Spatial vs. temporal effects on demographic and genetic structures: the roles of dispersal, masting and differential mortality on patterns of recruitment in *Fagus sylvatica*

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Abstract

Trees’ long lifespan, long-distance dispersal abilities and high year-to-year variability in fecundity are thought to have pervasive consequences for the demographic and genetic structure of recruited seedlings. However, we still lack experimental studies quantifying the respective roles of spatial processes such as restricted seed and pollen dispersal and temporal processes such as mast seeding on patterns of regeneration. Dynamics of European beech (*Fagus sylvatica*) seedling recruitment was monitored in three plots from 2004 to 2006. Six polymorphic microsatellite genetic markers were used to characterize seedlings and their potential parents in a 7.2-ha stand. These seedlings were shown to result from 12 years of recruitment, with one predominant year of seedling recruitment in 2002 and several years without significant recruitment. Using a spatially explicit mating model based on parentage assignment, short average dispersal distances for seed (δs = 10.9 m) and pollen (43.7 m < δp < 57.3 m) were found, but there was also a non-negligible immigration rate from outside the plot (ms = 20.5%; 71.6% < mp < 77.9%). Hierarchical analyses of seedling genetic structure showed that (i) most of the genetic variation was within plots; (ii) the genetic differentiation among seedling plots was significant (FST = 2.6%) while (iii) there was no effect of year-to-year seed rain variation on genetic structure. In addition, no significant effect of genetic structure on mortality was detected. The consequences of these results for the prediction of population dynamics at ecological timescales are discussed.

Keywords: contemporary gene flow, microsatellite, spatially explicit mating model, spatial genetic structure, tree

Introduction

Understanding the effects of demographic and genetic processes on the amount and spatial distribution of genetic variation in natural populations is one of the main objectives of modern evolutionary ecology. These issues have gained a renewed interest in the context of increased habitat fragmentation and rapid environmental change, which stress the need to understand and predict evolutionary trajectories of populations at ecological timescales. At short time and spatial scales, demographic (survival, growth, competition) and evolutionary forces (selection, gene flow and genetic drift) tightly interact to shape spatial patterns of allele frequencies across life stages or generations. In return, genetic variation affects population dynamics by determining individual capacities of survival, growth, and reproduction (Lande 1982). The study of this interplay between demographic and short-term evolutionary processes is sometimes referred to as demo-genetics and builds on the theoretical framework mainly developed by Lande (1982) and recently adapted by Coulson *et al.* (2006).
In trees, the period spanning from seed dispersal to early seedling recruitment is thought to be a major transition step where important demo-genetic interactions take place and have pervasive consequences on the structure and dynamics of tree populations (Petit & Hampe 2006). This step is characterized in particular by a massive mortality of seeds/seedlings produced during an individual lifetime, with typically only one seed in a million surviving as a reproductive adult (Petit & Hampe 2006). Moreover, recruitment studies highlight the role of various demographic and genetic processes and of their variation on patterns of regeneration. The major processes involved are the spatial distribution and density of reproductive plants, their seed outputs, the shape and form of their dispersal kernel and the spatial patterns of microsites favorable for seedling establishment (Clark et al. 1999, 2007; Nathan & Muller-Landau 2000). In the following section, we will focus in particular on: (i) dispersal limitation, which is a major factor shaping variation of recruitment through space; (ii) mast seeding (i.e. synchronous intermittent production of large seed crops) which is an important factor shaping the variation of recruitment through time particularly in temperate forests (Piovesan & Adams 2001; Kelly & Sork 2002); and (iii) mortality at early stages of the tree life cycle that causes major variation in the demographic and genetic structure across life stages.

The combined role of propagule (seed and pollen) production and dispersal on patterns of recruitment is widely acknowledged and studied in population dynamics and genetics. Seed dispersal and individual seed production shape both the initial spatial pattern of seedling abundance (Clark et al. 1999, 2007; Nathan & Muller-Landau 2000) and genetic relatedness among established individuals (Wright 1943). Unless pollen production is strongly limited or population density is very low (Sagnard et al. 2011), pollen dispersal is usually considered as driving mainly patterns of genetic relatedness. By contrast, the temporal component of the regeneration pattern due to inter-annual variation in seed production has received less attention, in particular from a population genetics perspective. It has been suggested that the genetic consequences of high variation in seed production among individuals are reduced over time because seed production ranking among trees varies strongly between years (Krouchi et al. 2004). This phenomenon tends to increase the effective population size and therefore decrease the spatial variation in genetic relatedness over the whole regeneration phase. From a population dynamics perspective, the few studies focusing on recruitment patterns across space and time show contrasting results. Some studies have found across-year consistency in seed-fall and seedling distribution with a strong site effect (Wright et al. 2005), while in others, across-year variation was higher than variation across sampling sites (Beckage et al. 2005). It thus remains largely unknown how spatial and temporal recruitment dynamics interact across heterogeneous landscapes, which of these components has a greater effect on patterns of regeneration in long-lived plants, and under which conditions (Alvarez-Buylla et al. 1996; Clark et al. 1999; Jones & Hubbel 2006; Hampe et al. 2008).

Massive seed and seedling mortality during recruitment has been shown to strongly affect tree population structure. At early life history stages, high density-dependent mortality due to seedling competition, predation and/or sensitivity to pathogens (Janzen 1970; Howe & Smallwood 1982; Nathan & Casagrandi 2004) can result in higher average distances between mothers and successfully established offspring than those expected from seed dispersal alone. The existence of Janzen–Connell effects in forest trees is supported by different studies across different forest systems including temperate deciduous forest (Hille Ris Lambers & Clark 2003). Janzen–Connell effects are expected to result in a decrease in structure and relatedness from the initial seed rain to recruited seedlings (Trapnell et al. 2008). Alternatively, an increase in genetic structure and relatedness from the initial seed rain to recruited seedlings can be expected when mortality is driven by microsite heterogeneity and genotype–microsite interactions, especially in spatially variable environments (Sagnard et al. 2010). Moreover, because the genetic load of trees is high (Petit & Hampe 2006), the purging of inbred individuals may contribute to seedling mortality (Ferriol et al. 2011). However, such processes are notoriously difficult to demonstrate in natural populations, and require monitoring the demo-genetics of naturally established seedlings through time (Kalisz et al. 2001). Most frequently, genetic structure studies taking a life-stage approach compare very distant cohorts, typically seeds, seedlings and adults (Alvarez-Buylla et al. 1996; Jones & Hubbel 2006).

Here we used a demo-genetic approach to investigate the consequences of the spatio-temporal patterns of regeneration on the demo-genetic structure of European beech (Fagus sylvatica L.). This wind-pollinated species is both gravity- and animal-dispersed (Jensen 1985) and produces beech nuts in irregular mast years. Previous demographic estimates of seed dispersal obtained through seed trapping and inverse modeling methods showed restricted dispersal abilities, with a median distance of seed dispersal of ~6.50 m (Sagnard et al. 2007). The European beech is described as a shade tolerant species able to survive under 1–2% of
full above-canopy light, but showing optimal growth potential at 30–40% of above-canopy light (Kunstler et al. 2007).

The originality of this study lies in the fact that we investigated the role of various genetic and demographic processes (inter-annual variation in seed production, dispersal, and mortality) on the distribution pattern of genetic diversity in three regeneration plots with different canopy closure in a beech stand where seedling recruitment was monitored from 2004 to 2006, and extrapolated back to 1993 by estimating seedling age. Because it is difficult to disentangle the different ecological factors shaping genetic structure, we also directly investigated the seed and pollen dispersal processes using parentage/paternity analyses. This approach allowed us to better test the consequences of a process (dispersal) and its variation in space (with stand density) or time (among year).

Based on this data set, the following specific questions were addressed: (i) how do patterns of seedling density and survival correlate in space and time? (ii) How spatially restricted are contemporary pollen and seed dispersal? (iii) Which spatial or temporal processes are the major factors shaping seedling genetic structure? (iv) Does mortality affect the genetic structure of the seedlings?

Materials and methods

Study species and sampling design

The European beech, Fagus sylvatica L. (Fagaceae), is a monoecious diploid (2n = 24) late-successional forest tree. It is a highly outcrossing wind-pollinated species (selfing <10%, Merzeau et al. 1994). Reproductive trees (typically older than 60–80 years in dense stands) produce beech nuts in irregular mast years, with an inter-mast interval of at least 2 years (Teissier du Cros 1981; Nilsson & Wastljung 1987). Seeds are primarily dispersed by gravity, and secondarily dispersed by rodents (Apodemus flavicollis, Clethrionomys glareolus) and birds (Glandus glandularis) that scatter hoard them (Jensen 1985).

The study site is a mixed beech-oak stand located within a large forest dominated by beech (Haye forest, North-Eastern France, Longitude: 06°06′36″E; Latitude: 48°38′23″N). Within the site (~7.8 ha), all 342 adult beech trees were mapped using a Rangemaster 900 Scan telemeter and a compass, with a precision of more than 5 m (Fig. 1). All were sampled for genetic analyses. Adult density varied across the site from <30 stem/ha to >90 stem/ha as a result of the 1999 Lothar storm.

Young seedlings were sub-sampled in 3 plots (A: 9 m²; B: 5 m² and C: 8 m² in area) corresponding to a gradient in the levels of photosynthetically active radiation (PAR) measured in micromol/m²/s with a Sun-Scan® system. Within each plot 5–6 measurements were averaged and converted into percentage of transmitted PAR by reference to the average measure of 157.9 µmol/m²/s obtained in the open (=incident PAR). Light availability under the canopy strongly decreased from 55% of transmitted light (plot A) to 31% of incident light (plot B) and finally to 6% of incident light (plot C), in relation to the variation in adult tree density in a 50-m radius around the plots (A: 56 trees/ha; B: 72 trees/ha; C: 92 trees/ha).

In total, 462 young seedlings were exhaustively mapped, among which 254 where sampled for genetic analyses (Table 1). Among the 462 seedlings, 371 were found in the first year of the survey (2004) while 91 new seedlings germinated in spring 2005 and were mapped in summer 2005. Survival of all seedlings was recorded from 2004 to 2006. Germination year was estimated retrospectively by counting node scars and ranged from 1993 to 2005. Finally, 372 seeds were collected in the autumn of 2004 in the crown of 29 fruiting trees (5–16 seeds/tree, mean = 12.8, SD = 2.98).

Fig. 1 Haye forest study plot, with exhaustive mapping of adult trees (plotted as circles: ○/●) and three seedling plots (plotted as squares: ■). Among all the 342 adult trees, 29 were chosen for collecting seed maternal progeny (●).
Genotyping

DNA was isolated from buds (adult trees and seedlings) and embryos (seeds) using the Qiagen DNeasy Plant kit. Individuals were genotyped using four nuclear microsatellite markers (FS1-03, FS1-25, FS3-04, FS4-46) developed for *Fagus sylvatica* (Pastorelli et al. 2003) and 2 nuclear microsatellite markers (FCM5 and SFC-0161) developed for *Fagus crenata* (Tanaka et al. 1999; Asuka et al. 2004) (Table 1), following PCR conditions given by the authors. Adult and seed PCR products were separated using an automated 96-capillary MegaBACE/C212 1000 sequencer (GE Healthcare). Genotypes were sized using the internal size standards ET400 and the MegaBACE/C212 Fragment Profiler version 1.2 software (GE Healthcare). Seedling PCR products were separated using a LICOR automated gel-sequencer (some adults were also genotyped on LICOR sequencer for homogenous sizing).

Null alleles and quality of the marker set

Null allele frequencies (NAF) were first estimated by direct counting in maternal progeny arrays (for details see Oddou-Muratorio et al. 2009). Additionally, we estimated NAF in adult and seedling cohorts using the maximum-likelihood method implemented in ML-NullFreq software and accounting for genotyping error (Kalinowski & Taper 2006).

The non-negligible prevalence of null alleles in European beech was confirmed, with four loci out of six affected (Table S1, Supporting information). NAF were >10% in 2 locus/cohort combinations (adults at locus FS1-25 and FS4-46) and >5% in 9 locus/cohort combinations out of 18. Adults tended to have higher NAF than seedlings at loci FS1-25 and FS4-46, but the opposite trend was observed at locus FS1-03. The difference in NAF between adult and seedling cohorts was highest (~0.13) at locus FS 4-46, despite low genetic differentiation among adult and seedling cohorts ($F_{ST}$ averaged across loci = 0.54%).

When high frequencies of null alleles were found ($P > 0.05$), we evaluated their impact on consanguinity and differentiation estimates using the ENA method proposed by Chapuis & Estoup (2007). This method consists (i) in correcting the original data set by statistical adjustment of genotype frequencies based on estimated null allele frequencies and then (ii) in re-estimating $F_{IS}$ or $F_{ST}$ based on visible alleles only. It is designed to yield unbiased $F_{ST}$ values, but not to yield unbiased $F_{IS}$-values, which are likely to be underestimated. Significantly positive $F_{IS}$ values were estimated at the four loci affected by null alleles (Table S1). However after correcting the data set for null alleles using the ENA method proposed by Chapuis & Estoup (2007), no significant heterozygote deficiency could be detected in either adults or seedlings (results not shown). By contrast, $F_{ST}$ values were of the same order of magnitude in the raw and corrected data sets, showing that genetic differentiation between groups of seedlings was not affected by null alleles.

According to these results, we later considered in our analyses that: (i) null alleles occurred at non-negligible frequencies in the data set, frequencies that were not estimated with precision as depicted by their variation across cohorts at the same locus; (ii) some of the observed positive $F_{IS}$-values were probably partly affected by null alleles, but also by the mating system; $F_{IS}$-values were thus assumed to be relevant in a comparative context; and (iii) genetic differentiation as estimated by $F_{ST}$ was not affected by null alleles.

Genetic diversity

Genotyping problems occurred at locus FCM5, and particularly for seedlings (81% missing data). Given that

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**Table 1** Effective recruitment patterns of beech seedlings in the three studied regeneration plots. Total count of seedlings in year 2004 (NS) and the number of seedlings used for genetic analyses (NG) per plot and per year of germination

| Plot | Year of germination | 1993 | 1995 | 1997 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005* | Total |
|------|---------------------|------|------|------|------|------|------|------|------|------|------|-------|
| Plot A | NS                  | 0    | 0    | 0    | 0    | 4    | 0    | 71   | 18   | 13   | 9    | 115   |
| PAR = 55% | NG                |      |      |      |      | 57   | 15   | 10   |      |      |      | 82    |
| Plot B | NS                  | 1    | 1    | 1    | 12   | 1    | 63   | 7    | 28   | 41   | 155   |
| PAR = 31% | NG                |      |      |      |      | 1    | 56   | 5    | 19   |      |      | 81    |
| Plot C | NS                  | 0    | 1    | 2    | 3    | 51   | 9    | 57   | 23   | 5    | 41   | 192   |
| PAR = 6% | NG                |      |      |      |      | 7    | 46   | 17   | 2    |      |      | 72    |
| Total  | NS                  | 1    | 1    | 3    | 4    | 67   | 10   | 191  | 48   | 46   | 91   | 462   |
| NG     | 8                  | 159  | 37   | 31   |      |      |      |      |      |      |      | 235   |

*Seedlings germinated in year 2005 were counted in summer 2005, while all the other were counted in summer 2004.
missing data affect the estimation of genetic differentiation, all analyses were run with only five loci, excluding FCSt.

Expected heterozygosity ($H_e$), allelic richness ($A$) and heterozygote deficiency (as measured by $F_{st}$) were estimated using the Fstat software (Goudet 2000). Allelic richness was rarefied to a minimum sample size of 10 individuals. Significance of $F_{st}$-values was assessed at the 5% confidence level after Bonferroni correction.

Spatio-temporal genetic structure

To investigate the spatial and temporal components of genetic structure in the seedlings, we used a hierarchical AMOVA design (Excoffier et al. 1992). We analysed the respective effects of spatial (plot) vs. temporal (year of germination) processes, by testing successively two two-level nested models (year within plot and plot within year) for the genotypic frequency vector $G_{ijk}$ of individual $k$ germinated in year $j$ and located in plot $i$:

$$G_{ijk} = \mu + f_i + t_{ij} + w_{ijk}$$  

and

$$G_{ijk} = \mu + f_i' + s_{ij} + w_{ijk}$$  

where $f_i$ is the average effect of plot $i$, $f_i'$ the average effect of year $j$, $t_{ij}$ is the average effect of year $j$ nested within plot $i$, $s_{ij}$ is the average effect of plot $i$ nested within year $j$ and $w_{ijk}$ is the replication error associated with the $k$th individual from the $i$th plot germinated in the $j$th year. For microsatellites assumed to follow a stepwise mutation model, Slatkin (1995) recommends measuring variation in allelic frequencies and genetic differentiation with alleles ordered according to their size rather than with unordered alleles (identity in state). Accordingly, we used both $F_{ST}$ and $R_{ST}$ as estimators of genetic differentiation. Significance of $F$-statistics was assessed by means of 5000 permutations. All computations were done using the Arlequin software (Schneider et al. 2000). Detailed AMOVA design is described in Appendix S1 (Supporting information).

Fine-scale spatial genetic structure within plots/cohorts. The classical analysis of spatial genetic structure (SGS) consists in plotting the variation in average genetic relatedness among individuals against distance (or logarithm of distance in a two-dimensional space). Under isolation by distance, this relationship is expected to be linear in a part of the distance range, and shows a decay rate proportional to $1/d_e$, where $d_e$ being the effective dispersal distance (Rousset 2000). Here, our sampling design with only three seedling plots was not conceived to investigate the variation of genetic relatedness among pairs of seedlings over a large range of distances. Instead, we focused on the ‘between-generation’ component of SGS, by computing coefficients of genetic relatedness ($F_{ij}$) among all pairs of individuals that involved one seedling ($i = 1$ to $N_s$, where $N_s = total number of seedlings) and one adult ($j = 1$ to $N_A$ where $N_A = total number of adults). Computed in this way, $F_{ij}$ coefficients reflect the parent-offspring component of genetic structure, with expected values equal to 0.25 when $i$ and $j$ are related (parent-offspring) or 0 when $i$ and $j$ are unrelated.

As proposed by Hampe et al. (2010), we analysed between-generation SGS for different group of seedlings, i.e. seedlings grouped by spatial plots (A, B and C) or by germination year. This allowed us to investigate the variation in SGS with spatial adult tree density (which decreased from plot C to A) and temporal adult tree density (high in the masting year 2002 and low in the other years).

All kinship analyses were performed using Spagedi 1.2 (Hardy & Vekemans 2002), which makes it possible to specify adult-seedling pairs to be compared. To measure genetic relatedness, we used the kinship coefficient ($F_{ij}$) of Loiselle et al. (1995). $F_{ij}$-values were estimated using five loci (excluding FCSt), and assumed not to be affected by null alleles, similarly to $F_{ST}$ (Rousset 2000). The allele frequencies from the whole population (i.e. grouping adults and seedlings) were used as a reference sample. To visualize SGS, $F_{ij}$-values were averaged over a set of distance classes ($d$) (with a minimum number of 80 pairs of individuals per distance class) and plotted against distance. To test SGS, $F_{ij}$ values were regressed on $\ln(d_{ij})$, where $d_{ij}$ is the spatial distance between individuals $i$ and $j$, to provide the regression slope $b_{log}$. Then, the spatial positions of all individuals were permuted 5000 times in order to get the frequency distribution of $b_{log}$ under the null hypothesis that $F_{ij}$ and $d_{ij}$ were uncorrelated. Approximate standard errors for the multi-locus estimates of $F_{ij}$ within each distance class were obtained through a jackknife procedure that consisted of deleting each locus one at a time. This assumes that the different loci provide independent replicates of the genetic structure process.

Impact of mortality on genetic structure. The genetic differentiation between dead and alive seedlings in year 2006 was first investigated using the hierarchical AMOVA design described above by equations (1) and (2), replacing the average effect of year by that of status (dead/alive) in $f_i$, $t_{ij}$ and $s_{ij}$ and considering that $w_{ijk}$ was the
replication error associated with the \( i \)th individual from the \( j \)th plot in state \( j \).

Then, fine-scale patterns of between-generation SGS were investigated as detailed above to test whether gene dispersal patterns from adults to seedlings differed among dead and alive seedlings.

**Estimation of the seed and pollen dispersal kernel based on established seedlings.** The spatially explicit mating model (SEMM) developed by Burczyk *et al.* (2006) and Oddou-Muratorio & Klein (2008) was used to estimate the shape and range of seed and pollen dispersal kernels from genotypes and positions of established seedlings and their potential parents. The model considers that each seedling can be mothered either (i) by a mother tree located outside the study site due to seed immigration (with probability \( m_s \)) or (ii) by a local mother tree located within the study site (with probability \( 1 - m_s \)). In the latter case, the model considers that offspring \( i \) may be the result either of self-pollination (with probability \( s \)), pollen flow from outside the neighbourhood (with probability \( m_p \)), or pollen from a sampled male (with probability \( 1 - s - m_p \)). The genotypes of seedlings and candidate mothers/fathers are used to define the compatible offspring–parent triplet, and to compute transition probabilities in a fractional parentage analyses design. The contribution of a sampled and genetically compatible mother tree \( j \) to the seedling rain at the location of seedling \( i \) is modelled as the product of the probability of a seed to disperse from \( j \) to \( i \) (the seed dispersal kernel), and of mother intrinsic fecundity. Similarly, the contribution of each sampled father tree \( k \) to the pollen cloud above mother tree \( j \) is modelled as the product of the probability of a pollen grain to disperse from \( k \) to \( j \) (the seed dispersal kernel), and of the father intrinsic fecundity. Here, we used the exponential power function to model the seed and pollen dispersal kernels:

\[
p(a, b; d) = \frac{b}{2na^2\Gamma(2/b)}\exp\left(-\left(\frac{a}{a}\right)^b\right)
\]

where \( d \) is the distance of interest (mother–seedling or father–mother) and \( \Gamma \) is the classically defined gamma function. The parameter \( b \) is the shape parameter affecting the tail of the dispersal function and \( a \) is a scale parameter homogeneous to distance. When \( b = 1 \) this model simplify to an exponential; when \( b < 1 \) the kernel is fat-tailed and when \( b > 1 \) the kernel is thin-tailed. Through parameter \( b \), this model thus allows to estimate whether long-distance dispersal events are respectively more or less important as compared to the exponential kernel.

The model allows for a simultaneous estimation of seed and pollen immigration levels (\( m_s \) and \( m_p \)), selfing rate (\( s \)) along seed and pollen dispersal kernels parameters \( (a_s, b_s, \theta_p, b_p) \), as detailed in Oddou-Muratorio & Klein (2008) and in online Appendix S2 (Supporting information).

SEMM requires as input the mapped locations of all sampled seedlings and all potential reproductive adult males and females within a local population, the multilocus genotypes of seedlings and adults, and allele frequencies of the same species in surrounding (background) populations. The genotypes and spatial positions of all the 342 adult trees found within the site and of the 221 seedlings were used for these analyses (33 seedlings were eliminated because they could not be genotyped at more than two loci and/or because of missing spatial position data). Note that locus FC5 was included in these analyses, as missing data do not bias this estimation procedure. Background allele frequencies were assumed to be similar to that of the local population.

**Estimation of the pollen dispersal kernel based on maternal progenies.** SEMM was also used to estimate the shape and the range of the pollen dispersal kernel from genotypes and positions of maternal trees, progeny arrays, and potential fathers. The model is simpler than the seedling model above (Oddou-Muratorio *et al.*, 2005 and Appendix S3, Supporting information) and considers that a given seed \( i \) collected on mother-tree \( j \) may be the result of self-pollination (with probability \( s \)), pollen flow from outside the neighbourhood \( N \) (with probability \( m_p \)), or pollen from a sampled male (with probability \( 1 - s - m_p \)). The genotypes and spatial positions of all 342 adult trees as well as the genotypes of the 372 seeds collected on 29 fruiting trees were used for these analyses.

**Results**

**Demographic dynamics in seedlings plots**

Effective recruitment patterns were highly variable among years, with on average 41.3% of seedlings germinated in 2002, and several years without significant recruitment (Table 1). For seedlings germinated before 2004, these variations can result either from low seed production and/or germination, or from high seedling mortality between the germination year and 2004. These effective recruitment patterns are consistent with the expected effect of the Lothar storm in 1999, as most of the seedlings observed alive between 2004 and 2005 germinated after the stand canopy was significantly opened by the storm. Overall, seedling density observed in 2004 was lower in plot A (12.7 m\(^2\)) than in plot B (31 m\(^2\)) or plot C (24 m\(^2\)) (Fig. 2).

The average mortality rate was 22.7% between 2004 and 2005, and 8.2% between 2005 and 2006. From year
2004 to 2005, there was a trend of higher mortality under low light conditions (high canopy closure), with mortality rate increasing from 15% in plot A to 21% in plot B and finally to 27% in plot C (\(\chi^2\) test: \(P\)-value = 0.10). By contrast, from year 2005 to year 2006, the mortality rate was lower in plot C (0.3%) than in plots A (9%) and B (12%) (\(\chi^2\) test: \(P\)-value = 0.01).

Overall, mortality from 2004 to 2006 (average mortality rate = 29%) tended to reduce variation in seedling density among plots (as measured by the coefficient of variation (CV) of seedling density, CV\(_{2004}\) = 0.36 vs. CV\(_{2006}\) = 0.31), with final seedling density in 2006 ranging from 9.8 m\(^2\) (plot A) to 16.9 m\(^2\) on plot C and to 21.2 m\(^2\) on plot B.

### Genetic diversity within seedling and adult cohorts

Levels of diversity did not differ among adult and seedling cohorts, among seedling plots, or among dead and alive seedlings (Table 2). Nei’s genetic diversity was high both in seedlings and adults (\(H_e = 0.71\) and 0.72, respectively), and allelic richness was also comparable in seedlings and adults (\(A = 5.71\) and 5.94, respectively). By contrast, the within-individual structure of genetic diversity differed between adult and seedling cohorts, with a higher heterozygote deficiency in the adults (\(F_{IS} = 0.131^{*}\)) than in the seedlings (\(F_{IS} = 0.069^{**}\)). However, when the three loci affected by null alleles (FS1-25, FS4-46, FS1-03) were removed, \(F_{IS}\)-values did not differ significantly from 0.

### Spatio-temporal genetic structure of seedlings

Spatial (among plots) vs. temporal (among year classes) components of seedling genetic structure were first investigated using two different two-level AMOVA models: (Model 1) years nested within plots (Table 3) and (Model 2) plots nested within years. The main effect for years was not significant (Model 2 see Table S2, Supporting information), while the main effect for plots was significant (Model 1). Detailed analyses of Model1 (Table 3) showed that the ‘among-plot’ component of genetic variation was not negligible (\(F_{RT} = 2.6\%\) of the total variation) considering the small spatial scale investigated (average distance among plots ~100 m). By contrast, year-to-year variation in a given plot was not significant (\(F_{SR} = 0.5\%\) but contributed to overall differentiation (\(F_{ST} = 3.1\%\)). Differentiation estimates using \(R\)-statistics were similar to these values.

### Table 2: Stratified genetic diversity indexes averaged over five loci

| Cohort Group | N  | \(N_{NA}\) | \(H_e\) | \(N_a\) | A  | \(F_{IS}\) |
|--------------|----|-----------|--------|--------|----|---------|
| Seedlings-Plot A | Live | 66 | 55.00 | 0.68 | 8.60 | 5.1306 | 0.034 |
|              | Dead | 16 | 15.00 | 0.75 | 6.80 | 6.0794 | 0.007 |
|              | All  | 82 | 70.00 | 0.70 | 9.80 | 5.3164 | 0.027 |
| Seedlings-Plot B | Live | 62 | 60.25 | 0.69 | 9.20 | 5.2766 | 0.053 |
|              | Dead | 19 | 18.00 | 0.68 | 6.00 | 4.9594 | 0.054 |
|              | All  | 81 | 78.25 | 0.68 | 9.40 | 5.18   | 0.052 |
| Seedling Plot C | Live | 56 | 48.25 | 0.73 | 8.60 | 5.7908 | 0.086 |
|              | Dead | 16 | 14.00 | 0.75 | 6.60 | 5.816  | 0.085 |
|              | All  | 72 | 62.25 | 0.73 | 9.20 | 5.7784 | 0.085 |
| All seedlings | Live | 184 | 163.50 | 0.71 | 12.40 | 5.7318 | 0.073\* |
|              | Dead | 51 | 47.00 | 0.73 | 8.60 | 5.7064 | 0.058 |
|              | All  | 235 | 210.20 | 0.71 | 13.20 | 5.714  | 0.069\* |
| Adult trees  |     | 342 | 327.80 | 0.72 | 15.80 | 5.9412 | 0.131\* |

\(N\) = number of genotyped seedlings; \(N_{NA}\) = sample size corrected for missing data, \(H_e\) = Nei’s expected heterozygosity, \(N_a\) = no. of alleles, \(A\) = allelic richness computed using rarefied sample of 10 individuals, \(F_{IS}\) = fixation index.

\*Significant at 5% confidence level.

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To investigate whether the lack of a significant among year/plot effect could be due to an over-representation of seedlings germinated in year 2002 in the data set (68% of the 234 seedlings), we rarefied the sample to balance sample size within year (so that year 2002 represented 47% of 139 seedlings) and ran the AMOVA analyses again (Table 3). Results were consistent with those obtained with the complete data set, with a significant main spatial effect ($F_{RT} = 3.2\%$).

Pairwise $F_{ST}$ among seedling plots (Table S3, Supporting information) showed that only plot C was significantly differentiated from plots B and A ($F_{ST} = 1.3\%$ in both cases). All seedling plot genetic frequencies significantly differed from that of the adult populations ($F_{ST}$ ranging from 0.9% for B plot to 2.3% for A plot).

**Fine-scale spatial genetic structure within plots and within cohorts**

The fine-scale variations of ‘between-generation’ SGS were analysed by plotting genetic relatedness among all seedling-adult pairs against distance (Fig. 3a). Then, similar plots were obtained by computing genetic relatedness among seedling-adult pairs for seedlings belonging to the same plot (Fig. 3b) or the same year class (Fig. 3c). Patterns of SGS were always markedly significant, with regression slopes of $F_{ij}$ on log(distance) different from zero in all cases ($P < 0.001$). Overall, the ‘between-generation’ SGS was strong and decreased rapidly with distance, with a marked peak of SGS in the first distance interval ($F_{10m} = 0.039$) followed by a notable decrease between 20 and 40 m and no more significant trend after 40 m (Fig. 3a). The adult SGS tended to be even stronger ($F_{10m} = 0.065$; Fig. 3a).

‘Between-generation’ SGS varied among plots (Fig. 3b). However, trends were not significant due to the high standard errors of $F_{ij}$ and $b$ estimates (Table S4, Supporting information). There was also no clear trend of increasing ‘between-generation’ SGS during years of low recruitment, as could have been expected with a reduced contribution of adults to reproduction in non-masting years. Year 2001 and 2004 (low recruitment) showed higher and lower ‘between-generation’ SGS, respectively, whereas year 2002 showed intermediate SGS (Fig. 3c).

**Direct gene flow estimates**

Maximum-likelihood estimates of seed and pollen dispersal as well as mating system parameters were obtained using SEMM (Table 4). Both for pollen and seed dispersal, the exponential kernel (i.e. fixing $b_p = 1$ and $b_s = 1$ and estimating solely $a_p$ and $a_s$) provided a better fit than the Gaussian kernel (i.e. fixing $b_p = 2$ and $b_s = 2$). The exponential power kernel with joint estimates of $b$ and $a$ parameters did not improve the fit (results not shown). We estimated a larger mean distance for pollen dispersal ($\hat{b}_p \approx 57$ m; CI = 30.6–123.4 m) than for seed dispersal ($\hat{b}_s \approx 11$ m; CI = 9.4–12.9 m). Also, the pollen immigration rate ($\hat{m}_p = 71.6\%$; CI = 60.2–85%) was significantly higher than the seed immigration rate ($\hat{m}_s = 20.5\%$; CI = 13.5–27.1%). The selfing rate was significantly positive ($s = 3.5\%$).

Independent estimates of pollen dispersal and selfing rate based on maternal progenies fell within the same range of that of estimates based on established seedlings. The best fit for pollen dispersal was also obtained with the exponential kernel (fixing $b_p$) with an estimated $\hat{a}_p$ value $\approx 44$ m. The selfing rate was significantly positive ($s = 2.1\%$).

**Impact of mortality on genetic structure**

Because there was strong among-plot genetic structure, the relationship between survival and genetic differentiation was tested using a two-level AMOVA design with status (alive/dead) nested within plot. Neither the main effect nor the interaction effect of status was significant (result not shown), showing that dead and alive seedlings were not genetically differentiated within plot.

The fine-scale variations of ‘between-generation’ SGS were analysed by plotting genetic relatedness among...
seedling-adult pairs against distance for dead and alive seedlings (Fig. 3d). The ‘between-generation’ SGS of dead and alive seedlings was very similar, with a marked peak in the first distance interval (>10 m), followed by a notable decrease between 20 and 40 m.

Discussion

Our results highlight some major spatial and temporal characteristics in the development of a demographic and genetic structure within beech populations. These results were based on a more than 10-year survey, with seedling establishment monitored from 2004 to 2006, and extrapolated back to 1993 by estimating seedling age. This 10-year timescale is relevant considering that in most forests across Europe, the management strategy of beech is high forest (even-aged stands resulting from natural regeneration). In this type of silviculture, stands are regenerated over a period spanning 10–20 years. At the beginning of the regeneration phase, adult trees are selectively logged to leave approx. 100–150 mature adult trees/ha. Because of masting and strong seedling vigour, all beech seedlings that will effectively contribute to the new reproductive stands are often recruited in less than 20 years.

Demographic and genetic structure show opposite spatio-temporal effects

Our results first indicate strong temporal and low spatial heterogeneity in recruited seedling density, contrasting with the strong spatial and low temporal heterogeneity in their genetic structure. Beginning with seedling density, we observed a strong temporal heterogeneity on early-recruitment patterns in European beech, with 41.3% of seedlings germinated in 2002, and
several years out of the 13 under study (from 1993 to 2005) without significant recruitment. These variations can result from low seed production, low germination, high mortality, or a combination of these factors. Many sites favourable for seedling establishment were opened by the 1999 Lothar storm, explaining the lack of recruitment before 1999. However, recruitment patterns between years 1999 and 2004 are consistent with a massive seedling germination event in 2002 and with the assumption that seed production is a limiting factor for recruitment in beech (Fioresean & Adams 2001).

By contrast, spatial heterogeneity in seedling density was weak, with a trend of lower initial density in the plot with open canopy (PAR = 55%, 12.7 seedlings/m² in 2004), compared to plots with intermediate canopy closure (PAR = 31%, 31 seedlings/m²) or high canopy closure (PAR = 6%, 24 seedlings/m²). However, our experimental design with only three plots was not conceived to address the impact of canopy closure on initial seedling density or to separate this effect from that of seed-tree density. Still, our results show that despite variable seed-tree density across the plot (from <30 to >90 trees/ha), there was a high density of seedlings even under unusually high canopy openness (>9700 trees/ha in 2006, which is several times higher than recommended for afforestation rates). This is consistent with other studies, which have found that seedling germination is almost independent of light availability (Szwagrzyk et al. 2001), contrary to subsequent growth and long-term survival (Szwagrzyk et al. 2001; Kunstler et al. 2005).

In contrast to their density, the genetic structure of recruited seedlings was significantly shaped by spatial processes and poorly affected by temporal heterogeneity of the seed rain. Here, the stand-level spatio-temporal genetic structure was investigated by testing successively the main and nested effects of spatial location and year of germination on genetic differentiation among seedlings as measured by $F_{ST}$. The main effect of spatial location was strongly significant, and translated into a significant level of differentiation between plots of $F_{ST} = 2.6%$. By contrast, genetic differentiation among temporal cohorts within plots was not significant. Moreover, genetic frequencies significantly differed between plot C and the two other plots ($F_{ST}$ C. A = $F_{ST}$ C. B = 1.3%) and between each seedling plot and adults (0.9 < $F_{ST}$ Adult-seedling < 2.3%; Table S2, Supporting information). These $F_{ST}$ values may look weak, but by comparison, genetic differentiation at allozyme loci measured over 389 populations across Europe were not larger than 5.9% (Comps et al. 2001). Using SSR markers to measure genetic differentiation among 10 populations across Europe, Buitenveld et al. (2007) reported pairwise $F_{ST}$-values ranging between 0.8% and 5.3%, with an overall $F_{ST}$ of 5.3%.

**Genetic structure revealed low levels of genetic drift despite restricted dispersal**

The major role of spatial vs. temporal processes in shaping plant genetic structure has been acknowledged in previous studies, usually by comparing genetic structure across different life-stages (Alvarez-Buylla et al. 1996; Chung et al. 2003; Jones & Hubbel 2006). In their pioneer demo-genetic study in the tropical tree *Cecropia obtusifolia*, Alvarez-Buylla et al. (1996) showed that patchy recruitment in gaps markedly affect the genetic composition of the seed rain, with higher differentiation among gaps than among life-stages. More recently, Jacquemyn et al. (2009) used multi-stage spatial genetic structure analyses combined with paternity analyses in the perennial *Orchis mascula* to show that patterns of SGS were mostly shaped by pollen and seed-mediated gene dispersal, and were consistent across life-stage.

By contrast however, we focussed here on a more fine-scale temporal structure. We investigated the genetic structure among seedlings recruited in consecutive years, and thus belonging to a single life-stage from the point of view of most previous studies. Because beech trees produce nuts in irregular mast years, we expected significant genetic differentiation among year-classes due to inter-annual variance in reproductive success. Such fine-scale temporal differentiation has been reported in marine perennial organisms (e.g. Planes & Lenfant 2002). In our case, the across-year genetic homogeneity of the seed rain suggest low levels of genetic drift, i.e. high effective population size and/or relatively even contributions of all adult trees to reproduction either as male or female, even in non-masting years. Fine-scale patterns of between-generation SGS confirmed (i) the across-year genetic homogeneity of the seed rain (Fig. 3C) and (ii) the major role of gene dispersal and effective population size on spatial patterns of allelic frequencies.

**Seed and pollen dispersal direct estimates**

Contemporary estimates of seed and pollen dispersal based on parentage/paternity analyses shed light on dispersal processes and their ecological determinants in European beech. Considering first seed dispersal patterns as estimated from established seedlings, our results reflected preferential short distance dispersal as depicted by the low average distance of seed dispersal ($\delta_r \approx 11$ m). However, events of long distance dispersal appeared not negligible and may contribute to higher effective population size in beech, as depicted by the seed immigration rate ($\hat{m}_s = 20.5%$) and the exponential-shaped dispersal kernel. These estimates convert into a median dispersal distance of 7.6 m and are
consistent with previous demographic estimates of seed dispersal in beech (median dispersal distance of 6.49 m in Sagnard et al. 2007), or with recent genetic estimates in beech species across different sites (Oddou-Muratorio et al. 2010). They are also consistent with life history traits of the beech dispersers. The rodents involved in secondary dispersal of beech seeds have been shown to remove seeds a few meters away from the source tree (4.1 m on average, Jensen 1985), whereas frequent 1 km dispersal events have been reported for jays (Nilsson 1985).

Our results suggest greater dispersal abilities for pollen than for seeds, with both a higher mean dispersal distance ($\delta_p = 43.7–57.2$ m vs. $\delta_s = 10.9$ m), and a higher immigration rate ($m_p = 71.6–77.9$% vs. $m_s = 20.5$%). The results based on established seedling or open-pollinated progeny were highly consistent (Table 4). This supports the accuracy of the SEMM. Moreover, it shows that early selective processes acting between seed release and seedling establishment may not be driven by the genetic origin of the pollen grain (no outbreeding or inbreeding depression).

From direct estimates of seed and pollen dispersal, we can estimate real-time, total gene flow estimates ($\sigma_{rt}$), as detailed in Oddou-Muratorio & Klein (2008). In a two-dimensional space, for hermaphrodite, outcrossing species $\sigma_{rt}^2 = \frac{1}{2} \sigma_{p-rt}^2 + \sigma_{s-rt}^2$, where $\sigma_{p-rt}^2$ and $\sigma_{s-rt}^2$ are the respective second moments of the pollen and seed dispersal kernels. In our case, $\sigma_{rt} = 51.35$ m (CI = 29–108 m); this is consistent with the shape of the between-generation auto-correlograms (Fig. 3) which show significant SGS up to ~50 m.

We reasonably assumed that differentiation ($F_{st}$) and SGS estimates were not biased by the frequencies of null alleles estimated in $F. sylvatica$ (between 0% and 14% depending on cohort and method of estimation, with all but two estimates <10%; see Table S1). However, attention should be put in evaluating the potential impact of null alleles on direct estimates of seed and pollen dispersal. Somewhat reassuringly, Dakin & Avise (2004) showed using simulations that the range of null allele frequency observed in this study (NAF <10%) equates to a less than 5% risk of falsely excluding an actual parent of a heterozygous offspring in parentage/paternity analyses. Additionally, we also estimated NAF using our mating model parameters, using a modified version of the SEMM and adult and seedlings genetic and spatial data as input (Chybicki & Burczyk 2010). Interestingly, ‘direct’ NAF estimates were lower (2% on average, see Table S1) than those obtained by traditional methods (between 4.3% and 6.7%). The reason for this discrepancy may be that ‘direct’ NAF estimates account for the SGS present in the population. By contrast, in the case of significant SGS and preferential local mating, biparental inbreeding can result in a deficit of heterozygotes (similar to a Wahlund effect) that traditional methods could misinterpret as a signature of null alleles.

**Evolutionary and ecological drivers of mortality**

The overall mortality rate over the two years of the survey (from 2004 to 2006) was low (28.8% for all seedlings, and 20% for those germinated in 2002). In a 10-year mortality survey, Szwagrzyk et al. (2001) reported mortality rates close to 100% after 4 years (but under lower light availability, with PAR ranging from <3% to 15%). During the year of high mortality (2004–2005, mortality rate 22.3%), low light availability tended to induce higher mortality, in agreement with previous results in European beech (Szwagrzyk et al. 2001).

To investigate evolutionary drivers of mortality, we first estimated genetic differentiation between dead and alive seedlings (as measured by hierarchical F-statistics within plots). We did not observe any significant differences between the two groups. Moreover, levels of heterozygote deficiency were consistent among groups ($F_{IS} = 0.073^{+}$ in alive seedlings vs. 0.058NS in dead seedlings, Table 2). Although the level of inbreeding estimated by $F_{IS}$ may be affected by null alleles, $F_{ST}$-values can still be used to compare dead and alive seedlings because of the absence of genetic differentiation between these groups. Thus, there was no evidence that mortality is driven by the purging of selfed individuals in the studied beech stand. Finally, between-generation patterns of spatial genetic structure (SGS) were also consistent for dead and alive seedlings (Fig. 3), indicating that levels of genetic relatedness within the stand did not significantly contribute to mortality. Overall, we did not find any evidence that mortality is driven by inbreeding or lack of local adaptation.

Interestingly, patterns of inbreeding and relatedness coefficients were actually stronger for adults compared to seedlings ($F_{IS} = 0.131$ in adults vs. 0.069 in seedlings). This indicates that massive recruitment during a single mast year does not reduce effective population size as could have been expected.

**Perspectives**

This study highlights different magnitudes of temporal vs. spatial effects on demographic and genetic patterns of early recruitment. The high heterogeneity among year classes in recruited seedling density revealed a major effect of mast seeding on demographic patterns of recruitment. By contrast, the low genetic differentiation among seedlings recruited in different years indicates balanced contribution of adult trees to
reproduction within year. The significant spatial genetic structure was consistent with the strong spatial limitation of both seed and pollen dispersal detected using parentage analyses and neighbourhood mating models. As a direct consequence for forest managers, our results highlight that genetic diversity within beech stands is mostly shaped by gene dispersal and adult tree density. Consequently, high levels of genetic diversity can be maintained within stand even if young seedlings are recruited in a reduced number of mast years.

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**Supporting information**

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

**Table S1** Null allele frequencies and heterozygote deficiency (FIS) per cohort and microsatellite locus.
Table S2 Nested analysis of molecular variation for genetic variation among four beech seedling temporal cohorts, sub-sampled in three spatial plots.

Table S3 Estimation of genetic differentiation among seedling plots and adult not using (No) and using (ENA) the ENA correction for null alleles as described in Chapuis & Estoup (2007).

Table S4 Estimates of between-generation SGS for each seedlings plots.

Appendix S1 AMOVA design.

Appendix S2 Estimation of the seed and pollen dispersal kernel using spatially explicit mating model (SEMM) for established seedlings.

Appendix S3 Estimation of the pollen dispersal kernel using SEMM for maternal progeny arrays.

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