

## Comparison of direct and indirect genetic methods for estimating seed and pollen dispersal in old-grown and expanding populations of *Fagus sylvatica*

Sylvie Oddou-Muratorio, Aurore Bontemps, Etienne K. Klein, Igor Chybicki, Giovanni G. Vendramin, Yoshihisa Suyama

### ► To cite this version:

Sylvie Oddou-Muratorio, Aurore Bontemps, Etienne K. Klein, Igor Chybicki, Giovanni G. Vendramin, et al.. Comparison of direct and indirect genetic methods for estimating seed and pollen dispersal in old-grown and expanding populations of *Fagus sylvatica*. 8. IUFRO International beech symposium, Sep 2008, Nanae, Japan. 8 p., 10.1016/j.foreco.2010.03.001 . hal-02756921

**HAL Id: hal-02756921**

**<https://hal.inrae.fr/hal-02756921>**

Submitted on 3 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1                   **Comparison of direct and indirect genetic methods for estimating**  
2                   **seed and pollen dispersal in *Fagus sylvatica* and *Fagus crenata*.**

3  
4                   Sylvie ODDOU-MURATORIO<sup>1\*</sup>, Aurore BONTEMPS<sup>1</sup>, Etienne K. KLEIN<sup>2</sup>, Igor  
5                   CHYBICKI<sup>3</sup>, Giovanni G. VENDRAMIN<sup>4</sup> and Yoshihisa SUYAMA<sup>5</sup>

6  
7                   <sup>1</sup> INRA UR 629, Ecologie des Forêts Méditerranéennes, Domaine St Paul, Site Agroparc, F-  
8                   84914 Avignon France

9                   <sup>2</sup> INRA, UR 546, Biostatistique et Processus Spatiaux , Domaine St Paul, Site Agroparc, F-  
10                   84914 Avignon France

11                   <sup>3</sup> Uniwersytet Kazimierza Wielkiego, Department of Genetics, Chodkiewicza 30, 85-064  
12                   Bydgoszcz, Poland

13                   <sup>4</sup> CNR, Istituto di Genetica Vegetale, Via Madonna del Piano 10, I-50019 Sesto Fiorentino  
14                   (Firenze), Italy

15                   <sup>5</sup> Graduate School of Agricultural Science, Tohoku University, 232-3 Yomogida, Naruko-  
16                   Onsen, Osaki, Miyagi 989-6711, Japan

17  
18                   \* To whom correspondence should be addressed: Phone: +33-490 135 914, e-mail:

19                   [oddou@avignon.inra.fr](mailto:oddou@avignon.inra.fr).

20  
21                   This manuscript is designed to be included in the special issue “Beech Ecology and  
22                   Management”

1

## 2 **Abstract**

3 The comparison between estimates of historical gene flow, using variance in allelic  
4 frequencies, and estimates of contemporary gene flow, using parentage assignment, is  
5 expected to provide insights into ecological and evolutionary processes at work within and  
6 among populations. Genetic variation at microsatellite loci was used to quantify genetic  
7 structure in two wind pollinated, gravity and animal-dispersed tree species (*Fagus sylvatica* L.  
8 and *Fagus crenata* Blum.) and to derive historical estimates of gene flow. The gene dispersal  
9 distances estimated assuming effective population density to be  $\frac{1}{4}$  of the observed density  
10 were  $\sim 77$  m in European beech and  $\sim 40$  m in Japanese beech. Parentage analyses and a  
11 neighbourhood model approach were used to estimate contemporary patterns of seed and  
12 pollen dispersal. Our results suggest restricted seed dispersal abilities in both European beech  
13 ( $\delta_s = 10.5$  m) and Japanese beech ( $\delta_s = 12.4$  m), with an exponential shaped seed dispersal  
14 kernel. A non-negligible rate of seed immigration ( $m_s = 27\%$ ) was detected in European beech  
15 sites but not in Japanese beech site. Pollen dispersal within studied sites also appeared limited  
16 ( $\delta_p = 41.63$  m in European beech and  $\delta_p = 79.4$  m in Japanese beech), despite high rate of  
17 pollen immigration ( $m_p = 68\%$  in European beech and  $m_p = 40\%$  m in Japanese beech).  
18 Interestingly, contemporary and historical estimates of gene flow were within the same order  
19 of magnitude (a few tens of meters).

1

**2 Introduction**

3 The impact of predicted climatic changes on forest ecosystems is expected to be acute,  
4 resulting in notable changes in species' ranges, ecosystem function and species interactions.  
5 Because trees are long-lived they can disappear, disperse to other places or adapt *in situ* to  
6 climate change over a few generations. For several tree species bioclimatic models predict  
7 geographical shifts in the range of suitable habitat of several hundreds of kilometres by 2100  
8 (without altitudinal compensation, Jump et al., 2009). The range of European beech (*Fagus*  
9 *sylvatica* L.) for example is predicted to strongly contract in the west while expanding  
10 northwards, due to inadequate chilling in the west as winter warms more than summer (Sykes  
11 et al., 1996). These important predicted distribution shifts partly account for the intense  
12 research effort dedicated to species dispersal in recent decades (Bullock and Nathan, 2008;  
13 Ronce, 2007).

14 A primary concern in plants is whether seed dispersal capacities will allow species to  
15 track 21<sup>st</sup>-century global warming. Analyses of paleoecological records are classically  
16 interpreted to suggest that tree populations are capable of rapid migration when climate  
17 warms, with rates of range expansion as high as 1000 m/yr during the early Holocene. Such a  
18 fast migration rate is possible by rare long-distance migration events (Clark, 1998) or by high  
19 latitude refugia reseeded the landscape (McLachlan et al., 2005). The latter means that post-  
20 glacial re-colonization may have been as much as an order of magnitude slower than  
21 previously thought (< 100 m/yr). Another major issue related to plant dispersal is whether  
22 gene migration either through pollen or seeds can support local adaptation to changing  
23 climatic conditions by mixing or adding well adapted genetic material to poorly-adapted gene  
24 pools (Davis and Shaw, 2001). The impact of migration on local adaptation depends on other  
25 important biological parameters (genetic effects, life cycle, mating system), and is

1 complicated to predict when realistic population demography estimates are included  
2 (Lenormand, 2002). For instance, while gene flow tends to oppose the effects of local  
3 selection and thus limits adaptation, intermediate rates of migration have been advocated to be  
4 optimal for local adaptation in low density populations at the range margin (Alleaume-  
5 Benharira et al., 2006). A more dynamic view of distribution ranges is now emerging in the  
6 literature which accounts for demographic and evolutionary processes associated to species  
7 expansions and retraction, and to marginal populations (Hampe and Petit, 2005).

8       Thus it is clear that predicting populations' responses to ongoing climate change  
9 requires fine characterisation of individual and gene dispersal processes at local, ecological  
10 scales as well as large, species-range scales. A way to address this issue is to use the rich  
11 toolbox available to estimate pollen and seed dispersal from genotype data at these different  
12 scales (Manel et al., 2005; Smouse and Sork, 2004). These approaches are generally termed  
13 "indirect" when using gene pool data or "direct" when using exact genotypes. The most  
14 indirect approaches estimate historical dispersal parameters from the observed genetic  
15 structure by fitting them to theoretical population models generally assuming drift– migration  
16 equilibrium. By contrast, the most direct approaches use genetic markers to monitor  
17 contemporary movements of individuals or propagules by reconstructing parentage  
18 relationships (Manel et al., 2005), as in paternity/parentage analyses which are model-free  
19 beyond assuming Mendelian inheritance of the markers.

20       Indirect approaches to assess historical gene flow within continuous populations employ  
21 the principle that Spatial Genetic Structure (SGS) displayed by neutral markers is essentially  
22 caused by local genetic drift, the effect of which is counterbalanced by gene dispersal. Under  
23 this isolation by distance model, the decay rate of genetic relatedness between individuals  
24 with distance has been shown to be inversely proportional to  $d_e \sigma_e^2$ , with  $\sigma_e^2$  equal to half of  
25 the mean-squared parent-offspring distance and  $d_e$  equal to the effective density of individuals

1 (Rousset, 2000). Intuitively, the product  $d_e\sigma_e^2$  expresses the degree of overlap between  
2 individual “gene shadows” (the spatial distribution of gene dispersal events around each  
3 parent). It implies that the intensity of genetic structuring decreases both with increasing  
4 dispersal and increasing individual density. Assuming that the genetic markers used in these  
5 analyses are not affected by natural selection, the summary parameter  $d_e\sigma_e^2$  reflects the  
6 importance of both gene flow and genetic drift in shaping local differentiation on a historical  
7 time-scale. When reliable estimate of effective population size is available refined methods  
8 have been proposed to estimate the gene dispersal distance  $\sigma_e$  from patterns of SGS  
9 (Vekemans and Hardy, 2004).

10       Alternatively, patterns of contemporary gene flow between the parental and offspring  
11 cohorts can be estimated by using genetic fingerprint and parentage analyses to assign  
12 seedlings to their parent trees (parentage inference), and to infer the pollen and seed dispersal  
13 curves (Oddou-Muratorio and Klein, 2008). In the case of plant populations, parentage  
14 analysis consists of genotyping a sample of dispersed seeds or established seedlings and all  
15 the reproductive plants within a circumscribed area for a set of shared polymorphic markers to  
16 detect the parent pair of each seedling (Meagher, 1986). To discriminate between male and  
17 female parentage of seed and seedlings, one can genotype maternally inherited tissues  
18 collected on dispersed seeds (Jones et al., 2005; Jordano et al., 2007). When dealing with  
19 established seedlings, where purely maternal tissues are no longer available, average effective  
20 pollen/seed dispersal distance can be directly estimated from parent-offspring genotype data  
21 using model fitting, such as the Neighbourhood model (Burczyk et al., 2006). These spatially  
22 explicit mating models have gained a broad acceptance among population geneticists and  
23 ecologists because they allow characterisation the seed and pollen dispersal processes and the  
24 heterogeneity in male/female fertility at an ecological time scale (Burczyk et al., 2006;  
25 Morgan and Conner, 2001), as well as the ecological factors that are likely to influence these

1 patterns, such as parental phenotypic traits (Gonzalez-Martinez et al., 2006), seed disperser  
2 behaviour (Jordano et al., 2007), or spatial environmental heterogeneity (Jones et al., 2005).

3 The comparison of historical versus contemporary estimates of gene flow is expected to  
4 provide interesting insights into the balance between and the time-scale of evolutionary forces  
5 at work within and among populations (Oddou-Muratorio and Klein, 2008). It has been  
6 argued that in undisturbed populations the consistency between historical and contemporary  
7 estimates of gene flow supports the drift-migration equilibrium hypothesis (Dunphy and  
8 Hamrick, 2005; Otero-Arnaiz et al., 2005), while divergence among real-time and effective  
9 migration rates has been used to highlight the consequence of sex-biased dispersal and social  
10 structure in small mammal species (Schweizer et al., 2007). In very recently disturbed  
11 populations, only contemporary estimates of gene flow should be affected because of  
12 temporal inertia of historical estimates for few (<10) generations (Leblois et al., 2004) ; the  
13 discrepancy among historical and contemporary estimates is thus expected to reflect the  
14 magnitude of the perturbation (Dutech et al., 2005). By contrast, consistent contemporary and  
15 historical estimates of gene flow in a recently perturbed area may indicate that compensatory  
16 mechanisms allow the maintenance of gene movement (Bacles et al., 2005).

17 European and Japanese beech (resp. *Fagus sylvatica* L. and *Fagus crenata* Blum.) are  
18 two related, deciduous, broad-leaved tree species that cover large portions of Europe and  
19 Japan., where they tend to grow in high density stands. Despite the high economic and  
20 ecological importance of forests dominated by European or Japanese beech, the processes  
21 shaping their ecological and evolutionary response in face of global changes are only starting  
22 to be understood. In particular, contemporary pollen and seed dispersal patterns in beech have  
23 begun to be studied only recently (Kramer et al 2008), showing that 90% of seeds disperse  
24 within 25m. Patterns of SGS show a tendency for strong family structure up to 30-40 m,  
25 which suggests restricted migration rates of both pollen and seeds (Chybicki et al., 2009;

1 Jump and Penuelas, 2007; Leonardi et al., 1996; Vornam et al., 2004). However, to our  
2 knowledge, the most recent available methods to estimate contemporary patterns of gene flow  
3 in these species have not been used, and historical and contemporary estimates have not been  
4 compared to date.

5 In the present study, we applied direct and indirect approaches to investigate both  
6 patterns of contemporary and historical gene flow in 4 sites (3 for European beech and 1 for  
7 Japanese beech). This study has several objectives: (1) to provide robust historical estimates  
8 of gene dispersal distance  $\sigma_e$  based on SGS, and contemporary estimates of seed and pollen  
9 dispersal based on spatially explicit mating models in European and Japanese beech ; (2)  
10 based on these estimates to investigate variation in the estimated dispersal abilities between  
11 sites (for European beech) and species and (3) to compare historical and contemporary  
12 estimates of dispersal within each site, with regard to the statistical specificities of both  
13 estimates and the expected effects of recent population demography within and among sites.  
14 Based on previous studies of SGS in beech, we expect rather restricted contemporary  
15 estimates of dispersal abilities in beech. However, pollen dispersal abilities are usually  
16 assumed important in wind-pollinated tree species. Also, our sampling design include recently  
17 disturbed forest stands, in which discrepancies between contemporary and historical gene  
18 flow estimates are susceptible to arise. Confronting contemporary and historical dispersal  
19 estimates will thus allow us to investigate how pollen and seed mediated gene flow  
20 respectively shape patterns of SGS in beech.

## 21 **Material and methods**

### 22 **Studied species and sampling design**

23 European and Japanese beech are described as highly outcrossing wind-pollinated trees  
24 (Asuka et al., 2005; Hanaoka et al., 2007; Merzeau et al., 1994). Reproductive trees (typically  
25 older than 40-50 years) produce beech nuts in irregular mast years (with an average interval



1 between mast years of ~5 or ~7 years; Tomita et al., 2002). Mature seed dissemination begins  
2 in September, primarily through gravity and secondarily assisted by various animals including  
3 rodents such as voles and wood mice that have been reported to scatter-hoard seeds (Jensen,  
4 1985).

5 For European beech three plots were studied within two different locations (Table 1). FS1 and  
6 FS2 are located on Mont Ventoux, South-Eastern France, where an expanding population of  
7 European beech mixed with *Abies alba* is re-colonising a pine forest (Bontemps et al.,  
8 unpublished). FS3 is located in the ancient Haye Forest, North-Eastern France (Oddou-  
9 Muratorio et al., unpublished). Within each plot, adult trees (i.e. with diameter > 10 cm or  
10 producing seeds) were exhaustively mapped (Table 1) and their diameter was measured (Fig.  
11 1). Within each site, both seedlings (seedlings, age <3 years) and saplings (< 1 m high, app.  
12 younger than 20 years, roughly estimated from scars counts) were sampled non exhaustively,  
13 and mapped (Fig 1).

14 For Japanese beech, the 170×170 m study plot was located in a primary beech forest in  
15 northern Japan on the southern foot of Mt Kurokima (see also Takahashi et al., 2000). All of  
16 the 287 adult trees were sampled, mapped, and their diameter was measured (Fig 1). 1387  
17 seedlings emerged in 2001 and were also mapped and sampled.

## 18 **Genotyping**

19 All Japanese beech individuals were genotyped at 7 highly variable microsatellite markers  
20 (Table 2), while in European beech, all individuals were genotyped at a set of either 5 (FS3)  
21 or 11 (FS1 and FS2) microsatellite markers (Asuka et al., 2004; Pastorelli et al., 2003; Tanaka  
22 et al., 1999).

23 High prevalence of null alleles has been reported for microsatellite markers developed for  
24 European beech (Chybicki and Burczyk, 2009; Oddou-Muratorio et al., 2009). Therefore, we  
25 carefully checked for presence of null alleles both in adult and seedling cohorts using the

1 maximum-likelihood method accounting for genotyping error implemented in ML-NullFreq  
 2 (Kalinowski and Taper, 2006).

3 **SGS and historical estimates of gene flow**

4 SGS was characterised by analysing variation of genetic relatedness, as measured by the  
 5 kinship coefficient ( $F_{ij}$ ) of Loiselle et al. (1995), as function of the logarithm of geographic  
 6 distance  $d_{ij}$  between every pair of adults/seedlings  $i$  and  $j$ .

7 To visualize SGS,  $F_{ij}$  values were averaged over a set of distance classes ( $d$ ), and plotted  
 8 against the logarithm of the distance. Approximate standard errors for the multilocus  
 9 estimates of  $F_{ij}$  within each distance class were obtained through a jackknife procedure that  
 10 consisted of deleting each locus one at a time. This assumes that the different loci provide  
 11 independent replicates of the genetic structure process. To test SGS, the  $F_{ij}$  values were  
 12 regressed on  $\ln(d_{ij})$ , where  $d_{ij}$  is the spatial distance between individuals  $i$  and  $j$ , to provide the  
 13 regression slope  $b$ . Then, the spatial positions of the individuals were permuted 5,000 times in  
 14 order to get the frequency distribution of  $b$  under the null hypothesis that  $F_{ij}$  and  $d_{ij}$  were  
 15 uncorrelated. Following Vekemans and Hardy (2004), the SGS intensity was quantified by  $Sp$   
 16  $= b/(F_1 - 1)$ , where  $F_1$  is the average kinship coefficient between individuals of the first  
 17 distance class (< 50 m).

18 An indirect estimate of the product  $d_e\sigma_e^2$ , where  $d_e$  is the effective population density, was  
 19 obtained through:

$$20 \quad d_e\sigma_e^2 = -(1-F_N)/4\pi b, \quad (1)$$

21 where  $F_N$  is the kinship coefficient between neighbouring individuals, estimated here by  $F_1$   
 22 (Rousset, 2000). Ideally,  $b$  should be computed as the restricted regression slope of  $F_{ij}$  on  
 23  $\ln(d_{ij})$  in the range  $\sigma_e > d_{ij} > 20\sigma_e$  for relationship (1) to be valid. However, in most data sets  
 24 investigated here the iterative procedure required for estimating this restricted regression  
 25 slope failed to converge. Therefore, all results reported here were based on regression over the

1 whole range of geographical distances between individuals. All computations were performed  
2 with the SPAGeDi software(Hardy and Vekemans, 2002).

### 3 **Neighbourhood model and contemporary estimates of gene flow**

4 We used a slightly modified version of the neighbourhood mating model described in  
5 Burczyk et al. (2006), where all potential parents are encapsulated within a single  
6 neighbourhood corresponding to the study site (Oddou-Muratorio and Klein, 2008). We  
7 assumed that each sampled seedling  $i$  could be mothered either (1) by a mother tree located  
8 outside the study site due to seed immigration (with probability  $m_s$ ) or (2) by a mother tree  
9 located within the studied site (with probability  $(1- m_s)$ ). In the latter case offspring  $i$  may be  
10 the result of self-pollination (with probability  $s$ ), pollen flow from outside the study site (with  
11 probability  $m_p$ ), or pollen from a sampled male (with probability  $1-s-m_p$ ). The probability of  
12 observing a multilocus diploid genotype  $G_i$  among the seedlings is thus:

$$13 \quad P(G_i) = m_s T(G_i|BAF) + (1-m_s) \sum_{j \in ISP} \Psi_{ij} \left[ s T(G_i|M_j, M_j) + m_p T(G_i|M_j, BAF) + (1-s-m_p) \sum_{k \in ISP} \Phi_{j,k} T(G_i|M_j, F_k) \right]$$

14 (2)

15 where  $T(G_i|BAF)$  is the transition probability that an offspring immigrating from a mother  
16 tree located outside of the study site has genotype  $G_i$  knowing the Background Allelic  
17 Frequencies (BAF) ;  $T(G_i|M_j, M_j)$ ,  $T(G_i|M_j, BAF)$ ,  $T(G_i|M_j, F_k)$  are the transition probabilities  
18 that an offspring has diploid genotype  $G_i$  when its mother tree of genotype  $M_j$  is respectively  
19 (1) self-pollinated (2) pollinated by a father drawn at random in a population with allelic  
20 frequencies BAF or (3) pollinated by a father of genotype  $F_k$ .

21 Parameter  $\Psi_{ij}$  is the proportion of seeds from mother tree  $j$  at the location of seedling  $i$  among  
22 the virtual seed pool originating from all known mother trees from the neighborhood  $N$ :

$$23 \quad \Psi_{ij} = \frac{\tau_{ij}}{\sum_{l \in N} \tau_{il}} \quad (3)$$

1 where  $\tau_{ij}$  is a function of one or more factors influencing female reproductive success,  
 2 including the seed dispersal kernel (see below).

3 Parameter  $\Phi_{jk}$  is the proportion of pollen from tree  $k$  in the pollen pool of mother tree  $j$  in the  
 4 total pollen pool from the known father trees in the neighborhood  $N$ :

$$\Phi_{jk} = \frac{\pi_{jk}}{\sum_{l \in N} \pi_{jl}} \quad (4)$$

5 where  $\pi_{jk}$  is a function of one or more factors influencing male reproductive success,  
 6 including the pollen dispersal kernel (see below).

7 *Models for pollen and seed dispersal kernel-* As in Oddou-Muratorio et al. (2005), pollen  
 8 dispersal was modelled using a dispersal kernel  $p_p(.,x,y)$  describing the probability for a  
 9 pollen grain emitted at position (0,0) to participate to the pollen cloud at position (x,y).

10 Following this scheme, we modelled seed dispersal using a dispersal kernel  $p_s(.,x,y)$   
 11 describing the probability for a seed emitted at position (0,0) to establish as a seedling at  
 12 position (x,y). We considered here the family of exponential power functions:

$$p(a,b;x,y) = \frac{b}{2\pi a^2 \Gamma(2/b)} \exp\left(-\left(\frac{\sqrt{x^2+y^2}}{a}\right)^b\right), \quad (5)$$

13 where  $\Gamma$  is the classically defined gamma function (Abramowitz and Stegun, 1964). The  
 14 parameter  $b$  is the shape parameter affecting the tail of the dispersal function and  $a$  is a scale  
 15 parameter homogeneous to distance. The mean distance ( $\delta$ ) traveled by a pollen grain/seed  
 16 under the kernel  $p(a,b;.)$  and the root-mean-square axial distance are given by Austerlitz et al.  
 17 (2004) and Rousset (2004):

$$\delta = a \frac{\Gamma(3/b)}{\Gamma(2/b)} \quad \text{and} \quad \sigma = a \sqrt{\frac{\Gamma(4/b)}{2\Gamma(2/b)}} \quad (6)$$

18 Both increase with  $a$  and decrease with  $b$ . When  $b < 1$ , the dispersal kernel is fat-tailed (Clark,  
 19 1998), i.e. the long-range decrease is slow, increasing the probability of long-distance

1 dispersal events. Conversely, when  $b > 1$  (for instance the Gaussian model, for which  $b = 2$ )  
 2 the dispersal is thin-tailed, with a rapid decrease of the dispersal function, implying fewer  
 3 long-distance dispersal events than when  $b < 1$ .

4 *Model for female/male reproductive success (the  $\tau_{ij}$ 's and the  $\pi_{jk}$ 's):* individual female  
 5 reproductive success  $\tau_{ij}$  of female  $j$  on seedlings location  $i$  was determined by two kind of  
 6 factors interacting multiplicatively: (1) the distance of seedling  $i$  from mother  $j$  through the  
 7 dispersal kernel described above and (2) adult tree diameter as a surrogate for female  
 8 fecundity. Here  $\tau_{ij}$  is expressed as:

$$9 \quad \tau_{ij} = e^{\gamma_f D_j} \times p_s(a_s, b_s; x_i - x_j, y_i - y_j) \quad (7)$$

10 where  $D_j$  is the diameter of mother  $j$ , and  $a_s$  and  $b_s$  the parameters of the seed dispersal kernel  
 11  $p_s$  described by equation (5). Classically, we chose an exponential function of parameter  $\gamma_f$  to  
 12 relate the fecundity surrogate (here  $D_j$ ) to reproductive success because it assures a positive  
 13 value for the reproductive success parameter. Additionally, this model of the selection  
 14 gradient is well-suited for a continuously distributed variable such as diameter.

15 Individual male reproductive success  $\pi_{jk}$  of tree  $k$  in the local pollen cloud of mother  $j$   
 16 depended on two independent factors: (1) the distance of mother  $j$  to father  $k$  and the pollen  
 17 dispersal parameters and (2) the diameter of tree  $k$  as a surrogate for male fecundity.

18 Therefore,  $\pi_{jk}$  was expressed as:

$$19 \quad \pi_{jk} = e^{\gamma_m D_k} \times p_p(a_p, b_p; x_j - x_k, y_j - y_k) \quad (8)$$

20 where  $D_k$  is the diameter of father  $k$ ,  $\gamma_m$  is the male fecundity parameter, and  $a_p$  and  $b_p$  are the  
 21 parameters of the pollen dispersal kernel  $p_p$ .

22 *Parameters inference-* We obtained maximum likelihood estimates for the seed and pollen  
 23 immigration rates ( $m_s$  and  $m_p$ ), selfing rate ( $s$ ), kernel parameters ( $a_s$  and  $b_s$  for seed dispersal

1 kernel;  $a_p$  and  $b_p$  for pollen dispersal kernel) and contextual gradients ( $\gamma_f$  and  $\gamma_m$ ), by  
 2 maximising the log-likelihood functions:

$$3 \quad \log L(a_p, b_p, a_s, b_s, s, m_p, m_s, \gamma_f, \gamma_m) = \sum_{i=1}^S \log [P(G_i)]. \quad (9)$$

4 where  $S$  is the total number of genotyped seedlings.

5 Parameters were fitted with the Newton-Raphson algorithm using NM+ software (Chybicki  
 6 and Burczyk unpublished). NM+ allowed us to estimate simultaneously the frequencies of  
 7 null alleles at each marker, and to eliminate markers significantly affected by null alleles (i.e.  
 8 with frequency of null alleles  $>0.10$ ).

## 9 Results

10 *Genetic markers and null alleles*- The frequency of null alleles was overall lower in the  
 11 Japanese beech data set than in any of the three European beech data sets (Table 2).  
 12 Frequencies of null alleles ranged from 0 (in 12 site/loci combination out of 33) to 0.125.

13 *SGS and historical estimates*- SGS was significant in the four data sets, with both adults and  
 14 seedlings more related than expected by chance for distances up to 30-40 metres (Figure 2).  
 15 The strength of SGS, as depicted by the  $Sp$ -statistics, significantly differed among sites: the  
 16 ancient Japanese beech stand showed the weakest pattern of SGS ( $Sp_{FC} = 0.0032$  in adults),  
 17 followed by the ancient European beech stand ( $Sp_{FS3} = 0.0087$  in adults), then by the high-  
 18 density recolonising European beech stand ( $Sp_{FS1} = 0.0218$  in adults), and finally by the low-  
 19 density recolonising European beech stand ( $Sp_{FS2} = 0.0354$  in adults). Within site, stronger  
 20 SGS was detected in adults compared to seedlings in the recolonising European beech stand  
 21 FS1 (FS1:  $Sp_A = 0.0218$  versus  $Sp_S = 0.0163$ , Table 3) while in FS2 SGS did not differ among  
 22 adults and seedlings ( $Sp_A = 0.0354$  versus  $Sp_S = 0.0205$ ). In mature populations of Japanese  
 23 beech, SGS was significantly weaker among adults compared to seedlings (FS1:  $Sp_A = 0.0032$   
 24 versus  $Sp_S = 0.0124$ ). In FS3, the sampling design of seedlings did not allow estimation of SGS  
 25 (Figure 1).

1 Using observed density  $d_{obs}/2$  to estimate  $d_e$ , SGS converted into axial variance of gene  
 2 dispersal ( $\sigma_e$ ) between 31 m (adult, FS2) and 73 m (seedlings, FS2, Table 3) for the European  
 3 beech, and between 19 m (seedlings) and 37 m (adult) for the Japanese beech. Fixing  $d_e =$   
 4  $d_{obs}/10$ ,  $\sigma_e$  increased markedly, ranging between 70 m and 163 m for the European beech and  
 5 between 42 m and 82 m for the Japanese beech. In Japanese beech,  $\sigma_{e\text{-seedlings}} < \sigma_{e\text{-adults}}$   
 6 whatever the assumed  $d_e/d_{obs}$  ratio (Table 3)

7 *Neighbourhood model and contemporary estimates*- Direct estimates of mating and dispersal  
 8 parameters are summarised in Table 4. For these analyses, the marker sfc1063 was removed  
 9 due to high frequency of null alleles (up to 12.5%). For the European beech, important seed  
 10 migration ( $21\% < m_s < 36\%$ ) and very high pollen migration ( $63\% < m_p < 72\%$ ) from outside  
 11 the studied site were detected. In the Japanese beech by contrast, seed immigration rate was  
 12 not different from zero, and pollen immigration rate was estimated to be 40%.

13 The average distance of seed dispersal within the study site fell within the same order of  
 14 magnitude across sites and species with  $\delta_s = 10.5$  m (for European beech on average on FS1  
 15 and FS3) and  $\delta_s = 12.4$  m (for Japanese beech). Average distance of pollen dispersal were also  
 16 not significantly different among species and sites with  $\delta_p = 38$  m on average in European  
 17 beech and  $\delta_p = 79$  m in Japanese beech. Some of the estimated values for  $\delta_s$  and  $\delta_p$  suffered  
 18 from low precision (large standard errors); this is an expected behaviour when the slope of the  
 19 dispersal kernel ( $b_s$  and  $b_p$ ) are well below 1 (Oddou-Muratorio et al., 2005). For both pollen  
 20 and seed dispersal, we observed a general tendency of slightly fat-tailed dispersal kernels (i.e.  
 21  $b \leq 1$ ). In FS3, the  $b$ -parameters could not be simultaneously estimated with the other  
 22 parameters due to lack of convergence, but the best fits were obtained with an exponential  
 23 kernel for seeds and pollen. The estimated seed dispersal kernel was exponential in FC, only  
 24 slightly more fatter-tailed than the exponential in FS3 ( $b_s = 0.72$ ), while it was strongly thin-

1 tailed in FS2 ( $b_s = 0.15$ ). For the pollen dispersal kernel the estimated shape ranged from 0.30  
2 (FC) to 1.03 (FS1).

3 The effect of diameter on female and male fertility was highly significant, with both male and  
4 female fecundities increasing with diameter ( $\gamma_s=0.86$  and  $\gamma_p=0.62$ ). The selection gradient  
5 tended to be steeper for female fecundity than for male fecundity. Selection gradients could  
6 not be estimated for FS3 (no data) or FS2 (due to lack of convergence).

## 7 **Discussion**

8 This study provided historical and contemporary estimates of gene flow in European and  
9 Japanese beech based on common, robust methods applied to four different parent-offspring  
10 genotype data sets. In the following we first discuss methodology related to each estimate  
11 (historical or contemporary) and investigate their variation in the observed range of species  
12 and sites. Finally, we discuss the implication of our results for the understanding of gene  
13 dispersal in beech, and the consequences for management of the genetic resources of this  
14 species.

## 15 **Patterns of SGS and historical estimates of gene flow**

16 In both European and Japanese beech seedlings and adults showed a significant pattern of  
17 SGS associated with historical dispersal rates ranging from ~47 m to ~105 m depending on  
18 the assumed ratio of effective versus observed density (respectively  $\frac{1}{2}$  and  $\frac{1}{10}$ ). For long-  
19 lived organisms such as trees it is notoriously difficult to estimate  $d_e/d_{obs}$ , or  $N_e/N$  (the ratio of  
20 effective versus census population size), as these quantities depend on the variation in lifetime  
21 reproductive success among individuals. Following Hardy et al. (2006) and assuming  
22  $d_e=d_{obs}/4$  in our long-lived hermaphrodite tree species the estimated gene dispersal distances  
23 were ~77 m in European beech and ~40 m in Japanese beech. Despite a slightly lower  $\sigma_e$   
24 estimate for the adults in FS1 ( $\sigma_{eFS1}=44.35$  m, significantly inferior to estimates from FS2 and  
25 FS3), gene dispersal distances were similar across sites and cohorts in European beech.



1 Moreover, our results overall reflect a trend towards restricted dispersal abilities in beech  
2 compared to tropical (Hardy et al., 2006) or temperate, low-density (Oddou-Muratorio and  
3 Klein, 2008) tree species, where  $\sigma_e$  ranged from 150 to 1200 m.

4 Interestingly, the comparison of SGS intensity between cohorts and between expanding and  
5 ancient populations highlights the interaction between population history, effective population  
6 density and dispersal in the development of SGS. First, the increasing SGS (and conversely  
7 decreasing  $d_e\sigma_e$  product) observed from adult to seedlings in Japanese beech contrasted with  
8 the more similar SGS patterns observed among cohorts in European beech. The reduction in  
9 the  $d_e\sigma_e$  product from adults to seedlings observed in the Japanese beech may be due to  
10 several causes : (1) a recent reduction in effective gene dispersal ( $\sigma_e$ ), (2) a recent reduction in  
11 effective population density ( $d_e$ ) in Japanese beech, and/or (3) a reduction of a substantial part  
12 of coancestry between nearest neighbours during stand maturation from seedlings to adults  
13 (Epperson, 1992). The fact that all the analysed Japanese beech seedlings correspond to a  
14 single year of reproduction (as compared to European beech seedlings, recruited across  
15 several year of reproduction) support hypothesis (2) above: single-year  $d_e$  is indeed expected  
16 to be lower (due to high inter-individual variance in reproductive success) as compared to  
17 average  $d_e$  accumulated over several reproductive events (Krouchi et al., 2004).

18 Second, SGS was significantly weaker in ancient stands (FS3 and FC) compared to re-  
19 colonising stands (FS1 and FS2). This variation was independent of observed adult density, as  
20 FS3 and FS1 showed roughly the same density (Table 1). Marked patterns of SGS have also  
21 been found in recently founded populations (Parker et al., 2001). In our case, it is likely that  
22 the strongly spatially aggregated distribution of adults in FS1, compared to the more regular  
23 distribution in FS3 (Figure 1), accounts for the higher intensity of SGS due to restricted  
24 overlap of seed shadows in FS1. By contrast, the higher SGS in FS2 compared to FS1 can be

1 explained by the lower population density in FS2, which also reduces the seed shadow  
2 overlap.

### 3 **Contemporary estimates of seed and pollen dispersal**

4 Direct estimates of seed and pollen dispersal curves using the spatially explicit mating model  
5 also highlight restricted seed dispersal abilities in European and Japanese beech, despite non-  
6 negligible long-distance dispersal events, and potentially important pollen-mediated gene  
7 flow.

8 Considering first seed dispersal patterns within studied sites, seed dispersal distances were  
9 consistent across sites and species, with a mean  $\delta_s$  value of 10.5 m for European beech and  
10 12.4 m for Japanese beech. Moreover the best fits were obtained either with exponential or  
11 fat-tailed kernels. The different values obtained for seed dispersal parameters in site FS2 may  
12 be partially explained by the age distribution of seedlings, because old seedlings (> 4 years  
13 old) were over-represented in this site compared to the 3 other sites. Estimates of the seed  
14 dispersal parameter for the sub-sample of young seedlings in FS2 fell within the same order  
15 of magnitude as the 3 others ( $\delta_s=7.68$  and  $b_s=1$ , Bontemps et al., unpublished). Thus, the seed  
16 rain generated within a single or few reproductive events and observed at the early stage of  
17 seedling recruitment reflects rather restricted seed dispersal abilities in beech compared to  
18 other species where such estimates are available (e.g.  $\delta_s \approx 135$  m in *S. torminalis*, Oddou-  
19 Muratorio and Klein, 2008);  $\delta_s = 277$  m in *Prunus mahaleb*, Robledo-Arnuncio and Garcia,  
20 2007). These discrepancies are consistent with life history traits of the species and suggest  
21 that seed dispersal in beech is primarily mediated by gravity and small rodents, as compared  
22 to fleshy-fruit species where dispersal is assisted primarily by birds but also bigger mammals  
23 (Jordano et al., 2007).

24 In contrast to seed dispersal within site, seed immigration rates in European beech ( $m_s=27\%$   
25 on average) indicate that “long-distance” seed dispersal events (i.e. from outside the study

1 site) are not negligible in the investigated sites. This was not the case for Japanese beech  
2 where we found a null seed migration rate. High rates of seed immigration could be  
3 associated with disturbances, as the three European beech sites all have a more recent history  
4 of perturbation than the Japanese beech sites, with recent expansion in FS1 and FS2, but also  
5 strong canopy opening in FS3 due to a storm occurred in 1999. However, the estimated values  
6 for immigration rates should be considered with caution, because they are also probably  
7 affected by different experimental biases: (1) mortality/logging of adults within the studied  
8 site could result in false migration events, as supported by high  $m_s$ -values observed in the  
9 saplings ( $m_s= 48\%$ ) compared to young seedlings ( $m_s=16\%$  for see Bontemps et al.,  
10 unpublished); (2) genotyping error and null alleles could also result in false parentage  
11 exclusion and overestimated migration rates.

12 Regarding pollen dispersal, the results for Japanese beech suggest greater abilities of pollen  
13 versus seed dispersal, with both a higher mean dispersal distance ( $\delta_p=79.04$  m versus  $\delta_s=12.44$   
14 m), and a fatter-tailed kernel ( $b_p = 0.31$  versus  $b_s = 1.06$ ). Pollen dispersal parameters ( $\delta_p$  and  
15  $b_p$ ) for European beech should be considered with caution as they were estimated based only  
16 on seedling for whom at least one compatible parent pair was found within the study site. Few  
17 seedlings met this requirement, as shown by the high  $m_s$  and  $m_p$ -values.

### 18 **Comparison of historical and contemporary estimates of gene flow**

19 We computed axial root mean squared  $\sigma_{p-rt}$  and  $\sigma_{s-rt}$  from our contemporary estimates of mean  
20 distance of seed and pollen dispersal ( $\delta_s$  and  $\delta_p$ , Table 4) using equation (6). This provided  
21 real-time, total gene flow estimates ( $\sigma_{rt}$ ) ranging between 19 m and 142 m in European beech  
22, and equal to 71 m in Japanese beech (Table 4). These are probably underestimates as they do  
23 not account for immigration from outside the study site. Interestingly, contemporary ( $\sigma_{rt}$ ) and  
24 historical ( $\sigma_e$ ) estimates of gene flow fell within the same order of magnitude (a few tens of  
25 meters). In FS2 and FC  $\sigma_{rt}$  fell within the confidence interval of  $\sigma_e$  estimated when assuming

1  $d_e = d_{obs}/10$ . In FS1 and FS3 contemporary estimates of gene flow tended to be lower than  
2 historical estimates, suggesting a possible recent reduction in effective dispersal abilities.  
3 However it is difficult to establish whether the observed discrepancy (1) is truly significant, (2)  
4 is due to the assumptions and statistical specificities of the two approaches or (3) whether the  
5 range of gene dispersal has actually changed. These results highlight the caution required  
6 when drawing quantitative conclusions from the comparison of contemporary and historical  
7 estimates because they both rely on different assumptions that affect the accuracy and  
8 precision of estimates in an incomparable way. Contemporary estimates may suffer from low bias at  
9 the scale of the study site, but they may underestimate long-distance dispersal (Oddou-  
10 Muratorio and Klein, 2008). By contrast, historical estimates have minimum precision, and  
11 suffer from high general uncertainty for the value of the effective density  $d_e$  value.

12 The comparison of historical versus contemporary estimates sheds some light on the different  
13 components of gene flow contributing to the development of SGS. For instance, the  
14 increasing SGS/ decreasing  $d_e \sigma_e$  product observed from adults to seedlings in Japanese beech  
15 is consistent with the restricted dispersal distances estimated using the contemporary  
16 approach, and in particular with the low pollen and seed migration rates ( $m_s \approx 0$ ,  $m_p = 40\%$ ). By  
17 contrast, in European beech, higher pollen and seed migration rates ( $m_s \approx 27\%$ ,  $m_p = 68\%$ ) may  
18 explain why SGS does not increase in the seedling cohort despite restricted seed and pollen  
19 dispersal distances within the study site. Thus, besides variation in the effective population  
20 density discussed above, our results suggest that long-distance seed and/or pollen flow can  
21 have a strong impact in the building of SGS within beech populations.

22 Finally, the roughly similar measures of gene flow obtained using historical or contemporary  
23 estimates in this study and others (Oddou-Muratorio and Klein, 2008) may provide guidance  
24 for researchers interested in measuring gene flow to select one of the two approaches,  
25 depending on the resources or constraints of the study. Historical approaches are clearly less

1 costly in term of sampling effort since they do not require exhaustive sampling and mapping.  
2 They can thus be useful in providing some preliminary estimates of total gene flow,  
3 particularly if the sampling design includes different cohorts among which estimates can be  
4 compared. By contrast, contemporary estimates are clearly more costly to obtain since they  
5 require exhaustive sampling of the parental population within a given area, as well as, ideally,  
6 measures of covariates of fecundity for these trees. Conversely, they yield a much more  
7 detailed description of gene flow, and in particular they allow the relative importance of seed  
8 versus pollen mediated dispersal and of long versus short distance dispersal to be gauged. A  
9 main conclusion of this study is that the combination of both historical and contemporary  
10 approaches may be the only solution when the objective is to detect recent changes in  
11 effective dispersal ability or to gauge the influence of the different components of gene flow  
12 in the building of SGS.

## 13 **Perspectives on beech's response to climate change and management of genetic** 14 **resources**

15 Estimates of seed dispersal in European and Japanese beech (a few tens of meters/ generation)  
16 obtained in this study are roughly one order of magnitude below what would be needed for  
17 beech to track 21<sup>st</sup>-century global warming (hundreds of meters for the next generation). This  
18 substantial discrepancy also supports recent re-interpretation of post-glacial expansion rates  
19 during the early Holocene in the light of chloroplast DNA surveys (McLachlan et al., 2005;  
20 Magri 2008). Rather than rapid range expansions from well-established refuges at rates of  
21 100-1000 m/yr, molecular studies suggested that for different tree species including beech  
22 post-glacial recolonisation relied on a mosaic of sparse, small populations scattered in  
23 multiple regions closer to modern range limit than previously thought. These authors  
24 proposed that average post-glacial migration rates of beech may have been slower than those  
25 inferred from fossil pollen, and closer to those estimated in this study. However, one can not

1 rule out that rare events of long-distance seed dispersal still contributed to post-glacial  
2 recolonisation, and may contribute to future response to climate change. The importance of  
3 these long-distance dispersal events is notoriously complicated to quantify, and requires to be  
4 addressed at spatial and temporal scales larger than in the present study.

5 Assuming that gene migration may favour a rapid adaptive response to the ongoing climate  
6 change by mixing or adding well adapted genetic material to poorly-adapted gene pools, this  
7 study suggests several ways in which forest management may enhance the beneficial effect of  
8 migration. Stand regeneration is clearly a crucial phase: to increase genetic diversity at local  
9 scale, management should (1) favour a regular spatial distribution of selected seed-trees (i.e.  
10 avoid clumping), (2) spread the recruitment process across several different years, even in  
11 masting species, (3) favour regeneration plots both close to and far from the seed trees. Note  
12 however that the final outcome of the interaction between gene flow and selection in the  
13 response of Beech population to global change can not be addressed without additional  
14 studies, comparing core and marginal populations, either at the front or at the rear edge of  
15 distribution range, and evaluating simultaneously the potential for genetic adaptation and gene  
16 flow.

## 17 **Acknowledgments**

18 We thank A. Roig and B. Jouaud (INRA Avignon) for sampling, genotyping and managing of  
19 lab work in *European beech* . We also thank F. Rei, O. Gilg, N. Thurion and F. Jean (UEFM,  
20 Avignon), as well P. Legroux, A. Nassau, F. Bonne, T. Paul and J.P. Lemaire (UEFL, Nancy)  
21 for field work (mapping and in situ measurement). For the study in *F. crenata*, we thank K.  
22 Maruyama, J. Takahashi, M. Tomita, N. Ueno, M. Takahashi, and K. Seiwa. This study was  
23 funded by the French Bureau des Ressources Génétiques (BRG grant AIP 223 N° 88 – 2003-  
24 2004), EVOLTREE NoE (BEECH project), and ECOGER.

## 25 **References**

- 1 Abramowitz, M., Stegun, I.A., 1964. *Handbook of mathematical functions with formulas,*  
2 *graphs, and mathematical tables* Nat. Bur. Standards, Appl. Math. Ser. 55., U.S.  
3 Government Printing Office, Washington D.C.
- 4 Alleaume-Benharira, M., Pen, I.R., Ronce, O., 2006. Geographical patterns of adaptation  
5 within a species' range: interactions between drift and gene flow. *J. Evol. Biol.* 19,  
6 203–215.
- 7 Asuka, Y., Tani, N., Tsumura, Y., Tomaru, N., 2004. Development and characterization of  
8 microsatellite markers for *Fagus crenata* Blume. *Mol. Ecol. Notes* 4, 101–103.
- 9 Asuka, Y., Tomaru, N., Munehara, Y., Tani, N., Tsumura, Y., Yamamoto, S., 2005. Half-sib  
10 family structure of *Fagus crenata* saplings in an old-growth beech-dwarf bamboo  
11 forest. *Mol. Ecol.* 14, 2565-2575.
- 12 Austerlitz F, Dick CW, Dutech C, Klein, E.K., Oddou-Muratorio, S., Smouse, P.E., Sork, V.  
13 L., 2004. Using genetic markers to estimate the pollen dispersal curve. *Mol. Ecol.* 13,  
14 937-954.
- 15 Bacles, C.F.E., Burczyk, J., Lowe, A.J., Ennos, R.A., 2005. Historical and contemporary  
16 mating patterns in remnant populations of the forest tree *Fraxinus excelsior*. *Evolution*  
17 59, 979–990.
- 18 Bullock, J.M., Nathan, R., 2008. Plant dispersal across multiple scales: linking models and  
19 reality. *J. Ecol.* 96, 567–568.
- 20 Burczyk, J., Adams, W.T., Birkes, D.S., Chybicki, I.J., 2006. Using genetic markers to  
21 directly estimate gene flow and reproductive success parameters in plants on the basis  
22 of naturally regenerated seedlings. *Genetics* 173, 363–372.
- 23 Chybicki, I. J., Burczyk, J., 2009. Simultaneous estimation of null alleles and inbreeding  
24 coefficients. *Journal of Heredity* 100, 106-113.

- 1 Chybicki, I.J., Trojankiewicz, M., Oleksa, A., Dzialuk, A., Burczyk, J., 2009. Isolation-by-  
2 distance within naturally established populations of European beech (*Fagus sylvatica*).  
3 Botany 87, 791–798.
- 4 Clark, J.S., 1998. Why trees migrate so fast: confronting theory with dispersal biology and the  
5 paleorecord. Am. Nat. 152, 204–224.
- 6 Davis, M.B., Shaw, R.G., 2001. Range shifts and adaptive responses to Quaternary climate  
7 change. Science 292, 673–679.
- 8 Dunphy, B., Hamrick, J.L., 2005. Gene flow among established Puerto Rican populations of  
9 the exotic tree species, *Albizia lebbek*. Heredity 94, 418–425.
- 10 Dutech, C., Sork, V.L., Irwin, A.J., Smouse, P.E., Davis, F.W., 2005. Gene flow and fine-  
11 scale genetic structure in a wind-pollinated tree species, *Quercus lobata* (Fagaceae)  
12 Am. J. Bot. 92, 252–261.
- 13 Epperson, B. K., 1992. Spatial structure of genetic variation within populations of forest trees.  
14 New Forests 6, 257-278.
- 15 Gonzalez-Martinez, S.C., Burczyk, J., Nathan, R., Nanos, N., Gil, L., Alia, R., 2006. Effective  
16 gene dispersal and female reproductive success in Mediterranean maritime pine (*Pinus*  
17 *pinaster* Aiton). Mol. Ecol. 15, 4577–4588.
- 18 Hampe, A., Petit, R.J., 2005. Conserving biodiversity under climate change: the rear edge  
19 matters. Ecol. Lett. 8, 461–467.
- 20 Hardy, O.J., Vekemans, X., 2002. SPAGeDi: a versatile compute program to analyse spatial  
21 genetic structure at the individual or population levels. Mol. Ecol. Notes 2, 618–620.
- 22 Hardy, O.J., Maggia, L., Bandou, E., Breyne, P., Caron, H., Chevallier, M.H., Doligez, A.,  
23 Dutech, C., Kremer, A., Latouche-Hallé, C., Troispoux, V., Veron, V., Degen B.,  
24 2006. Fine-scale genetic structure and gene dispersal inferences in ten Neotropical tree  
25 species. Mol. Ecol. 15, 559-571.



- 1 Hanaoka, S., Yuzurihara, J., Asuka, Y., Tomaru, N., Tsumura, Y., Kakubari, Y., Mukai, Y.,  
2 2007. Pollen-mediated gene flow in a small, fragmented natural population of *Fagus*  
3 *crenata*. *Botany* 85, 404-413.
- 4 Krouchi, F., Derridj, A., Lefevre, F., 2004. Year and tree effect on reproductive organisation  
5 of *Cedrus atlantica* in a natural forest. *Forest Ecology and Management* 197, 181-189.
- 6 Jensen, T.S., 1985. Seed-seed predator interactions of European beech, *Fagus sylvatica* and  
7 forest rodents, *Clethrionomys glareolus* and *Apodemus flavicollis*. *Oikos* 44, 149–156.
- 8 Jones, F.A., Chen, J., Weng, G.-J., Hubbell, S.P., 2005. A genetic evaluation of seed dispersal  
9 in the Neotropical tree *Jacaranda copaia* (Bignoniaceae). *Am. Nat.* 166, 543–555.
- 10 Jordano, P., Garcia, C., Godoy, J.A., Garcia-Castano, J.L., 2007. Differential contribution of  
11 frugivores to complex seed dispersal patterns. *Proc. Natl. Acad. Sci. U.S.A.* 104,  
12 3278–3282.
- 13 Jump AS, Penuelas J (2007) Extensive spatial genetic structure revealed by AFLP but not  
14 SSR molecular markers in the wind-pollinated tree, *Fagus sylvatica*. *Mol. Ecol.* **16**,  
15 925-936.
- 16 Jump AS, Mátyás C, Peñuelas J (2009) The altitude-for-latitude disparity in the range  
17 retractions of woody species. *Trends Ecol. Evol.* **24**, 694-701.
- 18 Kalinowski, S., Taper, M., 2006. Maximum likelihood estimation of the frequency of null  
19 alleles at microsatellite loci. *Conserv. Genet.* 7, 991–995.
- 20 Kramer, K., Buiteveld, J., Forstreuter, M., Geburek, T., Leonardi, S., Menozzi, P., Povillon,  
21 F., Schelhaas, M.J., Teissier du Cros, E., Vendramin, G.G., Werf, D.C., 2008.  
22 Bridging the gap between ecophysiological and genetic knowledge to assess the  
23 adaptive potential of European beech. *Ecol. Modell.* 216, 333–353.

- 1 Leblois, R., Rousset, F., Estoup, A., 2004. Influence of spatial and temporal heterogeneities  
2 on the estimation of demographic parameters in a continuous population using  
3 individual microsatellite data. *Genetics* 166, 1081-1092.
- 4 Lenormand, T., 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* 17,  
5 183–189.
- 6 Leonardi, S., Menozzi, P., 1996. Spatial structure of genetic variability in natural stands of  
7 *Fagus sylvatica* L. (beech) in Italy. *Heredity* 77, 359–368.
- 8 Loiselle, B.A., Sork, V.L., Nason, J., Graham, C., 1995. Spatial genetic structure of a tropical  
9 understory shrub, *Psychotria officinalis* (Rubiaceae). *Am. J. Bot.* 82, 1420–1425.
- 10 Magri, D., 2008. Patterns of post-glacial spread and the extent of glacial refugia of European  
11 beech (*Fagus sylvatica*). *J. Biog.* 35, 450-463.
- 12 Manel, S., Gaggiotti, O.E., Waples, R.S., 2005. Assignment methods: matching biological  
13 questions with appropriate techniques. *Trends Ecol. Evol.* 20, 136–142.
- 14 McLachlan, J.S., Clark, J.S., Manosa, P.S., 2005. Molecular indicators of tree migration  
15 capacity under rapid climate change. *Ecology* 86, 2088–2098.
- 16 Meagher, T.R., 1986. Analysis of paternity within a natural population of *Chamaelirium*  
17 *luteum*. I. Identification of most-likely male parents. *Am. Nat.* 128, 199–215.
- 18 Merzeau, D., Comps, B., Thiébaud, J.L., 1994. Estimation of *Fagus sylvatica* L. mating  
19 system parameters in natural populations. *Ann. For. Sci.* 51, 163–173.
- 20 Morgan, M.T., Conner, J.K., 2001. Using genetic markers to directly estimate male selection  
21 gradients. *Evolution* 55, 272–281.
- 22 Oddou-Muratorio, S., Klein, E.K., 2008. Comparing direct vs. indirect estimates of gene flow  
23 within a population of a scattered tree species. *Mol. Ecol.* 17, 2743–2754.

- 1 Oddou-Muratorio, S., Klein, E.K., Austerlitz, F., 2005. Pollen flow in the wildservice tree,  
2 *Sorbus torminalis* (L.) Crantz. II. Pollen dispersal and heterogeneity in mating success  
3 inferred from parent-offspring analysis. Mol. Ecol. 14, 4441–4452.
- 4 Oddou-Muratorio, S., Vendramin, G.G., Buiteveld, J., Fady, B., 2009. Population estimators  
5 or progeny tests: what is the best method to assess null allele frequencies at SSR loci?  
6 Conserv. Genet. 10, 1343–1347.
- 7 Otero-Arnaiz, A., Casas, A., Hamrick, J.L., 2005. Direct and indirect estimates of gene flow  
8 among wild and managed populations of *Polaskia chichipe*, an endemic columnar  
9 cactus in Central Mexico. Mol. Ecol. 14, 4313–4322.
- 10 Parker, K.C., Hamrick, J.L., Parker, A.J., Nason, J.D., 2001. Fine-scale genetic structure in  
11 *Pinus clausa* (Pinaceae) populations: effects of disturbance history. Heredity 87, 99–  
12 113.
- 13 Pastorelli, R., Smulders, M.J.M., Van't Westende, W.P.C., Vosman, B., Giannini, R., Vetori,  
14 C., Vendramin, G.G., 2003. Characterization of microsatellite markers in *Fagus*  
15 *sylvatica* L. and *Fagus orientalis* Lipsky. Mol. Ecol. Notes 3, 76–78.
- 16 Robledo-Arnuncio, J.J., Garcia, C., 2007. Estimation of the seed dispersal kernel from exact  
17 identification of source plants. Mol. Ecol. 16, 5098–5109.
- 18 Ronce, O., 2007. How does it feel to be like a rolling stone? Ten questions about dispersal  
19 evolution. Ann. Rev. Ecol. Evol. Syst. 38, 231–253.
- 20 Rousset, F., 2000. Genetic differentiation between individuals. J. Evol. Biol. 13, 58–62.
- 21 Schweizer, M., Excoffier, L., Heckel, G., 2007. Fine-scale genetic structure and dispersal in  
22 the common vole (*Microtus arvalis*). Mol. Ecol. 16, 2463–2473.
- 23 Smouse, P.E., Sork, V.L., 2004. Measuring pollen flow in forest trees: an exposition of  
24 alternative approaches. For. Ecol. Manage. 197, 21–38.

- 1 Sykes, M.T., Prentice, I.C., Cramer, W., 1996. A bioclimatic model for the potential  
2 distributions of north European tree species under present and future climates. J.  
3 Biogeogr. 23, 203–233.
- 4 Takahashi, M., Mukouda, M., Kono, K., 2000. Differences in genetic structure between two  
5 Japanese beech (*Fagus crenata* Blume) stands. Heredity 84, 103–115.
- 6 Tanaka, K., Tsumura, Y., Nakamura, T., 1999. Development and polymorphism of  
7 microsatellite markers for *Fagus crenata* and the closely related species, *F. japonica*.  
8 Theor. Appl. Genet. 99, 11–15.
- 9 Tomita, M., Hirabuki, Y., Seiwa, K., 2002. Post-dispersal changes in the spatial distribution  
10 of *Fagus crenata* seeds. Ecology 83, 1560–1565.
- 11 Vekemans, X., Hardy, O.J., 2004. New insights from fine-scale spatial genetic structure  
12 analyses in plant populations. Mol. Ecol. 13, 921–935.
- 13 Vornam, B., Decarli, N., Gailing, O., 2004. Spatial distribution of genetic variation in a  
14 natural beech stand (*Fagus sylvatica* L.) based on microsatellite markers. Conserv.  
15 Genet. 5, 561–570.

16

1 Figures legends

2

3 **Figure 1:** Sampling design within each studied site (FS1, FS2 and FS3 for *European beech*

4 and FC for *F. crenata*) . Adult trees (▲) were exhaustively mapped within the studied site.

5 Saplings (+, age  $\geq 3$ ) and/or seedlings (\*, age  $< 3$ ) were sampled preferentially in the central  
6 part of studied site.

7 **Figure 2:** Correlograms of kinship coefficient for A) adult trees and B) seedlings in the four

8 sites. Abscise values correspond to the upper limit of the distance intervals. Filled symbols  
9 represent values significantly different from the expected value under a random distribution of  
10 genotypes (95% confidence level). Confidence intervals around each  $F_{ij}$ -value were obtained

11 through a jackknife procedure over loci.

12

13

14

15

1 **Table 1: Information on study sites for each species**

Species	Site	Coordinates (UTM)		Number of genotyped adults	Observed adult density (tree.ha <sup>-1</sup> )	Number of genotyped seedlings
<i>Fagus sylvatica</i>	FS1 (Mont Ventoux)	44°10'45"N	5°14'01"E	96	50	256
<i>Fagus sylvatica</i>	FS2 (Mont Ventoux)	44°11'03"N	5°16'57"E	50	19	356
<i>Fagus sylvatica</i>	FS3 (Haye Forest)	48°45'30"N	6°20'29"E	350	44	221
<i>Fagus crenata</i>	FC (Kurokima)	38°55'30"N	140°47'50"E	287	379	1387

2

1

2 **Table 2:** Number of alleles (Na) and null allele frequencies (Fnull) per species, site and  
 3 microsatellite locus. Detailed information on microsatellite loci can be found in: <sup>1</sup> Pastorelli et  
 4 al., (2003) ; <sup>2</sup> Tanaka et al. (1999); <sup>3</sup> Asuka et al., (2004) and <sup>4</sup>Vornam et al. (2004)

Species	<i>Fagus sylvatica</i>						<i>Fagus crenata</i>	
	FS1		FS2		FS3		FC	
Locus	Na	Fnull	Na	Fnull	Na	Fnull	Na	Fnull
FS1-03a <sup>1</sup>							17	0.000
FS1_15 <sup>1</sup>	13	0.037	14	0.006				
FS4-46 <sup>1</sup>					24	0.094	14	0.000
FS3-04 <sup>1</sup>	3	0.000	4	0.000	5	0.000		
mfc5 <sup>2</sup>					24	0.084		
mfc7 <sup>2</sup>	7	0.057	7	0.058				
sfc0007 <sup>3</sup>	7	0.000	8	0.019				
sfc1105 <sup>3</sup>	14	0.000	9	0.031			33	0.002
sfc1143 <sup>3</sup>	12	0.012	11	0.000			23	0.000
sfc0036 <sup>3</sup>	10	0.000	11	0.000			24	0.000
sfc0018 <sup>3</sup>	9	0.006	11	0.013			19	0.002
sfc0378 <sup>3</sup>							27	0.010
sfc0161 <sup>3</sup>	14	0.003	14	0.000	18	0.000		
sfc1063 <sup>3</sup>	10	0.125	8	0.107				
mfs11 <sup>4</sup>	8	0.103	9	0.045				

5

1 **Table 3:** Estimates of SGS and gene dispersal parameters for each species by site and cohort  
 2 (A = Adult, S=Seedlings): average kinship coefficient between individuals separated by less  
 3 than 10 m (F1), SGS intensity (Sp) and its standard error (SE), neighbourhood size, (Nb),  
 4 gene dispersal distance ( $\sigma_e$ ) and 95% confidence interval (CI) for three effective densities  
 5 (De) estimated from the density of adults (D).  
 6

Species	Site	Stage	SGS parameter		Gene dispersal parameters			
			F1	Sp (SE)	Nb	$\sigma_e$ (CI) De=D/2	$\sigma_e$ (CI) De=D/4	$\sigma_e$ (CI) De=D/10
<i>Fagus sylvatica</i>	FS1	A	0.0821	0.0218 (0.0046)	45.80	31.36 (26.2-41.5)	44.35 (37.1-58.7)	70.12 (58.7-92.8)
		S	0.0328	0.0163 (0.0018)	61.43	40.62 (36.3-47)	57.44 (51.3-66.4)	90.82 (81.2-105)
	FS2	A	0.0785	0.0354 (0.0076)	28.22	64.69 (54.3-84.7)	91.48 (76.8-119.8)	144.64 (121.5-189.4)
		S	0.051	0.0205 (0.0026)	48.76	72.98 (66-82.9)	103.21 (93.3-117.2)	163.19 (147.5-185.3)
	FS3	A	0.0577	0.0087 (0.0012)	114.33	65.97 (58.4-77.4)	93.30 (82.6-109.5)	147.52 (130.7-173.2)
<i>Fagus crenata</i>	FC	A	0.0343	0.0032 (0.0007)	312.15	36.84 (30.6-49.8)	52.10 (43.2-70.4)	82.38 (68.4-111.3)
		S	0.0255	0.0124 (0.0012)	80.67	18.64 (17.1-20.7)	26.37 (24.2-29.3)	41.69 (38.2-46.4)

7

8



1 **Table 4:** Selfing rate ( $s$ ), seed and pollen migration rate ( $m_s$  and  $m_p$ ), average distance of seed  
 2 and pollen dispersal ( $\delta_s$  and  $\delta_p$ ), shape parameter of exponential-power kernel for seeds  
 3 ( $b_s$ ) and pollen ( $b_p$ ) and selection gradients ( $\gamma_s$  and  $\gamma_p$ ) estimated by the direct approach  
 4 in the different species and ISP. See text for details on real-time, total gene flow  
 5 estimates ( $\sigma_{rt}$ ).

Species	<i>Fagus sylvatica</i>						<i>Fagus crenata</i>	
	FS1		FS2		FS3		FC	
Site	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
$m_s$	0.23	0.03	0.36	0.04	0.21	0.04	0.007	0.0035
$m_p$	0.63	0.04	0.68	0.04	0.72	0.06	0.40	0.019
$s$	0.01	0.01	0.04	0.02	0.03	0.02	0.00*	NE
$\delta_s$ (m)	10.42	1.39	62.42	73.68	10.89	0.90	12.14	0.36
$\delta_p$ (m)	28.12	4.39	30.52	18.56	55.14	17.99	79.04	30.88
$b_s$	0.72	0.15	0.15	0.10	1.00*	NE	1.06	0.07
$b_p$	1.03	0.33	0.68	0.23	1.00*	NE	0.30	0.07
$\gamma_s$	0.86	0.08					0.85	0.06
$\gamma_p$	0.60*	NE					0.65	0.07
$\sigma_{rt}$ (m)	19.66		142.61		35.06		71.21	

6 \* fixed value for the parameter, NE= corresponding standard error not estimated

7