

# Effects of seed dispersal, adult tree and seedling density on the spatial genetic structure of regeneration at fine temporal and spatial scales

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**Abstract** Several demographic factors can produce family structured patches within natural plant populations, particularly limited seed and pollen dispersal and small effective density. In this paper, we used computer simulations to examine how seed dispersal, density, and spatial distribution of adult trees and seedlings can explain the spatial genetic structure (SGS) of natural regeneration after a single reproductive event in a small population. We then illustrated the results of our simulations using genetic (isozymes and chloroplast microsatellites) and demographic experimental data from an *Abies alba* (silver fir) intensive study plot located in the Southern French Alps (Mont Ventoux). Simulations showed that the structuring effect of limited dispersal on seedling SGS can largely be counterbalanced by high effective density or a clumped spatial distribution of adult trees. In addition, the clumping of natural regeneration far from adult trees, which is common in temperate forest communities where gap dynamics are predominant, further decreases SGS intensity. Contrary to

our simulation results, low adult tree density, aggregated spatial distribution of seedlings, and limited seed dispersal did not generate a significant SGS in our *A. alba* experimental plot. Although some level of long distance pollen and seed flow could explain this lack of SGS, our experimental data confirm the role of spatial aggregation (both in adult trees and in seedlings far from adult trees) in reducing SGS in natural populations.

**Keywords** Seed dispersal · Spatial genetic structure · Density · Demography · Temperate forest trees · Forest dynamics · *Abies alba*

## Introduction

The level and spatial organization of within-population genetic diversity is the result of evolutionary processes that include both genetic and demographic factors. Mating system, seed and pollen dispersal, gene migration among populations, natural selection, as well as spatial position of individual trees, density-dependant mortality, and annual variations of reproduction effort are all factors that interact to create and modify the level of diversity within a population and its spatial genetic structure (SGS; Wright 1978; Antonovics and Levin 1980; Epperson 1989; Epperson and Alvarez-Buylla 1997; Doligez et al. 1998; Latouche-Hallé et al. 2003). SGS is recognized as a key factor that determines the short-term evolutionary potential of a population (Epperson 1992). First, it affects the mating success between nearby individuals when inbreeding or self-incompatibility occurs. Also, patterns of genetic relatedness between interacting individuals affect the response to selection which determines the average fitness of competing individuals, and the type of competitive inter-

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This paper is in memory of our colleague Fabrice Sagnard (1974–2008).

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actions involved (from competition among sibs to competition among unrelated individuals in randomly distributed populations, see Rousset and Billiard (2000)). Therefore, gauging the strength of SGS and its impact on adaptive response has strong implications for conservation and forest management.

The building of SGS within a population can be interpreted using the theory of isolation by distance (Wright 1943), where geographically restricted gene flow generates a (neutral) genetic structure, as random genetic drift occurs locally. Considering a continuous population exhibiting isolation-by-distance, the decay rate of genetic relatedness between individuals with distance has been shown to be proportional to  $1/d_e\sigma_e^2$  —  $d_e$  being the effective density of individuals and  $\sigma_e^2$  the mean-squared axial dispersal distance (Rousset 2000; Vekemans and Hardy 2004). Intuitively, the product  $1/d_e\sigma_e^2$  expresses the degree of overlap between individual “gene shadows” (the spatial distribution of gene dispersal events around each parent). It implies that the intensity of genetic structuring decreases both with increasing dispersal distance and increasing individual density (Robledo-Arnuncio and Rousset 2010).

Theoretical work shows that SGS results from the combined effects of gene dispersal range and genetic drift through effective density (Wright 1943; Robledo-Arnuncio and Rousset 2010). Stand-forming forest tree species with wind-dispersed seeds and pollen are typical of temperate and boreal coniferous forests. Only a weak and often non-significant spatial autocorrelation of genotypes has been detected in these forest types (Vekemans and Hardy 2004), suggesting that gene flow is strong enough or density is high enough to prevent within-population spatial genetic structuring (Epperson and Allard 1989; Leonardi et al. 1996; Epperson and Chung 2001; Heuertz et al. 2003). In contrast, fine-scale genetic structures seem to be frequent in tropical forests or temperate forest tree species exhibiting low adult tree densities (Degen et al. 2001; Dutech et al. 2002; Latouche-Hallé et al. 2003; Lowe et al. 2003; Oddou-Muratorio et al. 2004; Vekemans and Hardy 2004) as well as in forest tree species with limited dispersal abilities (Berg and Hamrick 1995).

Besides dispersal range and effective population density, many other factors affect patterns of SGS, in particular the choice of genetic markers used (Streiff et al. 1998; Leblois et al. 2003) and temporal or spatial heterogeneities in individual distributions (Leblois et al. 2004). First, SGS is more difficult to observe when markers display low polymorphism (isozymes vs microsatellites, Streiff et al. 1998). Second, in long-lived woody species, effective population density can vary over time, depending on species status during succession (from pioneer to late-successional, from recolonizing to closed-canopy forest structures) but also at each reproductive event, depending

on fertility, which complicates the explanation of what causes SGS in perennial plants. Generally, SGS in younger cohorts is stronger than in older cohorts. Chung (2008), for example, showed that an early colonization stage induces SGS in the perennial herb *Hemerocallis taeanensis*, which disappears at the maturation stage. This can be attributed both to increasing self-thinning and changes in effective population density at reproduction time with maturation. Troupin et al. (2006), on the other hand, observed that SGS was found at later stages while not present at early stages of colonization in the pioneer tree species *Pinus halepensis*. The appearance of SGS during later colonization stages was explained by fine-scale environmental heterogeneity and variation in reproductive success which were not prevalent when adult tree density was very low at the earlier colonization stage. Jones and Hubbell (2006) attributed the fact that SGS was stronger in older trees than in young cohorts of the tropical tree *Jacaranda copaia* to the species' wide temporal variation in reproduction and low seedling survival rate. There, adult trees were the survivors of reproductive events involving few trees of the previous generation, which increased their relatedness.

Finally, spatial distribution of individuals has often been used to explain SGS (Latouche-Hallé et al. 2003; Oddou-Muratorio et al. 2004; Chung 2008). Using a simulation approach, Doligez et al. (1998) found larger SGS values in highly clumped populations than in uniformly distributed populations with similar levels of gene flow. Premoli and Kitzberger (2005) showed that old growth stands characterized by gap dynamics produced SGS although post-fire stands with few remaining seed-bearing trees did not in the evergreen angiosperm tree *Nothofagus dombeyi*. However, in a review comparing SGS in ten tropical tree species, Hardy et al. (2006) found no significant relationship between spatial aggregation of individuals and SGS. These conflicting results suggest that the effects of spatial aggregation on SGS may combine the effects of several processes, notably those of local dispersal and patch recruitment dynamics (Troupin et al. 2006).

Therefore, understanding short-term variation in spatial distribution from adults to seedlings (during recruitment) is indispensable to understand the building of SGS within continuous populations. Seedlings are often spatially aggregated in forest communities (e.g., Duchesneau and Morin 1999) as a result of density-dependant mortality (Janzen–Connell model, Janzen 1970; Connell 1971) or heterogeneous safe-site distribution (Houle 1992). In dense populations, seedling spatial distribution has also been shown to depend solely on seed dispersal (neutral model, Volkov et al. 2003). In this paper, we used a simple probabilistic isolation-by-distance model for continuous populations to test the impact of several demographic parameters on SGS at fine temporal and spatial scales.

Specifically, we tested the role of seed dispersal function, adult tree density and spatial structure, and seedling distribution in space on SGS in a modeled population. The biological characteristics of the modeled population were based on those of silver fir (*Abies alba* Mill.), a keystone, wind-pollinated, mixed-mating European Alpine conifer (Restoux et al. 2008; Wolf 2003). We then used experimental genetic (isozymes and chloroplast microsatellites) and demographic data: (1) to describe fine-scale SGS of a recolonizing *A. alba* population from southern France and (2) to illustrate how a spatially structured adult population with limited seed dispersal can (counterintuitively) produce a seedling population with very weak SGS.

## Material and methods

### The model

We developed a simple regeneration model that included demographic and genetic parameters. This model has four main characteristics:

- Realistic dispersal functions, such as the one demonstrated for *A. alba* (Sagnard et al. 2007), can be included.
- Initial variables (i.e., the demographic parameters density and spatial structure) can be analyzed separately.
- Seedling genotypes are attributed using a probability function, i.e., stochasticity for a single set of parameters can be included in the model.
- The model follows an inferential logic which is typical of experimental approaches where data are geographical coordinates and genotypes of adult trees and their natural regeneration.

### 1. Adult tree population

The simulated area was a 100×100 m (1 ha) plot.  $N$  adult trees were generated over this plot with either a random, regular, or clumped spatial distribution (see the “Sensitivity analyses” section below). For simplicity, genotypes were simulated at a single biallelic neutral locus, but the generalization at several alleles and loci is straightforward. Adult tree genotype frequencies were drawn probabilistically following Hardy–Weinberg proportions, with alleles  $A$  and  $a$  having frequencies  $p=0.7$  and  $q=0.3$  in the population, respectively, which correspond to the frequencies and expected heterozygosity ( $H_e=0.42$ ) observed for the most polymorphic isozyme loci in *A. alba* in southeastern France (Fady et al. 1999). No SGS was attributed to the simulated adult tree population. All adult trees produced the same number of seeds.

### 2. Seed rain

The number of seedlings was always 441, which corresponds to one seedling per node regularly distributed along a 5×5 m grid. The number of seeds found at a location  $(x, y)$  depends on geographic position of the seed trees, their fertility and their dispersal function (Ribbens et al. 1994). At location  $i(x_i, y_i)$ , the number of seeds  $n_{i(j)}$  from seed tree  $j$  is:

$$n_{i(j)} = Q_j \cdot f(d_{ij})$$

with  $Q_j$ , the number of seeds from seed tree  $j$  (i.e., the reproductive effort of seed tree  $j$ , considered identical for all trees in the simulations);  $f$ , the dispersal function (several models are available), and  $d_{ij}$ , the distance between location  $i$  and seed tree  $j$ . The total number of seeds found at  $i$ ,  $n_{i(T)}$  is thus:

$$n_{i(T)} = \sum_j Q_j \cdot f(d_{ij})$$

The contribution of seed tree  $j$  to the seed rain falling at  $i$ ,  $C_{i(j)}$ , is  $n_{i(j)}/n_{i(T)}$ . The probability that a seedling at location  $i(x_i, y_i)$  derives from a seed fallen from seed tree  $j$ ,  $P_{i(j)}$ , is equal to  $C_{i(j)}$ . We assumed the following mating parameters to be true: (a) the pollen cloud was homogeneous throughout the forest stand and (b) selfing did not occur more frequently than expected by chance in a random mating population (i.e.,  $1/2N$ ).

$M$  is the gametic association matrix during fertilization:

$$M = \begin{bmatrix} p & q & 0 \\ 1/2p & 1/2 & 1/2q \\ 0 & p & q \end{bmatrix}$$

and  $G$  is the binary vector representing the seed tree genotype,  $G=(1,0,0)$ ,  $(0,1,0)$ ,  $(0,0,1)$  if the seed tree has genotype AA, Aa, or aa, respectively. The vector of genotypic probabilities for a seedling found at location  $(x_i, y_i)$  is:

$$P_i(\text{AA, Aa, aa}) = \sum_j P_{i(j)} \cdot G_j \cdot M$$

The genotype of seedling  $i$  found at location  $(x_i, y_i)$  can be drawn from a multinomial function with the probabilities  $P_i(\text{AA})$ ,  $P_i(\text{Aa})$ , and  $P_i(\text{aa})$  given above, assuming an absence of selection (neutral locus).

### Sensitivity analyses

Census number and spatial distribution of seed trees, spatial distribution of seedlings, and seed dispersal functions were the initial variables used for the simulations. Each variable could be introduced separately into the simulations and its effect on the spatial structure of seedling genotypes analyzed. We ran

three sets of simulations to test the effects of (1) seed tree density and seed dispersal function on seedling SGS, (2) seed tree spatial distribution on seedling SGS, and (3) spatial distribution of natural regeneration on its SGS. Each simulation involving the same variables was repeated 100 times. The initial spatial positions and the genotypes of adult tree were drawn anew for each simulation replicate.

*Effects of seed tree density and seed dispersal function on seedling SGS* We tested several dispersal functions taken from the literature (Table 1), which covered many situations found in forests, at least at medium range distances (Ribbens et al. 1994; Clark et al. 1999; Sagnard et al. 2007). All functions were of the exponential type:

$$f(r) = \frac{1}{n} \exp^{-D \cdot r^c}$$

where  $D$  is the dispersal parameter that determines the speed of dispersal decrease with distance (decay coefficient),  $c$  is the parameter controlling the shape of the kernel (e.g., exponential:  $c=1$ ; Gaussian:  $c=2$ , etc.),  $r$  the distance between seedling  $i$  and seed tree  $j$  and  $n$  is the normalizer that bounds the dispersal function values between 0 and 1.

The simulated density of the seed trees was  $N_{ad}=2, 4, 9, 16, 25$ , or 49 per hectare (ha). Adult trees and seedlings were distributed regularly.

*Effect of seed tree spatial distribution on seedling SGS* Here, the number of adult trees per ha was  $N_{ad}=4, 9,$

16, and 25. The dispersal function used was that estimated for *A. alba* by Sagnard et al. (2007). Spatial distribution of seedlings was regular within the stand. We tested three possible spatial distributions of adult trees: regular, random, and clumped. To simulate clumping, adult trees were randomly distributed in a single 20×20 m plot located at the center of the 100×100 m stand.

*Effect of spatial distribution of natural regeneration on its SGS* Here, the number of adult trees per ha was  $N_{ad}=4, 9,$  or 16, and their position was random, i.e. those adult tree densities and position that could lead to a seedling SGS according to results shown in the “Results and discussion” section below. The dispersal function used was that estimated for *A. alba* by Sagnard et al. (2007). Five types of realistic spatial distributions were simulated for the progeny:

- RAND: seedlings were randomly distributed.
- DISP: seedlings were distributed according to seed rain and their spatial positions were drawn using random numbers. Seedling spatial distribution followed a non-homogeneous Poisson process (Sagnard et al. 2007).
- DISP10: same as DISP, but no seedling could be found less than 10 m from a seed tree (combination of non-homogeneous Poisson and “hardcore” processes).
- AGREG(>15 m): seedlings were clumped into four aggregates, each 20 m in diameter and containing 80 seedlings whose positions were drawn at random. The

**Table 1** Dispersal functions used in the simulations and their parameters

Decay coefficient ( $D$ )	Mean dispersal distance	Comments on why the dispersal function was selected	Examples of species for which the selected dispersal function fits best
0.02	50.0 m	Most frequent best-fitted function for those wind-dispersed tree species that dispersed furthest in Willson (1993)	<i>Betula uber</i> , <i>Eucalyptus regnans</i> , <i>Picea engelmannii</i> , <i>Tsuga heterophylla</i>
0.06	16.7 m	Upper boundary for $D$ in wind-dispersed tree species in Willson (1993)	<i>Pinus palustris</i>
0.104	9.6 m	Best-fitted dispersal function for a wind-dispersed conifer in Sagnard (2001)	<i>Abies alba</i>
0.2	5.0 m	Median value of $D$ for vertebrate-dispersed tree species in Willson (1993)	<i>Macrozamia riedlei</i>
Mixed exponential: 0.899/0.101 (Prod) 0.404/0.016 (Disp) <sup>a</sup>	8.1 m <sup>b</sup>	Best-fitted dispersal function for a wind-dispersed conifer in Higgins and Richardson (1999)	<i>Pinus pinaster</i>

All functions are of the negative exponential type, with parameter  $c=1$  (see text)

<sup>a</sup> The “prod” parameters in the mixed exponential function indicate that 89.9% of the seeds are dispersed locally and 10.1% at long distance, which corresponds to the dispersal parameters (Disp) of 0.404 and 0.016, respectively. Such a function could explain seed dispersal of a polychorous tree species, e.g., a wind-dispersed species that can also be dispersed by birds. It could also explain seed dispersal in a zoochorous tree species whose dispersal vector is not always the same, e.g., seeds dispersed by two separate bird species with totally different feeding habits or seeds sometimes dispersed after very strong winds (Higgins and Richardson 1999).

<sup>b</sup> This value is actually the mean spread rate, estimated in meters per year.

remaining 121 seedlings were randomly distributed. The center of each aggregate was at least 15 m away from a seed tree. This situation can be found when regeneration occurs in patches (e.g., falling of an old tree that generates a forest opening) far from remaining adult trees.

- AGREG(<15 m): same as AGREG(>15 m), but the center of each aggregate was less than 15 m away from the closest seed tree. This situation can be found when favorable regeneration sites are mostly close to adult trees, e.g., for species with limited ecological niches or when environmental conditions are very heterogeneous.

Output variable: SGS

Seedling SGS was evaluated using the Moran index (Cliff and Ord 1973), calculated using allele A (the most frequent here) for a given distance class ( $k$ ):

$$I_k = \frac{n \sum_i \sum_j \delta_{ij} z_i z_j}{2W \sum_i z_i^2}$$

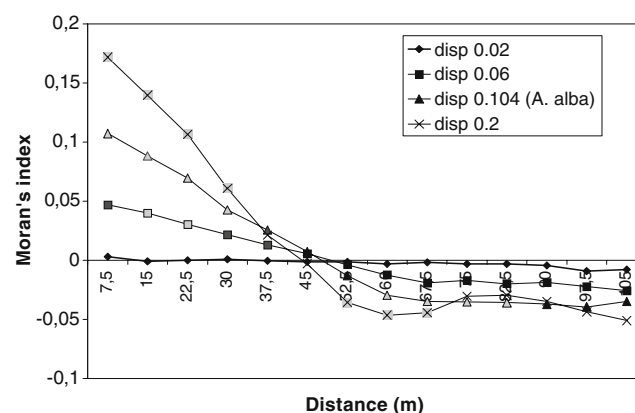
with  $z_i = x_i - x$ ,  $z_j = x_j - x$ ,  $\delta_{ij} = 1$  if  $d_{(k)} < d_{ij} < d_{(k+1)}$  and  $\delta_{ij} = 0$ , otherwise.  $n$  is the total sample size,  $W$  is the number of point pairs involved within a given distance class,  $x_i$  is the value of individual  $i$  (coded 2, 1, 0 for AA, Aa, aa, respectively),  $x_j$  is the value of individual  $j$  ( $i \neq j$ ) and  $x$  is the mean frequency of allele A across all distance classes. The Moran index was computed for each of 14 distance classes, including all pairs of individuals separated by a distance that fell within the distance interval under consideration (here 7.5 m). Results shown in the simulation outputs are the mean Moran index for each distance class over 100 simulations and the number of times that the simulations generated Moran index values significant at the 5% level. This test is conservative: its null hypothesis  $H_0$  “the spatial distribution of genotypes is random” is less frequently rejected than when tests based on  $p$  value (e.g., Bonferonni or Sidak tests) are used.  $H_0$  is rejected when less than five non-significant (5% level) simulations are randomly produced by the initial variables over 100 simulations.

The experimental data: case study using a recolonizing *A. alba* plot in Mont Ventoux (France)

We established an intensive study plot of 0.79 ha on the Mont Ventoux (44°10'56" N–5°17'01" E) at the southwestern-most tip of the Alps in France. The plot is located at an elevation of 1,400–1,450 m and belongs to the mountainous mid-European bioclimatic zone (Sagnard et al.

2002). The dominant forest tree species are *Pinus uncinata* (Mountain pine) and *Picea abies* (Norway spruce), both of which were planted some 60 to 80 years ago to restore a forest cover severely impacted by centuries-old grazing. A few *Fagus sylvatica* (beech) and *A. alba* adult trees are also found, which are assumed to have been naturally regenerated from a few nearby remnant trees used as pasture landmarks and shelters (National Forest Service photo archives) that have now disappeared. However, the plot is characterized by intense colonization by silver fir. Only five *A. alba* trees old enough to contribute significantly to seed rain were found within the plot (early stage of colonization), whereas it is the most common tree species within the natural regeneration cohorts of the plot (more than 1,100 individuals per ha, later stage of colonization). Other *A. alba* adult trees are found nearby, both as isolated trees within a 100-m radius of the plot boundaries (less than 20 trees in total) and as one dense stand located further away than 100 m to the plot boundaries (see Fig. 1 and text in Sagnard et al. (2007) for details). Thus, although pollen dispersal (Restoux 2009) and seed dispersal (Sagnard et al. 2007) are limited in *A. alba*, our experimental plot cannot be considered as isolated.

All *A. alba* trees and seedlings over 20 cm in height were mapped (Sagnard et al. 2007) and grouped into three height cohorts (that could roughly correspond to age cohorts although age  $\times$  height correlations are not straightforward in this species according to our own observations): C1, height <100 cm, 328 individuals; C2, height=100–300 cm, 229 individuals; C3, height >300 cm, 188 individuals. Using the methods of Goreaud and Pelissier



**Fig. 1** Correlograms showing the effect of seed dispersal function (dispersal mode) on the spatial genetic structure of regeneration. The number of adult trees is  $N=9$  and the negative exponential coefficient of the dispersal function appears in the legend (value after «disp»). Moran index values that differ significantly from 0 at the 5% confidence level appear in shaded gray and non-significant values appear in black. The spatial genetic structure resulting from the mixed exponential dispersal mode of Higgins and Richardson (1999) is not shown here as it was similar to that of the non-significant “disp 0.02” dispersal function

(1999), Sagnard (2001) showed that all cohorts were significantly aggregated and the smallest height cohort was aggregated away (farther than 15 m) from the highest height cohort. A total of 745 samples was genotyped at 15 isozyme loci (of which six were polymorphic: IDHb, GOTc, LAP, 6PGDa, 6PGDb, MDHb) following Fady et al. (1999) and at two chloroplast microsatellite loci (cpSSR, Pt 30204, and Pt 71936) following Vendramin and Ziegenhagen (1997). In addition to calculating standard population genetic measures (allele/haplotype frequencies, heterozygosity/gene diversity,  $F$  statistics), we estimated the fine-scale SGS of seedlings by calculating:

- (1) Moran's autocorrelation index (Cliff and Ord 1973) on the most frequent allele for each isozyme locus averaged over all loci (for comparison with our simulation approach). The Moran index was computed over all samples and for each height (age) cohort for each of nine distance classes with even sample size within class. We tested for the significance of the Moran index by permuting 5,000 times the spatial position of individuals to compute its frequency distribution under the null hypothesis that the Moran index and distance are not correlated.
- (2) To avoid biases linked to sampling design and choice of distance classes (Fenster et al. 2003), we calculated the slope of the regression of the Moran index against the log of distance ( $b_{\log}$ ) and tested for its significance as above. We also calculated  $Sp = b_{\log} / (F_1 - I)$ , where  $F_1$  is the average Loiselle kinship coefficient (Loiselle et al. 1995) between individuals of the first distance class ( $d < 10$  m).  $Sp$  is an indicator of the strength of SGS usable for comparison with other species (Vekemans and Hardy 2004).

All computations were done using Spagedi (Hardy and Vekemans 2002).

## Results and discussion

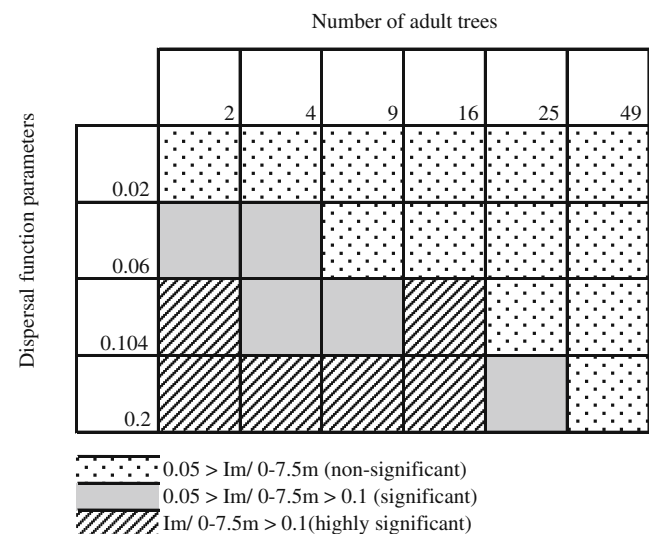
### Effect of seed tree density and seed dispersal type on seedling SGS

The Moran index decreased regularly with distance for all seed dispersal functions except those that dispersed furthest ( $D=0.02$  and mixed exponential functions; Fig. 1 for  $N_{ad}=9$  trees per ha). This typical isolation-by-distance structure decreased as average dispersal distance increased, for instance when a long distance component was added (Fig. 1). However, the spatial distribution of seedling genotypes was also strongly affected by the density of adult trees. When  $N_{ad}=49$  trees per ha, the Moran index was non-significant regardless of the seed dispersal func-

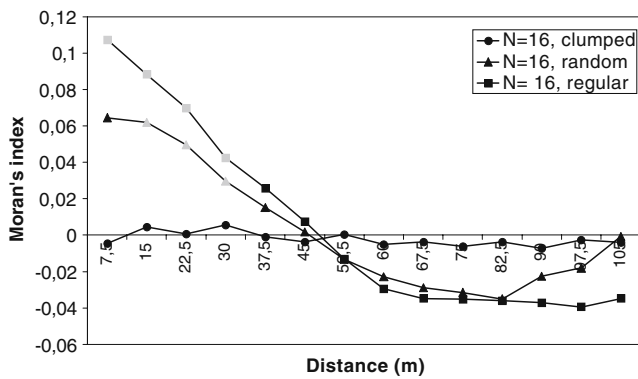
tions (Fig. 2, distance class 0 to 7.5 m). Spatial distribution of genotypes was not significantly different from a random distribution for the least localized dispersal functions (Fig. 1), regardless of the number of adult trees (Fig. 2). The Moran index was significant only for  $N_{ad} \leq 25$  trees per ha and for a decay coefficient higher than 0.02 (Fig. 2). Although dispersal range and shape are key factors for predicting SGS (Ohsawa et al. 1993), the density of reproductive individuals is at least as important (Robledo-Arnuncio and Rousset 2010). The densities of stand-forming broadleaf and coniferous forest trees generally exceed 25 adult individuals per ha. Such densities are thus sufficient to prevent the occurrence of strong SGS at short distance classes among naturally regenerated recruits in just one generation, assuming no prior SGS among adult trees. However, a significant SGS can be expected when population density becomes lower than 25 trees per ha and seeds are dispersed at very short range (e.g., gravity-dispersed seeds, Bittencourt and Sebbenn 2007) or when population density becomes lower than a few trees per ha such as in recolonizing populations (Chung 2008).

### Effect of seed tree spatial distribution on seedling SGS

Spatial autocorrelation of genotypes within the regeneration was highest for the regular distribution of adult trees, medium for the random distribution, and non-significant for the clumped distribution, regardless of adult tree density (Fig. 3 where  $N_{ad}=16$  trees per ha). In addition to density, adult population structure can thus also strongly influence seedling SGS. When seed trees are grouped into a single clump, seeds behave as if they came from a single source which cumulates the genetic diversity of all adult trees



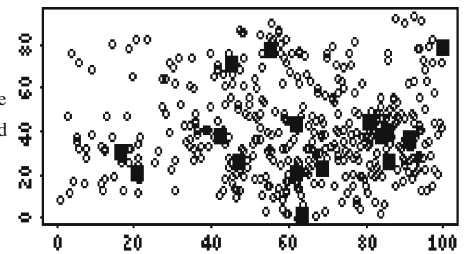
**Fig. 2** Moran index values for the first distance class simulated (0–7.5 m) for different adult tree densities and seed dispersal distances (dispersal mode)



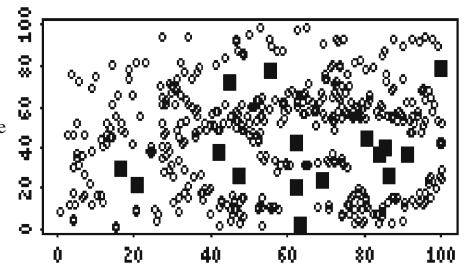
**Fig. 3** Correlograms showing the effect of seed tree spatial distribution on the spatial genetic structure of their regeneration. Values that differ significantly from 0 at the 5% confidence level appear in *shaded gray* and non-significant values appear in *black*. The dispersal function (Table 1) is that of Sagnard et al. (2007)

**Fig. 4** Example of simulated spatial distributions of seedlings for  $N_{tot}=16$ . The dispersal function (Table 1) is that of Sagnard et al. (2007). Four different spatial distributions are used: DISP, DISP10, AGREG(<15m) and AGREG(>15m)

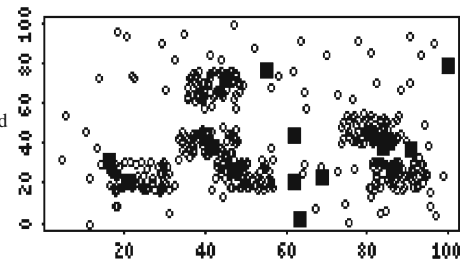
DISP: seedlings are distributed according to the seed rain modelled from an *Abies alba* seed trap experiment (Sagnard et al. 2007)



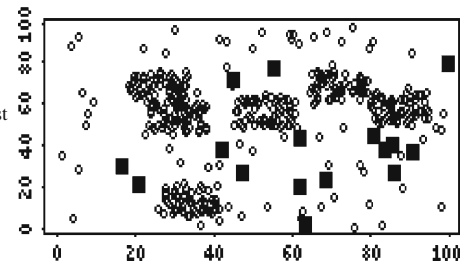
DISP10: same as DISP, but no seedling can be found at less than 10 m from a seed-tree.



AGREG(<15m): seedlings are clumped around seed trees.



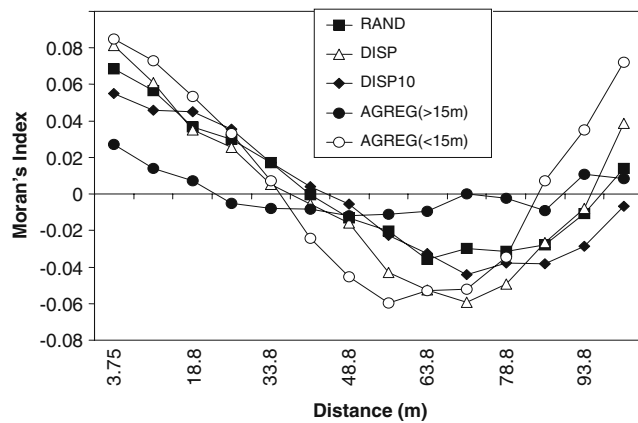
AGREG(>15m): seedlings are clumped at least 15 m away from seed trees



found in the source population (Hamrick et al. 1993). Each seed has roughly the same probability of coming from any adult tree at any location within the clumped stand. Rohlf and Schnell (1971) observed in their simulations that genetic structure decreased when adult trees were located at the center of a neighborhood structure.

Effect of spatial distribution of natural regeneration on its SGS

Spatial genetic autocorrelation of seedlings was maximum for the AGREG(<15 m) and DISP distributions (Figs. 4 and 5). In both cases, seedlings are more likely to establish and survive closer to their seed trees than in all other situations. At large distances, SGS was also maximum for these two seedling distributions which we interpret as an artifact due to the low number of individual pairs within the largest distance classes. At short distances, DISP10 had a significant but low



**Fig. 5** Correlograms showing the effect of the spatial distribution of seedlings on their spatial genetic structure ( $N_{\text{tot}}=16$ )

Moran's index, and AGREG(>15 m)'s Moran index was never significant for  $N_{\text{ad}}=16$  trees per ha (Fig. 5). A random distribution of seedlings (RAND) generated an intermediate SGS. The rare studies that compare seedling SGS with their spatial distribution (although not at the same spatial and temporal scales as ours) confirm the role of spatial aggregation on SGS. Doligez et al. (1998) found that Moran's index was positively correlated to spatial aggregation (measured by the distance of each individual from its closest neighbor using a spatial distribution corresponding to our DISP process, although, unlike ours, their model measured SGS at equilibrium after many generations). A positive correlation between Moran's index and spatial aggregation has also been found in the tropical tree species *Vouacapoua americana* by Dutech et al. (2002), although, again unlike ours, their experimental SGS was the result of drift and restricted gene flow over several generations.

Our simulations further demonstrate that, in an isolated population, it is not seedling aggregation per se that yields positive Moran index values. If this were the case, the Moran index of the AGREG(>15 m) would be as high as those of AGREG(<15 m) and DISP. What matters is seedling aggregation in comparison to adult tree spatial distribution. The AGREG(<15 m) and DISP spatial distributions are probably infrequent in natural forest tree populations (Houle

1992) as they do not take into account competition between adults and seedlings and density- and/or distance-dependant seedling survival in general (the Janzen–Connell hypothesis, Janzen 1970; Connell 1971). Regeneration dynamics, through adult–seedling competition and the Janzen–Connell effect, tend to reduce the seedling SGS.

A case study: unexpectedly low fine-scale genetic structure within a recolonizing silver fir (*A. alba* Mill.) plot

We initially expected to find a strong seedling SGS within our intensive *A. alba* study site from the results of our simulation tests. The plot had low adult tree density, aggregated spatial distribution of seedlings at all height classes close to seed trees, and limited seed dispersal (median dispersal distance <20 m; Sagnard et al. 2007) restricting the contribution of surrounding trees. In addition, the mating system of *A. alba* was found to be significantly selfed at low density in Mont Ventoux (Restoux et al. 2008), another reason to expect strong SGS (Fenster et al. 2003; Vekemans and Hardy 2004). However, SGS for isozymes was very weak in our plot and never significant for cpSSRs (Table 2). The slope of the regression of kinship coefficients against the log of the distance ( $b_{\text{log}}$ ) was significant overall ( $-0.004$ ,  $p<0.01$ ), but when samples were divided into three height cohorts,  $b_{\text{log}}$  was only significant for the intermediate height (age) cohort ( $100<h<300$  cm). In this cohort, the Moran index reached a maximum value of 0.031 in the first distance class (Fig. 6). Our Sp value (0.005 for isozymes) was among the lowest in Vekemans and Hardy (2004). It was lower than the average value reported for trees and for wind-dispersed plants (Sp=0.0102 and 0.012, respectively) and consistent with the value reported for wind-pollinated plants (Sp=0.0064).

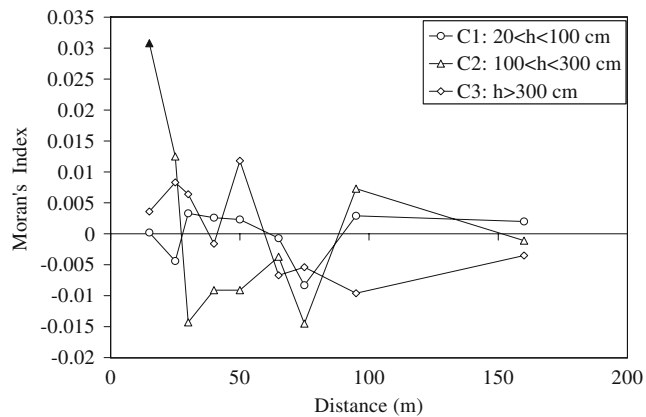
One reason for detecting a very weak SGS in the experimental plot could be that our isozyme markers have low resolution (Streiff et al. 1998; Hardy et al. 2006). Isozymes typically display low variation, with one major allele and one or a few low frequency alleles per locus. In this population, the expected heterozygosity was 0.174 and the mean number of alleles per locus was 2.2. However, we

**Table 2** Value ( $b_{\text{log}}$ ) and 95% confidence interval (95% low–95% high) of slope of regression of the Moran index against log of distance and value of SGS strength (Sp) for three different height cohorts and two different genetic markers in the *Abies alba* experimental plot of Mont Ventoux

	$b_{\text{log}}$	95% Low	95% High	Sp
Isozymes (all samples)	-0.004**	-0.002	0.002	0.005
Isozymes (20<h<100)	0.000	-0.005	0.004	-0.000
Isozymes (100<h<300)	-0.008*	-0.007	0.005	0.006
Isozymes (h>300)	-0.005	-0.007	0.005	0.004
cpSSR (all samples)	-0.001	-0.008	0.005	0.000

Significance (one-sided test): \* $p<0.005$ ; \*\* $p<0.001$





**Fig. 6** Correlogram of pairwise Moran's index (calculated using isozyme data) and distance for three cohorts (C1, C2, and C3) in the *Abies alba* experimental plot of Mont Ventoux. Filled symbols represent values significantly different from values expected under a random distribution of genotypes (95% confidence level)

did not detect SGS using cpSSRs either. These markers are highly polymorphic in *A. alba* in general (Vendramin et al. 1999) and in this population in particular (56 different haplotypes detected). As pollen dispersal is restricted in this population (mean dispersal distance less than 10 m, Restoux 2009), there are good reasons to believe a significant SGS should have been detected using cpSSRs if one was present.

The lack of SGS in the experimental plot can also be explained by different demographic processes, some because the experimental population differed from the simulated population, others in line with findings from our simulations. First, the studied stand was recently colonized so that more generations may be needed for significant SGS to appear (Troupin et al. 2006). Second, dispersal may have been less restricted at the early stage of the colonization process than at the later stage from which the seed dispersal kernel was estimated (Sagnard et al. 2007). Inbreeding was significant in the height (age) class smaller than 100 cm ( $F_{is}=0.157$ ,  $p<0.001$ ) and non-significant ( $p>0.05$ ) in the height classes between 200 and 300 cm ( $F_{is}=0.055$ ) and above 300 cm ( $F_{is}=0.036$ ). The effective number of cpSSR haplotypes was 6.13 in the smaller, 8.28 in the intermediate, and 7.86 in the highest height class. Overall differentiation between height classes was non-significant ( $F_{st}$  and  $R_{st}=0.00$ ). Taken together, these results indicate that all seedlings come from a common gene pool. Those that established at the earliest stage of colonization came from outside the plot as no mature adult trees were present in the plot. Inbred seedlings that established at later stages mostly came from the few local adult trees that established during the earliest stage of colonization. However, inbreeding alone in the later stages was insufficient to lead to a noticeable SGS in the most recent cohorts.

Third, the pollen contribution of surrounding trees may have been higher than expected, thus significantly increasing gene flow from outside the experimental stand. Pollen has been shown to travel long distance (several tens of meters on average) in natural (e.g., Oddou-Muratorio and Klein 2008) as well as planted populations (Jones et al. 2008). In very isolated small populations, pollen can travel even further although long distance dispersal accounts for very few of the seeds produced (Robledo-Arnuncio and Gil 2005). However, in our low density, but less isolated, experimental population, pollen was found to be dispersed over short distances (mean dispersal distance of less than 10 m) and seeds sired by few pollen trees during each reproductive event (Restoux 2009), making it improbable that long distance dispersal of pollen could account for a high proportion of fertilizations.

Fourth, and as highlighted in our simulations, the respective spatial distribution of adult and seedlings may contribute to very weak SGS observed in our experimental plot. Sagnard et al. (2007) showed that a competition process between adult trees and seedlings exists at short distances, limiting the emergence and survival of seedlings close to adult trees (Janzen–Connell effect). Finally, the adult trees were clumped within the plot (Sagnard et al. 2007), which was also shown to decrease seedling SGS in our simulations.

#### Advantages and limitations of the model

Our simulations show that, besides adult population density and dispersal parameters, other demographic factors may influence SGS within a small plot, i.e., (1) spatial distribution of adult trees within the plot and (2) spatial distribution of natural regeneration at a certain distance from adult trees (the Janzen–Connell hypothesis). Thus, a lack of spatial autocorrelation among genotypes should not be systematically taken as an indication of high seed and/or pollen flow. Although significant positive values of Moran's index do indicate gene flow limitations, an absence of significant values does not make it possible to hypothesize on the role of mating system or seed dispersal without additional information on population demographic dynamics.

Our model may diverge from real situations for several reasons. The simulated populations were small and considered to be isolated (no migration), which over-estimates seedling SGS. However, SGS is probably not at equilibrium over just one generation (Leblois et al. 2004), and therefore underestimated in our simulated population. Further, our modeled mating system excluded significant selfing, which is known to occur to some extent in *A. alba* (Restoux et al. 2008), and by excluding selfing, we underestimated offspring SGS. Finally, there was no SGS in our simulated

adult trees which may also result in an underestimation of seedling SGS.

A more realistic but complex model for long-lived species would include cumulative reproductive events and the stochasticity of flower phenology, seed production, and germination over time, which might affect seedling SGS over time. In addition, considering different spatial scales may be important to decipher the effect of spatial and genetic aggregation of adult trees on SGS in the regeneration as trees are often grouped into populations at scales larger than our own. However, we feel that this simpler model sheds light on important demographic processes affecting SGS and may help explain the occurrence or absence of SGS in experimental situations. This simple model can also be used to test or discuss the effects of management techniques in low density stands, e.g., when mature trees are naturally thinned (Kenkel 1988) or cut to make way for natural regeneration in natural or plantation forests, or when populations are isolated at range margins and managing for conservation is required.

#### Potential implications for forest management

Our study demonstrates that both adult tree and seedling spatial distributions are key factors for the regeneration SGS. This is particularly important since the spatial distribution of adult trees is controlled by forest management in most forest ecosystems. Thus, forestry management practice can influence (Degen et al. 2006) and possibly control the spatial structure of a forest stand, and thus the SGS of its regeneration. In monospecific forest stands, adult tree density is generally high (over 49 trees per ha as in our simulations), gene flow significant, and no SGS should be expected at the local scale after a single generation. There, long-term and large-scale demographic processes are the ones of importance. However, in mixed forests or in marginal and isolated stands, adult tree density is often lower than this threshold value. Using simulations, we showed that SGS in the regeneration increases when seedlings are close to the adults. If we assume that SGS may be an indication that an evolutionary process leading to loss of genetic diversity through inbreeding at the population level is under way, a forest management practice that reduces the occurrence of SGS by controlling adult tree and seedling spatial structure would in fact minimize the effects of consanguinity at population level and maximize the effects of natural selection by increasing the competition between related and unrelated individuals (Mitton and Grant 1984; Bush and Smouse 1992; Epperson and Alvarez-Buylla 1997). Effects of spatial aggregation on SGS are temporally and spatially scale-dependent and our simulations explore only one aspect of these. Without going as far as drawing practical management guidelines from our

simple simulation study, including demographic strategies in forest management that will affect SGS based on our analysis would help further sustain low density and isolated stands in times of severe environmental and climatic uncertainty.

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