

# Impacts of gene flow and logging history on the local genetic structure of a scattered tree species, *Sorbus torminalis* L. Crantz

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## Abstract

*Sorbus torminalis* L. Crantz is a colonizing tree species usually found at low density in managed European forests. Using six microsatellite markers, we investigated spatial and temporal patterns of genetic structure within a 472-ha population of 185 individuals to infer processes shaping the distribution of genetic diversity. Only eight young stems were found to be the result of vegetative reproduction. Despite high levels of gene flow (standard deviation of gene dispersal = 360 m), marked patterns of isolation by distance were detected, associated with an aggregated distribution of individuals in ~100-m patches. This spatial structure of both genes and individuals is likely to result from patterns of seedling recruitment combined with low tree density. Our results suggest that landscape factors and logging cycles markedly shape the distribution of favourable sites for seedling establishment, which are then colonized by sibling cohorts as a result of joint seed transportation by frugivores. These combined genetic and demographic processes result in similar genetic structure both within and among logging units. However, conversion to high forest may enhance genetic structuring.

**Keywords:** microsatellite, Ripley's *K*-function, silviculture, SPAGED1, spatial auto-correlation, spatial genetic structure

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## Introduction

The spatial distribution of genetic variation within local plant populations is the result of various genetic and demographic processes acting at specific temporal and spatial scales. Most theoretical studies focused on the long-term or equilibrium structure of genetic variation as revealed by neutral loci. They allowed the prediction of the expected spatial pattern of genetic structure (SPGS) under drift–dispersal equilibrium (beginning with Wright 1943, 1946; see also Hardy & Vekemans 1999; Rousset 2000). Moreover, field surveys have often supported these theoretical expectations and emphasized the impact of seed and pollen dispersal on local SPGS (Sork *et al.* 1999; Vekemans &

Hardy 2004), although the assumption of a true equilibrium is unlikely to hold in nature.

However, in some cases SPGS is likely to be affected by demographic instability through space and time. Only a few theoretical studies have examined the potential consequences of demographic instability in continuous populations evolving under isolation by distance. Doligez *et al.* (1998) suggested that spatial aggregation produces a slight increase in SPGS, though their model did not clearly allow the effects of an individual's distribution and those of its dispersal abilities to be disentangled. In a simple case where genetic structure is analysed within a local area included in a large habitat with either lower or higher density, Leblois *et al.* (2004) showed that SPGS depends primarily on the local density at the place of sampling. However, the impact of the surrounding density can be important depending on the sampling design. Leblois *et al.* (2004) also studied diverse sources of temporal variation in density.

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Their results suggest that reductions in population density (bottlenecks) can be imprinted in SPGS within a few tens of generations, whereas recent increases in population density (demographic flushes) can generally not be detected in the present genetic structure.

In empirical surveys on the other hand, rigorous inferences about demographic impact on local SPGS remain rare. Moreover, the few available marker-based cases illustrate a wide range of demographic effects on SPGS (reviewed in Epperson 2000; see also Kalisz *et al.* 2001; Chung *et al.* 2003). A key demographic process seems to be whether or not the population is increasing in density (Epperson 2000). In addition, SPGS also strongly depends on life stage, particularly in species with long life cycles such as trees.

In forest tree species, empirical surveys within the last decades have produced evidence of the impact of spatial and temporal demographic instabilities on SPGS. Spatial heterogeneity in density is frequently acknowledged within populations (Goreaud 2000). Many studies suggest that, together with adult density, the spatial distribution of individuals controls the levels of spatial genetic structure through the degree of overlap in individual seed shadows (Hamrick *et al.* 1993).

Besides spatial processes, two main sources of temporal changes in population density were identified. First, in many woody species, seedlings are found in much higher densities than adults, resulting in a typical L-shaped age distribution (Chung *et al.* 2003). In most cases, the strong levels of spatial genetic structure observed in seedlings have been shown to decrease in adult stages because of self-thinning processes (Hamrick *et al.* 1993; Epperson & Alvarez-Buylla 1997; Chung *et al.* 2003).

Second, various studies investigated the impact on SPGS of fluctuations of population density caused either by natural disturbances (e.g. fires; Parker *et al.* 2001) or anthropogenic disturbances (e.g. logging Boyle *et al.* 1990; Knowles *et al.* 1992; Young & Merriam 1994; Epperson & Chung 2001). These studies classically compared SPGS among two or more populations with different population densities resulting from different disturbance histories. A variety of SPGS were observed among disturbed sites, from complete spatial mixing of genotypes (Knowles *et al.* 1992) to high spatial structuring (Boyle *et al.* 1990; Knowles *et al.* 1992; Parker *et al.* 2001), with all possible variations in between (Epperson & Chung 2001). The contrast with SPGS on an undisturbed site was not always straightforward, and could even be contradictory within the same study depending on the spatial scale being considered (Young & Merriam 1994). A main conclusion of these studies is that the effects of disturbance history on SPGS depend on the nature of the disturbance and the characteristics of the populations.

A major source of disturbance across European forests since the beginning of the Neolithic period is the

fragmentation induced by human deforestation for agriculture. Within the remnant forest areas, silviculture has been more or less rationalized to ensure the production of high-diameter stems for at least 400 years. Silvicultural practices are now characterized in European forests by a regular cycle of thinning (through selective logging), sometimes ending with clear-cutting (high forest). Long-term impact of selective logging on gene pools has been addressed by some authors (Ledig 1992). However, to our knowledge, the demographic impact of logging cycles themselves on SPGS has received little attention.

The present paper examines the combined effects of logging cycles, gene flow and age structure on the build-up of SPGS within a population of the temperate forest tree *Sorbus torminalis* L. Crantz. This low-density species (usually less than 1 individual/ha) has good abilities to colonize forest clearings. However, as a sapling, it can scarcely compete with other tree species at undisturbed sites. Using foresters' records, we managed to trace the logging history of stands over the last 70 years across the 22 management units (MU) of the studied site. These records showed that *S. torminalis* was not systematically eliminated during logging operations, but rather was selectively cleared to favour a more valuable individual in the neighbourhood (either from the same or from another species). Also, they allowed us to identify patches of forest (i.e. groups of MU) that showed similar logging histories, thereafter denoted logging units. Our objective was to investigate the impact of logging cycles on SPGS. Indeed, under the hypothesis that logging events create favourable conditions for *S. torminalis* seedling establishment by opening clearings within forest stands, we can expect higher levels of genetic relatedness within units than among units once spatial effects are removed. Combined with restricted dispersal, logging patterns could even lead to strong differentiation among logging units through a spatio-temporal Wahlund effect.

We used six highly variable microsatellite markers to characterize local genetic variation in all of the 185 adult *S. torminalis* in the study site, and to identify eventual clones that were the result of vegetative reproduction (Rameau *et al.* 1989). Then, we partitioned the observed genetic variation across spatial and temporal dimensions (see Kalisz *et al.* 2001). The spatial component of genetic structure was extracted using standard auto-correlation tools (Hardy & Vekemans 2002; Vekemans & Hardy 2004). To analyse the effects of population age structure on SPGS, individuals were partitioned into temporal groups (cohorts) after we had calibrated the relation between age and diameter. Finally, to analyse the effects of logging history on SPGS, individuals were assigned to eight logging units. Spatio-temporal patterns of genetic structure were then characterized by studying the decrease in kinship coefficients among pairs of individuals with geographical distance, considering

(i) all pairs of individuals, (ii) pairs of individuals of the same temporal group and (iii) pairs of individuals within/among logging units. We also compared SPGS with spatial patterns of individual distribution.

## Materials and methods

### Biological material

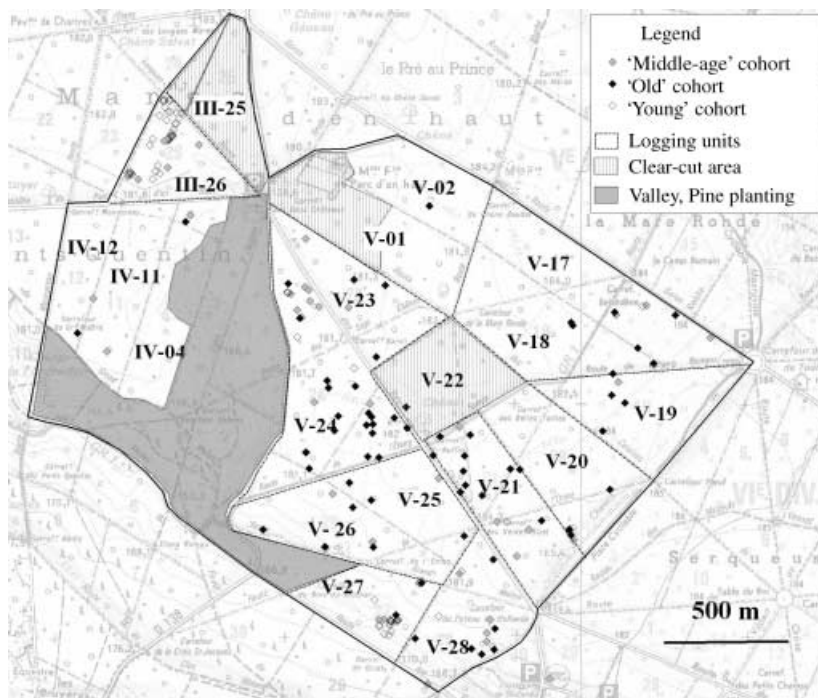
**Studied organism.** The wild service tree, *Sorbus torminalis* (L.) Crantz, is a primarily outcrossing species of the Rosaceae family (subfamily Maloideae). It is widely distributed across Europe (from northern Africa to the south of Sweden and from the east of Great Britain to the north of Iran). Pollinated by a wide range of hymenopteran and dipteran insects, this species has good abilities of long-distance pollen dispersal (Oddou-Muratorio *et al.* 2003; Oddou-Muratorio *et al.* submitted for publication). Efficient dispersal of its fleshy fruits by birds (Snow & Snow 1988) and mammals (Grime *et al.* 1988) probably accounts for its good colonizing abilities (Oddou-Muratorio *et al.* 2001a). Traditionally favoured for bird hunting and for use in wood manufacturing, this valuable species is increasingly appreciated in the veneer industry for furniture.

**Study site and individual sampling.** The study site is located at the northeast part of the Rambouillet Forest (France). It is composed of 22 MU of mixed oak and broad-leaved stands, covering 472 ha (Fig. 1). We identified 185 reproductive trees after an exhaustive survey during the flowering

period (Fig. 1). Generally, only trees with a diameter > 10 cm were reproductive, but flowers and fruits could be observed on smaller *S. torminalis* with few other trees in their neighbourhood, typically edge trees or trees in small clearings. Each tree was labelled with three identifiers (e.g. V-21.01): the first identifier corresponded to large-scale management zones (three 'divisions': III, IV and V), the second to the MU (from 04 to 29), the last one to the individual tree.

**Silvicultural context.** Up to about 1960, each MU was composed of a mixed-deciduous understorey mostly regenerated by sprouting (coppice), overtopped by an uneven-aged, low-density, oak-dominated canopy layer (reserve trees). The logging records since 1932 indicate that about every 30 years, c.30% of the reserve trees were logged in each MU, while coppice stems were more or less completely removed, except for a few high-quality stems that were selected to enter the reserve population. The objective was to ensure a roughly stable number of reserve trees through time. The good sprouting abilities of *S. torminalis* were likely to favour this species in the understorey, but logging records also show that few *S. torminalis* were regularly selected as reserve trees.

In the 1960s, foresters decided to renounce this silvicultural system (known as 'coppice under reserve') and to switch instead to 'high forest', that is even-aged stands regenerated every 180–200 years by clear-cutting. In high forest, regeneration occurs from seeds produced by a few seed trees (typically 70 trees/ha) that are given better



**Fig. 1** Study site and *Sorbus torminalis* distribution. Bold numbers correspond to management units. The 185 exhaustively mapped reproductive trees were split into three cohorts on the basis of d.b.h. Alternatively, 157 trees among these 185 were grouped in eight logging units, corresponding to management units harvested in the same years. No *S. torminalis* were found in the grey area because of specific environment characteristics and silvicultural treatments.

opportunities for fruit outset during the first 10–20 years after clear-cutting. In the early 1960s, seed trees were often chosen from among the dominant species (oak in our case) to obtain homogeneous stands. However, now, regeneration of secondary species is generally highly favoured: e.g. no *S. torminalis* seed tree has been cut for 15 years on any MU of our study site.

To convert 'coppice under reserve' stands into high forest, from 1960, coppice cutting was discontinued, and tree diameters were progressively homogenized by selective logging, beginning with the largest individuals and low-quality coppice stems. As this conversion began 40 years ago, most MUs of the study site have never been clear-cut (Fig. 1).

### Genetic markers

**Genotyping.** For each sample, DNA was isolated from five or six frozen buds or from one or two frozen leaves per tree following the procedure given in Oddou-Muratorio *et al.* (2001a). The genotypes of all individuals were scored at six microsatellite loci: MSS1, MSS5, MSS6, MSS9, MSS13 and MSS16, described in Oddou-Muratorio *et al.* (2001b). We used fluorescently labelled primer pairs to perform amplification by polymerase chain reaction (PCR). The PCR fragments were then separated by electrophoresis on a sequencing polyacrylamide gel, and detection was achieved under various wavelengths with an FM-BIO II scanner (Hitachi Software). Three adult trees showing three-banded genotypes (Oddou-Muratorio *et al.* 2003) were eliminated from genetic analyses.

**Identity probability.** Microsatellite markers were used to detect the possible occurrence of vegetative reproduction. As is usual in this type of study, adult trees sharing exactly the same multilocus genotype and located close to each other were suspected of being clones.

To validate this decision, we computed the probability of observing a group of  $k$  or more individuals sharing the same multilocus genotype without vegetative reproduction, following Frascaria *et al.* (1993). The probability of occurrence of genotype  $A_i$  at locus  $i$  can be estimated by the frequency of this genotype in the observed sample. It requires the assumption that parents of the sampled trees mated at random and also that allelic frequencies were similar in the parental and present populations. Assuming independence among loci, the probability  $P_A$  of occurrence of the multilocus genotype  $A$  is:

$$P_A = \prod_{i=1}^l P_{A_i}$$

where  $l$  is the number of scored loci.

The probability  $P_A(k)$  of occurrence of  $k$  individuals with the same genotype  $A$  in a group of  $N$  individuals is a binomial probability:

$$P_A(k) = C_N^k (P_A)^k (1 - P_A)^{N-k}$$

### Demographic data analyses

**Relation between diameter and age in *S. torminalis*.** Diameter was measured for each of the 185 individuals at a height of around 1.30 m (diameter at breast height, or d.b.h.) to avoid irregularities caused by the basal development of roots.

For a subset of 34 trees, the relation between d.b.h. and age was assessed by counting the annual rings on wood core samples. Those 34 trees were chosen to represent a large panel of d.b.h. and of stand local densities. One radial increment core per tree was collected 50 cm from the ground. One radial X-ray profile was obtained from each core, and the number of rings on each X-ray profile was counted using WINDENDRO software, following the methods described in Rozenberg *et al.* (2001).

**Spatial patterns of individuals' distribution.** Ripley's  $K$ -function provides a standardized measure for describing the second-order property of a given point pattern at different ranges simultaneously (Ripley 1976). Considering a homogeneous (invariant by translation) and isotropic (invariant by rotation) point process of density  $\lambda$ , Ripley's  $K$ -function is defined so that  $\lambda K(r)$  is the expected number of neighbours in a circle of radius  $r$  centred on an arbitrary point of the pattern (Ripley 1976). The  $K$ -statistic can be used to compare the observed point process to a Poisson process of density  $\lambda$ , which corresponds to the null hypothesis of complete spatial randomness (CSR).

Here we used the modified  $L$ -function, defined by Besag & Diggle (1977) as:

$$\hat{L}(r) = \sqrt{\frac{K(r)}{\pi}} - r$$

This  $L$ -function has a more stable variance than the  $K$ -function, and is easier to interpret:  $L(r) = 0$  under CSR;  $L(r) < 0$  indicates inhibition, i.e. there are fewer neighbours within a distance  $r$  of an arbitrary point of the pattern than expected under CSR, so that the pattern tends to be regular;  $L(r) > 0$  indicates aggregation, i.e. there are more neighbours within a distance  $r$  of an arbitrary point of the pattern than expected under CSR, so that the pattern tends to be clustered. As the theoretical distribution of the estimator of the function  $L(r)$  is unknown, Monte Carlo simulations were used to test significant deviation at the 5% significance level (Besag & Diggle 1977).

Spatial coordinates of 181 trees among 185 were obtained with 1-m resolution using a Trimble GPS unit (Trimble®). Analyses were performed using ADS in the ADE-4 statistical package (<http://pbil.University-lyon1.fr/ADE-4/ADSWebUS.html>; Thioulouse *et al.* 1997). This software integrates edge effect corrections for irregularly shaped sampling plots (Goreaud & Pélissier 1999). We grouped all MUs in division

V (from MU 17 to MU 28, see Fig. 1) to constitute a roughly homogeneous area with respect to ecological and silvicultural factors, and  $L$ -function was computed every 10 m over this 1200-m range.

*Spatial auto-correlation of tree diameter.* We conducted spatial auto-correlation analysis of tree diameter; using the function  $Kcor(r)$  of Goreaud (2000), which is very similar to the  $Kmm$  function of Penttinen *et al.* (1992). The  $Kcor$  function measures the mean correlation between the values of a quantitative variable borne by an arbitrary tree  $i$  of the pattern and one of its neighbours  $j$  within a distance  $r$ :

$$\hat{K}cor(r) = \frac{1}{\hat{Var}(X)} \cdot \frac{1}{N} \sum_i \sum_j (X_i - \bar{X}) \cdot (X_j - \bar{X}) \cdot k_{ij}$$

where  $X$  is a quantitative random variable measured over the  $N$  individuals of the pattern, here the d.b.h. Parameter  $k_{ij}$  is the same weighting factor as in  $K(r)$  that allows an edge-effect correction for irregularly shaped sampling plots (Goreaud & Pélissier 1999).

The distribution of  $Kcor(r)$ -values was tested at a 5% significance level using Monte Carlo simulations of the null-hypothesis of no correlation, obtained by reallocating at random the values of  $X$  over the points of the pattern (Goreaud 2000). The analyses were performed with a computer program written by F. Goreaud and R. Pélissier (available upon request from R.P.).

#### *Spatial component of genetic structure*

The spatial component of fine-scale genetic structure was assessed using a spatial auto-correlation analysis of genetic relatedness computed between pairs of reproducing individuals.

*Kinship coefficients ( $F_{ij}$ ).* The kinship (or coancestry) estimator of Loiselle *et al.* (1995) measures the correlation in frequencies  $p_{ila}$  and  $p_{jla}$  of the homologous allele  $a$  at locus  $l$  in pairs of mapped individuals  $i$  and  $j$ . It is computed as:

$$F_{ij} = \frac{\sum_l \sum_a (p_{ila} - p_{la})(p_{jla} - p_{la})}{\sum_l \sum_a p_{la}(1 - p_{la})} + \frac{1}{n_i - 1}$$

where  $p_{ila}$  is the frequency of allele  $a$  at locus  $l$  in the reference sample,  $n_i$  is the number of genes defined in the sample at locus  $l$  (the number of individuals times the ploidy level minus the number of missing alleles). The denominator of the first term weights allele contributions and allows this estimator not to suffer particular bias in the presence of low-frequency alleles. The second term adjusts for the bias attributable to finite sample size.

Though  $F_{ij}$  is a function of the probability that the two homologous genes are identical by descent, we assumed

that mutation can be neglected relative to migration and genetic drift, and we used identity in state to estimate identity by descent (Rousset 1996). Note that  $F_{ij}$  coefficients are computed relative to the studied population.

*Fraternity coefficients ( $\Delta_{ij}$ ).* To investigate the impact of logging on SPGS (see below), genetic relatedness between individuals was also measured using fraternity coefficients ( $\Delta_{ij}$ ). Whereas  $F_{ij}$  is a 'two-genes' coefficient, in the sense that it is based on comparisons between two homologous genes, the 'fraternity' coefficient  $\Delta_{ij}$  is a 'four-genes' coefficient, based on the simultaneous comparison of all four homologous genes of two diploids under the assumption of random mating (Lynch & Ritland 1999). For two diploids  $i$  and  $j$ ,  $\Delta_{ij}$  is defined as the probability that the two genes of  $i$  are identical by descent to each of the genes of  $j$ . Hence, a positive  $\Delta_{ij}$  coefficient means that there is a double genetic link between  $i$  and  $j$ . In a random mating population,  $F_{ij} = 0.25, 0.25, 0.125$ , and  $\Delta_{ij} = 0, 0.25, 0$ , for a couple  $(i, j)$  being parent-offspring, full-sibs, or half-sibs, respectively.

*Test of isolation by distance.* The hypothesis of isolation by distance was tested by regressing the matrix of pairwise  $F_{ij}$  coefficients against the matrix of the logarithm of the pairwise geographical distances. The overall presence/absence of isolation by distance can be assessed using as statistics the slope of this regression, thereafter denoted  $b$  (Vekemans & Hardy 2004). A randomization procedure was carried out to evaluate the significance of  $\hat{b}$  (by 10 000 random permutations of spatial locations among individuals). We applied the same procedure to the matrix of pairwise  $\Delta_{ij}$  coefficients, and we denoted the slope of the regression of  $\Delta_{ij}$  coefficients against geographical distances as  $b'$ .

Computation of the statistics and randomization procedures was performed using the software SPAGED1, version 0.1, developed by Hardy & Vekemans (2002). SPAGED1 allowed us to obtain approximate standard errors for the multilocus estimates of  $F_{ij}/\Delta_{ij}$  within each distance class using a jackknife procedure that comprises deleting each locus at a time. This assumes that the different loci provide independent replicates of the genetic structure process. Trees issued from vegetative reproduction were eliminated from the analyses. Remaining individuals were grouped into 10–20 distance classes, to ensure a minimum of 30 pairs of individuals per distance class, and a more or less balanced number of pairs.

#### *Impact of gene flow on SPGS*

The slope of the regression of multilocus kinship estimates against the logarithm of the distance provided an indirect estimate of gene dispersal distance (Hardy & Vekemans 1999; Rousset 2000). For outcrossing species in a two-dimensional

space, defining the neighbourhood size as  $N_b = 4 \pi D \sigma^2$ , where  $D$  is the 'effective' population density and  $\sigma^2$  is the variance of gene dispersal distances (distances measured on an axial scale),  $N_b$  can be estimated as:  $N_b = -(1 - F_I) / \hat{b}$ , where  $\hat{b}$  is the estimated slope of the regression of pairwise  $F_{ij}$  on logarithm of distance, and  $F_I$  is Wright's inbreeding coefficient. We took as  $F_I$  estimate the intraindividual value of the kinship coefficient provided by SPAGED1 (Vekemans & Hardy 2004).

Rousset (2000) underlined the fact that the estimate of  $D\sigma^2$  is less biased when only pairs of individuals separated by more than  $\sigma$  are considered in the analysis. As we had no *a priori* knowledge of  $s$ -value in our case, we used a stepwise procedure, in which the first estimated value of  $s$  was used to determine the sample size appropriate for spatial analyses (Rousset 2000). This procedure was repeated until convergence of  $N_b$  estimates.

#### Impact of age structure on SPGS

The relation between d.b.h. and age (see above) was used to group the nonclonal adult trees in temporal groups on the basis of their d.b.h. (see Results). SPGS were then characterized within each cohort, by computing kinship coefficients between pairs of individuals of the same cohort, but relative to the whole population. Within each cohort, the hypothesis of isolation by distance was tested as described above. Comparisons of SPGS among cohorts were based on the confidence interval for the multilocus estimates of  $F_{ij}$  computed within each distance class and within each cohort using the jackknife procedure explained above.

Classical nonspatial analyses of genetic structure were also conducted within and among cohorts using FSTAT (Goudet 1995). We computed the values of  $F_{IS}$  within each cohort and tested their significance through two specific tests (respectively for heterozygote excess or deficiency). Multilocus pairwise estimates of differentiation ( $F_{ST}$ ) between cohorts were estimated by a weighted analysis of variance (Weir & Cockerham 1984). Finally, the genetic homogeneity of the different cohorts was tested through the G-based exact test for population differentiation proposed by Goudet *et al.* (1996).

#### Impact of logging history on SPGS

**Sampling design.** Logging records revealed that some MU of the study site were systematically logged at the same year between 1932 and today (see Appendix for detailed logging history). This allowed us to distinguish eight logging units with more than five *S. torminalis* adult trees (Fig. 1): III-26 (42 trees), V-17 and V-18 (12 trees), V-19 (five trees), V-20 and V-21 (19 trees), V-23 and V-24 (25 trees), V-25 and V-26 (21 trees), V-27 (21 trees) and V-28 (12

trees). Only these 157 trees were used in the following analyses.

**Statistical test.** Kinship coefficients ( $F_{ij}$ ) were computed between pairs of individuals (i) belonging to MU logged in the same year (i.e. within logging units) or (ii) belonging to MU logged in different years (i.e. among logging units). We thus obtained  $F_{ij}$ -correlograms within and among logging units, and their significance was assessed using a randomization procedure and the  $b$ -statistics. To compare  $F_{ij}$ -values within and among logging units, we computed standard errors for the multilocus estimates of  $F_{ij}$  as explained above.

Also, to examine in detail the nature of the genetic links between individuals, we computed fraternity coefficients ( $\Delta_{ij}$ ) between pairs of individuals within and among logging units, we tested the significance of each  $\Delta_{ij}$  correlogram, and we compared them. For these tests,  $F_{ij}/\Delta_{ij}$  values within and among logging units were always computed relative to the whole sample.

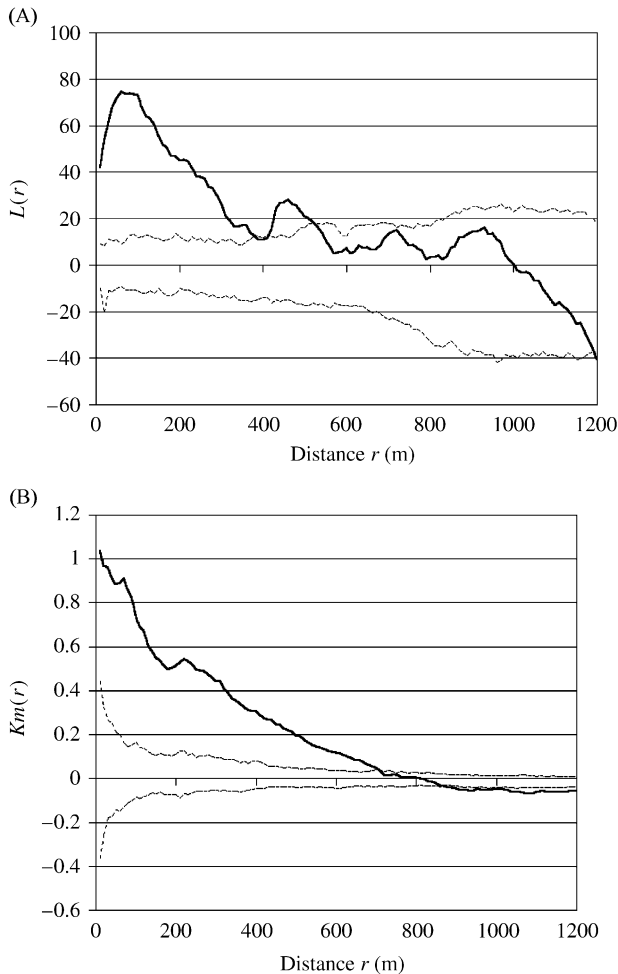
## Results

#### Importance of vegetative reproduction

The six microsatellite markers were highly variable within the study site (Table 1; allelic frequencies can be seen in Oddou-Muratorio *et al.* 2003); although 13 individual trees showed nonunique genotypes, grouped into four genets. In three genets, two to four young stems (around 10 cm d.b.h.) were located in the close neighbourhood (within a 2–16-m radius) of a larger tree (30, 38, or 48 cm d.b.h.). The probabilities of obtaining these groups of individuals by chance ranged from  $3.10^{-30}$  to  $4.10^{-23}$ . In these three cases, we considered the young stems as originating from sprouting from the larger tree, and we thus eliminated these eight young stems from the following analyses. For the last genet, two distant trees shared exactly the same genotype A, with a probability  $P_A(2) = 4.10^{-14}$ . Considering the large distance between these trees (595 m), we excluded the hypothesis of vegetative reproduction, and kept these two individuals in the following analyses.

**Table 1** Observed ( $N_a$ ) and effective ( $A_e$ ) numbers of alleles at each microsatellite locus

Locus	$N_a$	$A_e$
MSS16	21	11.6
MSS9	15	8.8
MSS6	19	8.1
MSS1	12	4.6
MSS5	8	3.6
MSS13	6	3
Mean	13.5	6.62

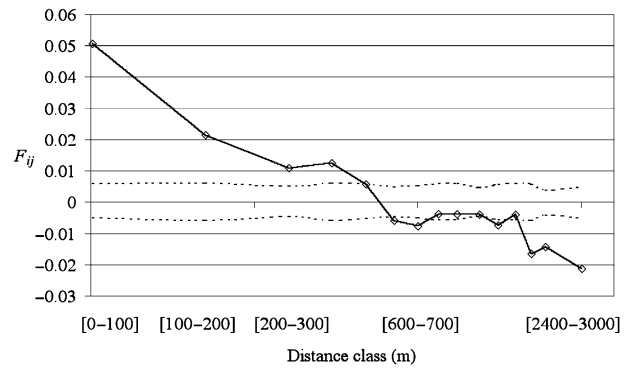


**Fig. 2** Variations of (A)  $L(r)$ -function and (B)  $Km(r)$ -function across division V (encompassing MU from V-17 to V-28). The lines correspond to the observed data. Broken lines correspond to the 95% confidence intervals computed by Monte Carlo simulations under the hypothesis of (A) complete spatial randomness or (B) random distribution of diameter across individual location.

#### Spatial patterns of individual distribution

Across division V, the variations of  $L$ -function depicted a divergence from the null-hypothesis of random distribution of trees (Fig. 2A). Positive  $L$ -values showed that *Sorbus torminalis* distribution tended to be clustered. Moreover, the  $L$ -function reached a primary maximum at c.100 m. A secondary peak was observed at c.450 m, indicating significant aggregation at different scales ranging between these two values. The major peak suggested a predominant aggregate size with a radius of c.100 m.

The variations of the  $K_{cor}$  function showed a significant positive auto-correlation of tree d.b.h. with distance across division V (Fig. 2B): nearest neighbours were more similar in d.b.h. than two randomly chosen individuals.



**Fig. 3** Correlogram of  $F_{ij}$  coefficients across the whole study site (logarithmic scale). Broken lines correspond to the confidence interval at 95% for the null hypothesis of complete spatial randomness of genotypes, constructed by 10 000 permutations of genotypes across individual positions.

#### Impact of gene flow on SPGS

**SPGS across the study site.** Pairwise kinship coefficients were computed between all the 170 mapped individuals showing two-banded genotypes and originating from sexual reproduction (Fig. 3). SPGS was significant overall at the 5% level, as depicted by the slope of the regression of pairwise  $F_{ij}$  against the logarithm of geographical distance ( $\hat{b} = -0.017$ ;  $P < 0.05$ ). The correlogram showed a steady, sharp decrease for distance up to 300 m:  $F_{ij}$  coefficients decreased from 0.054 (SE = 0.006) to 0.014 (SE = 0.007) between the first (0–100 m) and the third (200–300 m) distance classes.

**Gene flow estimate.** The intraindividual value of the kinship coefficient did not reveal a significant departure from Hardy–Weinberg equilibrium. The effective neighbourhood size ( $N_b$ ) was thus simply estimated as  $-1/\hat{b}$ . The iterative procedure yielded a value for  $N_b$  of 63 individuals. Using the census number of reproductive trees (185) within the 472-ha study plot to estimate population density (that is 0.39 individuals per ha), the mean standard deviation of gene dispersal ( $\sigma$ ) was found to be ~360 m.

#### Impact of age structure on SPGS

**Age structure within *S. torminalis* population.** Among the 34 radial increment cores collected for ring counting, 14 had to be eliminated either because they did not include tree heart, or because they were incomplete. The remaining 20 samples yielded the distribution of d.b.h. as a function of age shown on Fig. 4. A logarithmic model fitted relatively well to this observed distribution (d.b.h. =  $16 \times \ln(\text{age}) - 39$ ;  $R^2 = 0.73$ ).

Using this relation, the 170 individuals were arbitrarily partitioned into three temporal groups, thereafter denoted

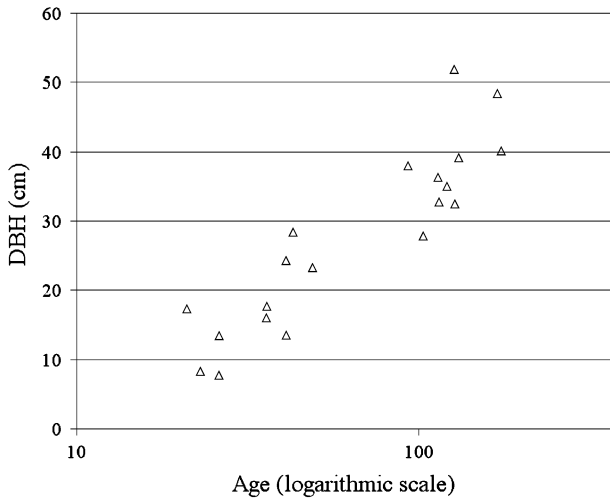


Fig. 4 Relation between diameter and age. Age was estimated for a subset of 29 individuals by counting the annual rings on wood core samples after X-ray radiography.

as cohorts: (i) 60 'young' trees (age < 40 years) with d.b.h. < 20 cm, (ii) 47 'middle-aged' trees (age  $\geq$  40 but < 110 years) with d.b.h.  $\geq$  20 cm but < 37 cm and (3) 63 'old' trees (age  $\geq$  110 years) with d.b.h.  $\geq$  37 cm. In doing this, we assumed that young trees were established after the conversion to high forest.

**Non-spatial genetic structure.** An  $F_{IS}$ -value of  $-0.074$  (significantly negative at the 5% level) was detected in the old cohort, whereas  $F_{IS}$ -values in the young ( $F_{IS} = 0.044$ ) and middle-aged cohorts ( $F_{IS} = -0.023$ ) were not significantly different from zero. Based on Goudet's  $G$ -test (Goudet *et al.* 1996), differentiation between cohorts was overall significant at the 5% level but considering pairwise estimates, only young and old cohorts were significantly differentiated ( $P < 0.1\%$ ), with a multilocus estimate of  $F_{ST}$  of 1.3%.

**SPGS within cohorts.** SPGS was overall significant at the 5% level within each cohort:  $\hat{b}_{YOUNG} = -0.016$ ;  $\hat{b}_{MEDIUM} = -0.016$ ; and  $\hat{b}_{OLD} = -0.018$ . The global shape of the correlogram was similar across cohorts (Fig. 5), with significant  $F_{ij}$ -values only in the two first distant classes. However,  $F_{ij}$ -values differed slightly among cohorts within these two first distant classes. Below 200 m, pairs of 'old' individuals were on average less related ( $\bar{F}_{ij} = 0.03$ ) than pairs of 'young' or 'middle-aged' individuals ( $\bar{F}_{ij} > 0.05$ ). Moreover, between 200 and 400 m, pairs of 'young' individuals were less related than expected by chance ( $\bar{F}_{ij} = -0.01$ ) whereas pairs of 'middle-aged' or 'old' individuals remained more related than expected by chance ( $\bar{F}_{ij} > 0.02$ ).

Indirect estimates of gene flow were derived for each cohort using  $\hat{b}_{YOUNG}$ ,  $\hat{b}_{MEDIUM}$  and  $\hat{b}_{OLD}$ , and taking  $F_{IS}$ -values provided by  $F_{STAT}$  to estimate Wright's inbreeding

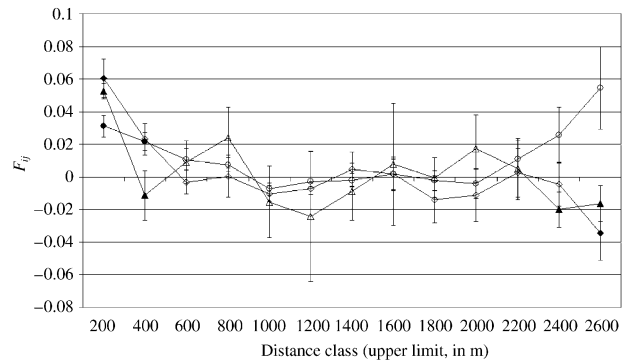


Fig. 5 Correlograms of  $F_{ij}$  coefficients for young ( $\Delta$ ,  $\blacktriangle$ ), middle-aged ( $\diamond$ ,  $\blacklozenge$ ) and old ( $\circ$ ,  $\bullet$ ) cohorts. Abscissa values correspond to the upper limit of the 200-m-wide distance intervals. Filled symbols represent values significantly different from the expected value under a random distribution of genotypes (95% confidence level). Confidence intervals around each  $F_{ij}$ -value were obtained through a jackknife procedure over loci.

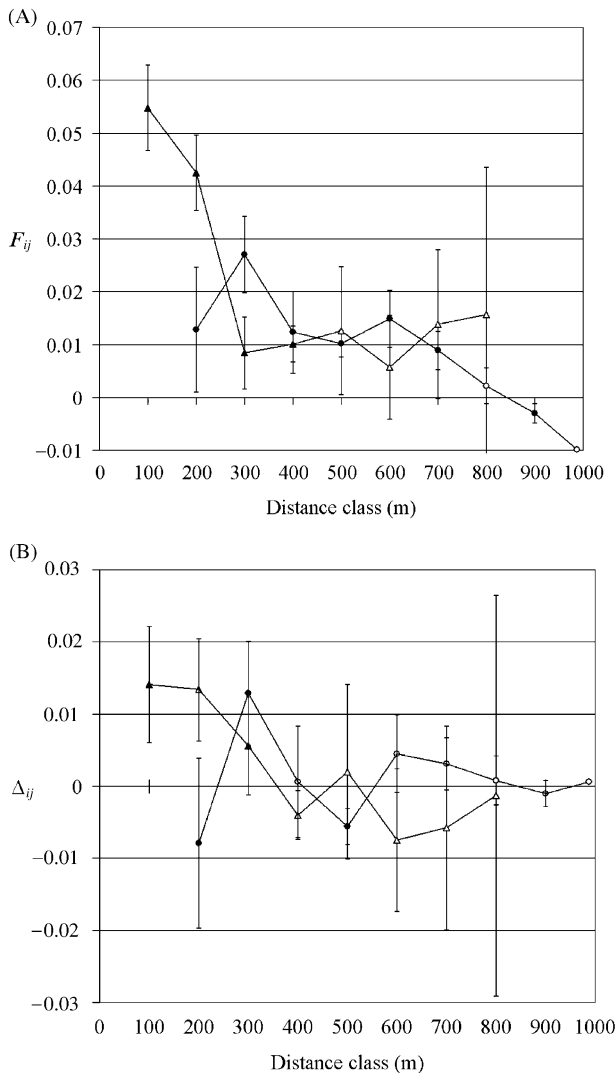
coefficient. Estimated values of  $N_b$  were 58, 63 and 59 individuals, respectively, in young, middle-aged and old cohorts. Still using the census number of reproductive trees to estimate population density (that is 0.39 individuals per ha), these  $N_b$  estimates converted into  $\hat{\sigma}_{YOUNG} \sim 345$  m,  $\hat{\sigma}_{MEDIUM} \sim 360$  m and  $\hat{\sigma}_{OLD} \sim 350$  m (old cohort).

#### Impact of logging history on SPGS

The kinship coefficients of Loiselle *et al.* (1995) (Fig. 6A) revealed significant SPGS at the 5% level both within ( $\hat{b}'_{WITHIN} = -0.018$ ) and among logging units ( $\hat{b}'_{AMONG} = -0.020$ ). However, the shapes of the correlograms significantly differed within and among logging units. The pattern of pairwise  $F_{ij}$  within logging units was very similar to that of the whole population. In contrast, pairwise  $F_{ij}$  computed among logging units revealed low levels of relatedness among individuals separated by 100–200 m, and high levels of relatedness among individuals separated by 200–300 m. The average  $F_{ij}$ -value among logging units was not computed for the first distance class, because the sample included only three pairs of individuals with distances < 100 m that belonged to different logging units. Similarly,  $F_{ij}$ -values were not computed within logging units for distances > 800 m.

Using the fraternity coefficients of Lynch & Ritland (1999; Fig. 6B), we also observed significant SPGS at the 5% level both within ( $\hat{b}'_{WITHIN} = -0.008$ ) and among logging units ( $\hat{b}'_{AMONG} = 0.002$ ). The surprising positive value of  $\hat{b}'_{AMONG}$  was probably caused by the very low  $\Delta_{ij}$ -value ( $\Delta_{ij} = -0.007$ ) observed in the 100–200 m distance class, combined with the very high  $\Delta_{ij}$ -value ( $\Delta_{ij} = 0.013$ ) observed in the 200–300 m distance class. The correlogram of  $\Delta_{ij}$  coefficients within logging units was more consistent with classical patterns of isolation by distance, with higher





**Fig. 6** Correlograms of  $F_{ij}$  coefficients (A) and  $\Delta_{ij}$  coefficients (B) computed within logging units ( $\Delta$ ,  $\blacktriangle$ ) and among logging units ( $\circ$ ,  $\bullet$ ). Abscissa values correspond to the upper limit of the 100-m-wide distance intervals. Confidence intervals around each  $F_{ij}$ -value were obtained through a jackknife procedure over loci.

levels of relatedness at short distances ( $\Delta_{ij} = 0.014$  and  $0.013$ , respectively, in the first two distance classes).

We translated these  $\Delta_{ij}$ -values into expected proportions of full-sibs, assuming that  $\Delta_{ij} = 0.25$  corresponds to 100% full-sibs. The observed  $\Delta_{ij}$ -values of  $\sim 0.014$  indicated roughly the same proportions of full-sibs (5.9%) within logging units (in the two first distance classes) as among logging units (in the third distance class).

## Discussion

This study illustrates that SPGS in this population of *Sorbus torminalis* is the result of various genetic and demographic

processes, among which demographic instability plays an important role.

### Vegetative reproduction is limited

A prerequisite for the investigation of SPGS is an evaluation of the respective importance of vegetative vs. sexual reproduction in the species under study. Indeed, these two mechanisms of gene dispersal have specific effects on SPGS, and should thus be accounted for separately (Epperson 2000).

Microsatellite markers are a powerful tool with which to assess vegetative reproduction, because the probability  $P(k)$  that  $k$  sexually reproduced individuals have the same multilocus genotype at such highly variable markers is extremely low (here it was always below  $10^{-14}$ ). However, we did not reject the hypothesis of sexual reproduction for two ramets located  $\sim 600$  m apart and showing a  $P(k)$ -value of  $10^{-14}$  (the highest value found). Indeed, the hypothesis of successive vegetative propagation from a single individual appeared unrealistic in that case. It is also possible that these two trees originated from apomictic seeds produced by the same mother tree, though apomixis is unlikely to be very efficient in *S. torminalis* (Rasmussen & Kollmann, 2004). Apomixis has been found several times in the *Sorbus* genus (e.g. Nelson-Jones *et al.* 2002) but preferentially for the hybrid species of this group. However, keeping these two ramets among the 'sexually reproduced' individuals did not markedly change the results of analyses (results not shown).

When using both genotypes and spatial distribution of trees to detect clones, only eight individuals among 185 were found to originate from vegetative reproduction ( $< 4\%$ ). This is a small number, given the extensive abilities of basal stem off-shoots and sprouting reported for *S. torminalis* (Rameau *et al.* 1989). Vegetative reproduction thus appeared particularly limited in the studied population, perhaps because low-density stands allowed the successful establishment of sexually reproduced seedlings. However, it is also possible that the present trees are the lone surviving stems of a genet whose other ramets disappeared during stand evolution. Only specific investigations of vegetative reproduction and of its ecological determinants (such as levels of inter- and intraspecific competition) could fully address this issue.

### Despite high gene flow, low population density resulted in strong genetic structure

Spatial auto-correlation of kinship coefficients computed between pairs of sexually reproduced individuals revealed a typical isolation-by-distance pattern. The strength of isolation by distance can be evaluated comparatively through the  $S_p$ -statistics proposed by Vekemans & Hardy (2004)

and defined as  $-\hat{b}/(1-F_{(1)})$ , where  $\hat{b}$  is the regression slope of pairwise  $F_{ij}$  coefficients against logarithm of distance ( $\hat{b} = -0.017$  in *S. torminalis* across the study site) and  $F_{(1)}$  is the average  $F_{ij}$  value at short distance. In the light of the  $Sp$ -based SPGS review performed by Vekemans & Hardy (2004), SPGS in *S. torminalis* ( $Sp \sim 0.017$ ) appears to be relatively strong compared to other tree species ( $\bar{Sp} = 0.010$ ) and to other plant species with animal-dispersed seeds ( $\bar{Sp} = 0.009$ ), but is consistent with other entomophilous plant species ( $\bar{Sp} = 0.017$ ).

Because of low population density, the rather strong values for SPGS observed in *S. torminalis* were nevertheless associated with high levels of gene dispersal, as depicted by  $\hat{\sigma} \sim 360$  m. Note that this  $\hat{\sigma}$ -value was probably an underestimate because it was computed using the observed tree density, which is likely to be an overestimate of the effective population density ( $\hat{d}_e$ ). This high  $\hat{\sigma}$ -value is consistent with the pollen dispersal abilities observed in *S. torminalis* in a companion study (Oddou-Muratorio *et al.* submitted for publication). Indeed, assuming either a Gaussian or exponential model for pollen dispersal, reasonable estimates of the standard deviation of pollen dispersal distance ( $\sigma_p$ ) ranged between  $\sim 200$  m and  $\sim 400$  m. Considering that in our hermaphrodite, outcrossing species,  $\sigma^2 = 0.5\sigma_p^2 + \sigma_s^2$ ,  $\sigma_s^2$  being the variance of seed dispersal (Crawford 1984), a  $\sigma_s$ -value between  $\sim 200$  m and  $\sim 300$  m would explain the observed SPGS. These rough estimates reflect similar dispersal abilities for seed and pollen, which is consistent with a previous survey of SPGS in *S. torminalis* on a large geographical scale (Oddou-Muratorio *et al.* 2001a).

Our results thus illustrate that high dispersal abilities can be more than counterbalanced by low population density, resulting in rather strong levels of spatial genetic structure. Though this connection between population density and SPGS is explicitly formulated for instance in the model of Rousset (2000), it is interesting to note that these patterns have a strong biological basis, as direct measures of pollen dispersal showed that pollinator flight distance increases when population density decreases (e.g. Dick 2001).

#### SPGS changed across temporal cohorts

In long-lived species with overlapping generations, partitioning the genetic structure across the temporal dimension can provide clues as to the causes of SPGS (Kalisz *et al.* 2001). We used the age–diameter relationship assessed on a subset of 20 trees to partition all individuals into three temporal cohorts. Moreover, we neglected the effects of local environmental conditions on tree diameter, though spatial auto-correlation of tree diameter may reflect similar growing conditions and stand density for nearer-neighbours than for more distant pairs. In addition, our

'cohorts' included a wide range of ages (from 40 to > 70 years), and the limit between the middle-aged and old cohorts was arbitrary. Despite these limitations, our results showed that this partition was pertinent to the analysis of SPGS.

First, these temporal groups showed a clear differentiation of allelic frequencies, though only the young and old cohorts exhibit a significant  $F_{ST}$  of 1.3%. Assuming neutrality for microsatellite markers, these temporal changes in allelic frequencies could result from various forms of non-random mating; such as the variation of individual reproductive success among years, or the differences in relative recruitment of immigrant vs. resident individuals.

Second, a slightly positive (though nonsignificant) fixation index was detected in the young cohort, whereas the negative  $F_{IS}$ -value observed in the old cohort indicated a significant heterozygote excess. Such patterns of increasing heterozygosity with age were reported in various tree species (Bush & Smouse 1992), and are generally acknowledged to result from selection acting against inbred genotypes.

Finally, spatial analyses revealed contrasted SPGS among cohorts. The lower level of relatedness at short distances in old cohorts may indicate that selection against the inbred genotype became smoother in the later generations, because of efficient elimination of deleterious alleles. As compared to the smoothly decreasing correlograms in the middle-aged and old cohorts, the sharp decrease of kinship values in the young cohort between 0 and 200 m ( $\bar{F}_{ij} = 0.05$ ) and 200–400 m ( $\bar{F}_{ij} = -0.01$ ) is likely to reflect the change in silvicultural system. Indeed, the young cohort theoretically includes trees that were established after the conversion to high forest in 1960, whereas trees from old and middle-aged cohorts were all established before this conversion. Our results thus suggest that as compared to the coppice-under-reserve system, the conversion to high forest may hamper the successful establishment of seedlings at distances > 200 m from the seed trees. It would thus be interesting to conduct a similar study in a long-established high forest, to test whether this silvicultural system really leads to higher genetic structuring among logging units.

#### Spatial aggregation of individuals reflected patterns of seedling establishment

Ripley's method of analysis (Ripley 1976) detected a significant spatial aggregation of trees. The predominant level of aggregation ( $\sim 100$  m) grouped highly genetically related trees, as depicted by SPGS. The lower size of individual aggregates is usually interpreted as the seed dispersal distance from a single maternal tree (Ueno *et al.* 2002; Latouche-Halle *et al.* 2003). In our case, this would indicate lower abilities of seed dispersal than reflected by SPGS ( $200 \text{ m} < \hat{\sigma}_s < 300 \text{ m}$ ). One could also argue that SPGS-based  $\hat{\sigma}$ -estimates usually show low precision

(Rousset 2000). In addition, it is likely that the abilities of seed dispersal are not the only factor shaping the spatial distribution of individuals.

Indeed, various postdispersal processes control seedling establishment and mortality, in particular in animal-dispersed species. In a related species with similar life-history traits, *Prunus mahaleb*, Godoy & Jordano (2001) showed that effective patterns of seed dispersal were markedly affected by landscape features, in particular the availability of favourable patches for seedling establishment, and by the nonrandom movements of frugivores, which strongly select microhabitats. These authors observed a thrush species that typically flies to perch on pine trees after feeding on *P. mahaleb*. *Sorbus torminalis* is also strongly dependent on frugivores for seedling establishment, because the transit of the fruit through the animal intestine probably allows the seed to acquire germination capacity (Oddou-Muratorio *et al.* 2001a). Thrushes feeding on *S. torminalis* are thought to perch on large oaks, as suggested by the abundance and grouping of young seedlings under them (S. Oddou-Muratorio, personal observation).

It is thus likely that the ~100-m patches of *S. torminalis* individuals did not reflect dispersal abilities, but rather patterns of frugivore movements and the patchy distribution of favourable sites for seedling establishment. Moreover, the strong spatial auto-correlation of tree diameter showed that these ~100-m patches correspond to even-aged groups of individuals. These spatio-temporal patterns suggest that ecological constraints primarily determine the opening of favourable sites for seed delivery and germination, that are then colonized through a distance-limited seed rain from adjacent mature trees.

#### Logging cycles decreased genetic structure among logging units

We investigated whether human management could contribute to this pattern of seedling recruitment in ~100-m patches of related individuals. In particular in a mixed silvicultural treatment with coppice overtopped by reserve trees, we suspected that coppice removal could open favourable sites for seedling establishment, while the persistence of reserve oak trees would ensure protective shade for seedlings to develop, and would also provide thrushes with somewhere to perch.

Under this scenario, we expected higher levels of relatedness within than among logging units, because of preferential mating and recruitment within logging units. Our results clearly support this expectation. However, we also showed that logging events did not lead to strong differentiation among logging units. In particular, the significant isolation-by-distance patterns observed among logging units showed that trees established on different logging units shared the same parents, and thus belonged to the

same reproductive unit. In addition, we even observed higher levels of relatedness (as expressed by the kinship coefficient  $F_{ij}$ ) among than within logging units for pairs of individuals in the 200–300 m distance class, and using the fraternity coefficient  $\Delta_{ij}$ , we could estimate that ~5.9% of these pairs were full-sib pairs.

Note however, that these  $\Delta_{ij}$ -values should be considered with caution. Indeed, as  $\Delta_{ij}$  coefficients were computed assuming a random mating population, the conversion of  $\Delta_{ij}$ -values into proportions of full-sibs is subject to bias because of the complex patterns of family relationships in the long-lived species under study. For instance, in the case of two offspring from the same mother tree but sired by two different males we would expect:  $\Delta_{ij} = 0$  (half-sibs); but if the two male parents are full-sibs, the expected  $\Delta_{ij}$ -value becomes 0.125. Therefore, we only used  $\Delta_{ij}$ -values for comparative purposes, which allowed us to conclude that the proportion of full-sibs could be as high among as within logging units.

Observed SPGS within and among logging units supported our hypotheses that logging events favour *S. torminalis* establishment. First, higher levels of relatedness were observed for pairs of individuals within logging units in the first two distance classes. In the distance class 100–200 m in particular, a high  $F_{ij}$ -value ( $\bar{F}_{ij\text{WITHIN}} = 0.043$ ) was associated with a significantly positive  $\Delta_{ij}$ -value, indicating sib-relationships. By contrast, the corresponding lower  $F_{ij}$ -value among logging units ( $\bar{F}_{ij\text{AMONG}} = 0.013$ ) was associated with a significantly negative  $\Delta_{ij}$ -value, excluding sib-relationships. This result can be understood as follows: when a MU 'A' was logged, and favourable sites for *S. torminalis* were opened, the trees that were given the best opportunities to send seeds to these patches were (i) the nonlogged mature trees of 'A' and (ii) trees on the border of adjacent nonlogged MU 'B'. In case (ii), we expect parent-offspring relationships across logging units, and sib-relationships within logging units, as depicted by the correlogram in the first two distance classes.

Following this scheme, the values of  $F_{ij}$  and  $\Delta_{ij}$  in the 200–300 m distance class can be interpreted as follows: within logging units, the probability of sib-relationship decreased rapidly with the distance between individuals because of preferential seed dispersal in the neighbourhood (resulting in  $\bar{\Delta}_{ij\text{WITHIN}} = 0.006$ ). By contrast, the higher  $F_{ij}$ -values among logging units ( $\bar{F}_{ij\text{AMONG}} = 0.027$  vs.  $\bar{F}_{ij\text{WITHIN}} = 0.008$ ) resulted in part from a parent-offspring relationship that persists at greater distance, because of long-distance pollen dispersal and low parent density. Moreover, this high  $F_{ij}$ -value was also contributed to by sib-relationships, as depicted by the significant positive  $\Delta_{ij}$ -value ( $\bar{\Delta}_{ij\text{AMONG}} = 0.013$ ) which can be explained as follows: if logging occurred in turn on MU 'B' within the next few years, we would expect seedlings establishing on MU 'B' to be sired by the same parents as those established on MU 'A'.

## Conclusions

Our results showed that a diverse set of genetic and demographic processes contribute to the marked SPGS detected in *S. torminalis*. First, SPGS classically reflected the balance between high dispersal abilities (for both seed and pollen) and low adult density. In addition, this study highlighted the original role of logging cycles and seedling recruitment in a colonizing, animal-dispersed species. In the case of *S. torminalis*, it is probable that logging cycles, combined with landscape features, primarily shape the distribution of favourable sites, which are then colonized by sib cohorts as a result of joint seed transportation and deposition by frugivores. Considerable seed dispersal abilities, combined with low tree densities, allow seed-trees to colonize adjacent logging units, resulting in similar genetic structuring among and within logging units.

The spatial and temporal distribution of logging events thus appears as a major tool for forest managers to promote high levels of genetic diversity in *S. torminalis* across management units. Our results suggest that changes in logging cycles directly affect SPGS, as detected in the young cohort which became established during stand conversion to high forest.

Besides demographic instability, temporal stochasticity is also likely to shape SPGS. Indeed, trees are among the species most susceptible to showing highly stochastic patterns of reproductive success, because they combine a long life cycle with massive production of seeds. Thus, the genetic composition of seedlings colonizing a favourable site may be significantly unbalanced by the massive contributions of trees that have, by chance, produced significant numbers of seeds at the time of the site opening.

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## Supplementary material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2373/MEC2373sm.htm>

**Table S1.** Silvicultural treatment and logging history within management units (MU) MU logged in the same years were grouped in the same logging units (see Fig. 1). For each logging

unit, we give the present number of individuals (*N*) used for spatial auto-correlation analyses.

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This study was performed at the Genetic Conservatory of Forest Trees of the French National Forest Office. Sylvie Oddou-Muratorio is particularly interested in human impact on the population genetics of forest trees. Brigitte Musch is a population geneticist at ONF working on forest trees. Raphaël Pélissier is a (tropical) forest ecologist particularly interested in methodological aspects. Pierre-Henri Gouyon is interested in evolutionary processes in natural populations of various organisms.

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**Appendix**

Silvicultural treatment and logging history within management units (MU). MU logged in the same years were grouped in the same logging units (see Fig. 1). For each logging unit, we give the present number of individuals (*N*) used for spatial auto-correlation analyses.

Logging unit	Logging year	Silvicultural treatment	Logging purpose	<i>N</i>
III-26	1951	Coppice under reserve trees	Coppice removal + reserve thinning	42
	1965	Even-aged stand	Thinning	
V-17 and 18	1939	Even-aged stand	Thinning	12
	1965			
V-19	1941	Even-aged stand	Thinning	5
	1966			
V-20 and 21	1941	Coppice under reserve trees	Coppice removal + reserve thinning	19
	1967	Even-aged stand	Thinning	
V-23 and 24	1944	Coppice under reserve trees	Coppice removal + reserve thinning	25
	1968	Even-aged stand	Thinning	
V-25 and 26	??	Coppice under reserve trees	Coppice removal + reserve thinning	21
	1969	Even-aged stand	Thinning	
V-27	1937, 1941, 1957, 1970	Even-aged stand	Pine thinning	21
	1970	Even-aged stand	Thinning	
V-28	1970	Even-aged stand	Thinning	12