of human impact on forests in this area (Magri and Sadori 1999). We found no evidence for recent demographic bottlenecks in our populations, even in Remote-Abyssal ones that are now characterized by small population size. This result suggests that the putative strong reduction in size experienced by our Remote-Abyssal populations occurred a longtime ago so that the effects on genetic structure are no longer detectable. The method we used to test the evidence of genetic bottlenecks is based on transitory excess of gene diversity detectable in the generations following a demographic bottleneck (Piry et al. 1999). Using the same method, Jump and Penuelas (2006) found evidence of a recent bottleneck in fragmented populations of beech in Spain dating back 600 years. We used a different type of markers, but Cornuet and Luikart (1996) argued that isozymes associated with an IAM are more suitable than microsatellite markers for detecting less recent bottlenecks.

In conclusion, our results highlight that population size rather than distance from the main biogeographic range affects genetic variability both within and among populations. Random genetic drift has a larger impact on the most fragmented group, whereas gene flow seems to balance genetic drift in the 2 less fragmented ones. The genetic structure and impoverished genetic variability of Remote-Abyssal populations, their ecological marginality, and ongoing climatic changes put these peripheral populations at risk of local extinction. Because it is likely that these populations have evolved some degree of adaptation to their environmental conditions (drier and warmer than optimum for beech), their extinction could represent an important loss for beech biodiversity.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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