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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 life span, 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 (δ_s = 10.9 m) and pollen (43.7 m < δ_p<57.3 m) were found, but there was also a non-negligible immigration rate from outside the plot (m_s=20.5%; 71.6%<m_p<77.9%). Hierarchical analyses of seedling genetic structure showed that (1) most of the genetic variation was within plots; (2) the genetic differentiation among seedling plots was significant (F_{ST} = 2.6%) while (3) 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 time-scales are discussed.

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 time scale. 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 allelic 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 characterised in particular by a massive mortality of seeds/seedlings produced during an individual life time, 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; major processes 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.*, 2007; Clark *et al.*, 1999; Nathan & Muller-Landau, 2000). In the following section, we will focus in particular on: (1) dispersal limitation, which is a major factor shaping variation of recruitment through space; (2) 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 (Kelly &Sork, 2002; Piovesan &Adams, 2001); (3) 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.*, 2007; Clark *et al.*, 1999; 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 due to the fact that 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; Hampe *et al.*, 2008; Jones & Hubbel, 2006).

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 (Howe & Smallwood, 1982; Janzen, 1970; 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: (1) how do patterns of seedling density and survival correlate in space and time? (2) How spatially restricted are contemporary pollen and seed dispersal? (3) Which spatial or temporal processes are the major factors shaping seedling genetic structure? (4) Does mortality affect the genetic structure of the seedlings?

Material 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(Nilsson & Wastljung, 1987; Teissier du Cros, 1981). Seeds are primarily dispersed by gravity, and secondarily dispersed by rodents (*Apodemus flavicollis, Clethrionomys glareolus*) and birds (*Glandus glandularius*) 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 meters (Figure 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.

[Figure 1 around here]

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 SunScan ® system. Within each plot 5-6 measurements were averaged and converted into percentage of transmitted PAR ($\frac{Transmited\ PAR}{Incident\ PAR}$) by reference to the average measure of 157.9 micromol/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 meter radius around the plots (A: 56 trees ha^{-1}; B: 72 trees ha^{-1}; C: 92 trees ha^{-1}).

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, S.D. = 2.98).

Genotyping: DNA was isolated from buds (adult trees and seedlings) and embryos (seeds) using the Qiagen DNeasy Plant kit. Individuals were genotyped using 4 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* (Asuka *et al.*, 2004; Tanaka *et al.*, 1999) (Table 1), following PCR conditions given by the authors. Adult and seed PCR products were separated using an automated 96-capillary MegaBACE™ 1000 sequencer (GE Healthcare). Genotypes were sized using the internal size standards ET400 and the MegaBACE™ Fragment Profiler ver. 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 A1). 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 (1) in correcting the original data set by statistical adjustment of genotype frequencies based on estimated null allele frequencies and then (2) 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 A1). 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 datasets, 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: (1) 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; (2) 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; (3) 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 missing data affect the estimation of genetic differentiation, all analyses were run with only 5 loci, excluding FCM5.

Expected heterozygosity (*He*), allelic richness (*A*) and heterozygote deficiency (as measured by F_{IS}) were estimated using the Fstat software (Goudet, 2000). Allelic richness was rarefied to a minimum sample size of 10 individuals. Significance of F_{IS}- values was assessed at the 5% confidence level after Bonferonni 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 analyzed the respective effects of spatial (plot) versus 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*:

$G_{ijk}=\mu+f_i+t_{j(i)}+w_{ijk}$ (1)

and $G_{ijk}=\mu+f'_j+s_{i(j)}+w_{ijk}$ (2)

where f_i is the average effect of plot *i*, f'_j the average effect of year *j*, $t_{j(i)}$ is the average effect of year *j* nested within plot *i*, $s_{i(j)}$ 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 online appendix A1.

Fine-scale spatial genetic structure (SGS) within plots / cohorts: The classical analysis of 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 $1/d_e\sigma^2_e$, d_e being the effective population density, and σ^2_e the axial variance in gene 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 analyzed 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). Fij-values were estimated using 5 loci (excluding FCM5), 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 5,000 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'_j , $t_{j(i)}$ and $s_{i(j)}$ and considering that w_{ijk} was the replication error associated with the k_{th} individual from the i_{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 i can be mothered either (1) by a mother tree located outside the study site due to seed immigration (with probability m_s) or (2) 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* at 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:

$$p(a,b;d) = \frac{b}{2\pi a^2 \Gamma(2/b)} \exp\left(-\left(\frac{d}{a}\right)^b\right) \qquad (3)$$

where *d* is the distance of interest (mother-seedling or father-mother) and Γ 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, a_p, b_p), as detailed in Oddou-Muratorio & Klein (2008) and in online Appendix A2 (supplementary material).

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 2 loci and/or because of missing spatial position data). Note that locus FCM5 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 (online Appendix A3 supplementary material). The model is simpler than the seedling model above (Oddou-Muratorio *et al.*, 2005) 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}) (Figure 2).

[Table 1 around here]

[Figure 2 around here]

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 (χ^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%) (χ^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 coeeficient of variation (CV) of seedling density, CV_{2004}= 0.36 versus 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.

[Table 2 around here]

Spatio-temporal genetic structure of seedlings

Spatial (among plots) versus temporal (among year classes) components of seedling genetic structure were first investigated using two different two-level AMOVA models: (Model1) years nested within plots (Table 3) and (Model2) plots nested within years. The main effect

for years was not significant (Model2 see Table A2), while the main effect for plots was significant (Model1). 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 3 around here]

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 A3) 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 (SGS) within plots and within cohorts

The fine-scale variations of 'between-generation' SGS were analyzed 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

383 with distance, with a marked peak of SGS in the first distance interval (F_{10m} = 0.039)
384 followed by a notable decrease between 20 and 40 m and no more significant trend after 40 m
385 (Fig 3A). The adult SGS tended to be even stronger (F_{10m} = 0.065; Fig 3A).
386 [Figure 3 around here]
387 'Between-generation' SGS varied among plots (Fig 3B). However, trends were not significant
388 due to the high standard errors of F_{ij} and *b* estimates (Table A4). There was also no clear trend
389 of increasing'between-generation' SGS during years of low recruitment, as could have been
390 expected with a reduced contribution of adults to reproduction in non-masting years. Year
391 2001 and 2004 (low recruitment) showed higher and lower 'between-generation' SGS,
392 respectively, whereas year 2002 showed intermediate SGS (Fig 3C).
393 **Direct gene flow estimates**
394 Maximum-likelihood estimates of seed and pollen dispersal as well as mating system
395 parameters were obtained using SEMM (Table 4). Both for pollen and seed dispersal, the
396 exponential kernel (*i.e.* fixing b_p = 1 and b_s = 1 and estimating solely a_p and a_s) provided a
397 better fit than the Gaussian kernel (i.e. fixing b_p = 2 and b_s = 2). The exponential power kernel
398 with joint estimates of *b* and *a* parameters did not improve the fit (results not shown). We
399 estimated a larger mean distance for pollen dispersal ($\hat{\delta}_p \approx 57$ m; CI = 30.6 – 123.4 m) than for
400 seed dispersal ($\hat{\delta}_s \approx 11$ m ;CI = 9.4 -12.9 m). Also, the pollen immigration rate ($\hat{m}_p$ =71.6%;
401 CI = 60.2 – 85%) was significantly higher than the seed immigration rate ($\hat{m}_s$ =20.5%; CI =
402 13.5 -27.1%). The selfing rate was significantly positive (*s*= 3.5%).
403 [Table 4 around here]
404 Independent estimates of pollen dispersal and selfing rate based on maternal progenies fell
405 within the same range of that of estimates based on established seedlings. The best fit for

406 pollen dispersal was also obtained with the exponential kernel (fixing b_p) with an estimated
407 $\hat{\delta}_p$ value ≈ 44 m. The selfing rate was significantly positive (s= 2.1%).

408 **Impact of mortality on genetic structure**

409 Because there was strong among-plot genetic structure, the relationship between survival and
410 genetic differentiation was tested using a two-level AMOVA design with status (alive/dead)
411 nested within plot. Neither the main effect nor the interaction effect of status was significant
412 (result not shown), showing that dead and alive seedling were not genetically differentiated
413 within plot.
414 The fine-scale variations of 'between-generation' SGS were analyzed by plotting genetic
415 relatedness among seedling-adult pairs against distance for dead and alive seedlings (Fig 3D).
416 The 'between-generation' SGS of dead and alive seedlings was very similar, with a marked
417 peak in the first distance interval (>10 m), followed by a notable decrease between 20 and 40
418 m.
419

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 time scale 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 to 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 vigor, 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 favorable 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 (Piovesan & 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.m^{-2} in 2004), compared to

plots with intermediate canopy closure (PAR= 31%, 31 seedlings.m^{-2}) or high canopy closure (PAR=6%, 24 seedlings.m^{-2}). 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 per ha^{-1}), there was a high density of seedlings even under unusually high canopy openness (>9,700 ha^{-1}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 (Kunstler *et al.*, 2005; Szwagrzyk *et al.*, 2001).

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 ($F_{ST\ C\text{-}A} = F_{ST\ C\text{-}B}$= 1.3%) and between each seedling plot and adults ($0.9<F_{ST\ Adult\text{-}seedling}<2.3\%$; Table A2). 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, Buiteveld *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 versus 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 parentage 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 (1) the across-year genetic homogeneity of the seed rain (Fig 3C) and (2) 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 ($\hat{\delta}_s \approx 11$ m). However, events of long distance dispersal appeared not negligible and may contribute to high 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 (δ_p=43.7 – 57.2 m versus δ_s=10.9 m), and a higher immigration rate (m_p = 71.6% to 77.9% versus 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 (σ_{rt}), as detailed in Oddou-Muratorio & Klein (2008). In a two dimensional space, for hermaphrodite, outcrossing species: $\sigma_{rt}^2 = ½\sigma_{p\text{-}rt}^2 + \sigma_{s\text{-}rt}^2$, where $\sigma^2_{p\text{-}rt}$ and $\sigma^2_{s\text{-}rt}$ are the respective second moments of the pollen and seed dispersal kernels. In our case, σ_{rt} = 51.35 m (CI = 29-108m); this is consistent with the shape of the between-generation auto-correlograms (Figure 3) which show significant SGS up to ~50m.

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 A1). However, attention should be put in evaluating the potential impact of null alleles on direct estimates of seed and pollen dispersal. Somehow 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 A1) 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 versus 0.058^{NS} in dead seedlings, Table 2). Although the level of inbreeding estimated by F_{IS} may be affected by null alleles, F_{IS}-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 versus 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 versus 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 neighborhood 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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Figure legend

Figure 1. Haye forest study plot, with exhaustive mapping of adult trees (plotted as circles: o /●) and 3 seedling plots (plotted as squares : ■). Among all the 342 adult trees, 29 were chosen for collecting seed maternal progeny (black circles)

Figure 2. Patterns of mortality across year and plots. Density of alive seedlings were monitored during 3 years (2004 in black, 2005 in grey, 2006 in white) on 3 plots (A, B, C) differing for light availability under the canopy as measured by percentage of photosynthetically active radiation (PAR). Hatched bars correspond to result based on seedlings germinated in year 2002 (masting year), whereas filled bars correspond to all seedlings cohorts.

Figure 3. Between-generation patterns of spatial genetic structure : A. for all seedling-adult pairs (bold line), with the kinship values among all adult pairs plotted as a reference (broken line); B. among the different spatial plots; C. among the different temporal cohorts; D. for dead and live seedlings.

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

		Year of germination										
Plot		*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*

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734 *Seedlings germinated in year 2005 were counted in summer 2005, while all the other
735 were counted in summer 2004 .

Table 2: Stratified genetic diversity indexes averaged over 5 loci. 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.

Cohort	Group	N	N_{NA}	H_e	N_a	A	F_{IS}
Seedlings-	Live	66	55.00	0.68	8.60	5.1306	0.034
Plot A	Dead	16	15.00	0.75	6.80	6.0794	0.007
	All	*82*	*70.00*	*0.70*	*9.80*	5.3164	*0.027*
Seedling-	Live	62	60.25	0.69	9.20	5.2766	0.053
Plot B	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-	Live	56	48.25	0.73	8.60	5.7908	0.086
Plot C	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**

* significant at 5% confidence level

Table 3. Nested analysis of molecular variation for genetic variation among 3 beech seedlings plots, each sampled in 3 successive years. 234 seedlings were used for these analyses.

	234 individuals				**135 individuals**			
Source of variation	**df ***	Sum of squares	**Est. Var.**	**% total variance**	**df ***	**Sum of squares**	**Est. Var.**	**% total variance**
Among subplots	2	17.07	0.05	2.59	2	13.77	0.06	3.21
Among Years/subplots	7	14.03	0.01	0.50	7	11.78	0.01	0.44
Within years	352-452	709.60	1.75	96.91	225 - 269	433.56	1.75	96.36
Total		740.69	1.80			459.11	1.82	

* df = degree of freedom for the within year component. df varied among loci due to missing data.

751 **Table 4:** Direct estimates of selfing (*s*), pollen and seed migration rates (resp. m_p and m_s) and
752 pollen and seed dispersal distance (δ_p and δ_s) through spatially explicit mating models
753 (SEMM) applied either on seedlings (e.g. Oddou-Muratorio and Klein 2008) or seeds (e.g.
754 Oddou-Muratorio et al 2005).

	Seedlings SEMM		**Seeds SEMM**	
Parameter	Estimate	Confidence Interval	Estimate	Confidence Interval
s	3.5%	4.2-23.8%	2.1%	0.4-3.8%
m_p	71.6%	60.2-85.0 %	78.0%	72.1-83.0 %
δ_p (m)	57.25	30.6-123.4	43.5	25.4-61.7
m_s	20.5%	13.5-27.1 %	-	-
δ_s (m)	10.92	9.4-12.9	-	-

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