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1 **Fine-scale spatial genetic structure and intra-specific interactions of *Populus nigra* within a natural river**  
2 **corridor along the lower Allier River (France)**

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4  
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40

41 **Abstract**

42 Spatial genetic structure (SGS) studies contribute to our understanding of gene flow and species dispersal. Only a  
43 few studies have linked the spatio-temporal pattern of SGS and intra-specific interactions. Black poplar (*Populus*  
44 *nigra* L.) is a threatened pioneer riparian tree species along many rivers across Europe. We studied its SGS in  
45 cohorts of varying ages at a fine-scale (i.e. at distances including the zone of influence between individuals) to  
46 better understand local patterns of dispersal and intra-specific interactions during early life-stages. We genotyped  
47 349 *P. nigra* individuals in three gravel bars along a 1.6 km reach of the Allier River in the Réserve Naturelle du  
48 Val d'Allier, central France. We found high genetic diversity values ( $H_e = 0.860$ ) thus identifying this site as  
49 important for the conservation of *P. nigra* genetic diversity. We also found significantly more clones in locations  
50 exposed to the river flow compared with less exposed locations. These clones offer mechanical protection and  
51 habitat improvement (through facilitation) to individuals in close proximity. A significant fine-scale SGS was  
52 observed in the youngest cohort at the gravel bar scale while none was observed in the older cohorts or at the river  
53 reach scale. This pattern was confirmed by a reanalysis of published data on the Garonne River. A shift in intra-  
54 specific interactions, from facilitation to competition through self-thinning, could explain the loss of SGS in older  
55 cohorts. This highlights the importance of shifts in intraspecific interactions through life and their consequence on  
56 population genetic structure.

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63           **1. Introduction**

64           Spatial genetic structure (SGS), defined as the non-random spatial distribution of genotypes, characterizes  
65 the relationship of relatedness estimates between pairs of individuals and their physical proximity (Loiselle et al.,  
66 1995; Hardy, 2003) with positive spatial genetic structure commonly interpreted as a sign of restricted gene flow.  
67 Thus, patterns of dispersal of pollen and seeds have important consequences for the SGS (Banks et al., 2013).  
68 However, density-dependent, biotic interaction processes may alter the SGS of plant populations generated by  
69 dispersal. For example, the thinning process in maturing forests may change their genetic composition and  
70 structure by removing groups of siblings (Yoda et al., 1963; Harper, 1977). Biotic interactions are likely to change  
71 over life stages, especially when the environment changes as well. The stress gradient hypothesis (Bertness and  
72 Callaway, 1994) predicts that different stress levels will change the type of interaction between individuals from  
73 positive (i.e. facilitation in stressful environments) to negative (i.e. competition in benign environments). As most  
74 plants are not capable of movement, they will be in contact with their direct neighbours throughout their lifetime,  
75 and plant interactions occur where plants overlap their ‘zones of influence’, e.g. among immediate neighbours at  
76 fine spatial scales (Stoll and Weiner, 2000). In addition, the SGS pattern could in turn influence what type of biotic  
77 interaction will take place among the immediate neighbours on plants (Fajardo et al., 2016). Few studies have  
78 linked intra-specific interactions and the pattern of SGS within populations (Till-Bottraud et al. 2012; Segovia et  
79 al., 2015). Studies of SGS in plants generally address large geographic areas (Hardy et al., 2005; Kettenring et al.,  
80 2019; González-Robles et al., 2020) and sampling often neglects the scale of the “zone of influence” of the studied  
81 species. Over smaller areas, SGS is often referred to as “fine scale genetic structure” (FSGS), but the smallest  
82 distances studied vary greatly among plant types. In herbaceous plants, studies are at the scale of metres (Ning et  
83 al., 2018; Loh et al., 2020) whereas in trees it is usually the scales of kilometres (Sagnard et al., 2011; Born et al.,  
84 2008; Deng et al., 2020). In the past few years, FSGS studies in trees have been conducted on smaller scales  
85 (Kitamura et al., 2018; Twyford et al., 2020) but little is known at the scale of a few meters or less. In the following,  
86 we will use “fine-scale spatial genetic structure” (FSGS) to refer to scales including the distance at which  
87 individuals interact with each other (i.e. including the zone of influence).

88           *Populus nigra* L. (Salicaceae), the black poplar, is a pioneer tree growing in natural river corridors in  
89 Europe. It is considered as a priority for research and conservation because human activities have severely reduced  
90 population sizes and increased population isolation (Lefèvre et al., 1998; Villar and Forestier, 2006). Additionally,  
91 poplar populations in their early life stages (<15 years) can have a significant effect on hydrogeomorphological  
92 processes, fluvial landforms and related habitat conditions by trapping huge amounts of fine sediment, nutrients  
93 and organic matter (Hughes and Rood, 2003; Corenblit et al., 2016a). This ecosystem engineering effect (*sensu*  
94 Jones et al., 1994) is maximised where saplings grow in high densities. Sediment and organic material accretion  
95 improve resistance to mechanical stress but also improve poplar survival and growth (Corenblit et al., 2014). Intra-  
96 specific facilitation is supposed to be a major driver at the establishment stage in densely populated recruitment  
97 bands or patches (Barsoum, 2002; Corenblit et al., 2014, 2018), and may correspond to a positive niche  
98 construction effect *sensu* Odling-Smee et al. (2003). In this context, the fine scale genetic structure could provide  
99 valuable information on the colonization process of alluvial bars by *P. nigra* within the fluvial corridor. Moreover,  
100 the feedbacks between FSGS and intra-specific interactions and their impact on seedling establishment and  
101 population genetic diversity and structure remain unexplored.

102           The aims of this study were to understand local patterns of dispersal and intra-specific interactions under  
103 natural conditions during the early life-stages of *P. nigra*. To do so, we explored the FSGS (at a meter scale or  
104 less) of three different cohorts of black poplar over a 1.6 km reach of the Allier River (France) located in a nature  
105 reserve. We used microsatellite markers that allowed us to estimate the genetic diversity of the population. As  
106 vegetative propagation was identified in the population, we investigated its geographic distribution, in particular  
107 in relation to topography using a digital elevation model (DEM) of this reach of the Allier River. The originality  
108 of the present study lies in the very fine scale (metric) genetic structure approach combined with the comparison  
109 between different cohorts.

110

## 111           **2. Material and Methods**

### 112           **2.1. Study Species**

113 *Populus nigra* L. (Salicaceae) is a fast-growing tree species with a good tolerance to submersion, sediment burial  
114 and high temperatures (Chamaillard, 2011). The species is dioecious and wind pollinated. Seed dispersal combines  
115 a wind-mediated phase often followed by a secondary hydrochorous phase (Barrat-Segretain, 1996; Karrenberg  
116 and Suter, 2002). The seeds are short-lived (they cannot survive more than a few weeks), and germination only  
117 occurs immediately after arrival on suitable bare moist alluvial bars (Barsoum and Hughes, 1998). As a pioneer  
118 species, the eco-hydrological conditions for regeneration include freshly disturbed, competitor-free sediments.  
119 Prime seedling recruitment habitat in the active alluvial zone occurs at the immediate margins of the main or  
120 secondary channels and is highly dependent on water table level fluctuations (Corenblit et al., 2014). Harsh  
121 summer (drought) and winter (submersions, erosion and/or burial) conditions on alluvial bars allow seedlings to  
122 establish only during restricted time periods, not necessarily every year, and on areas of the alluvial bars that are  
123 close enough to the water resource to permit the roots to track the water table during the dry season (i.e. recruitment  
124 box *sensu* Mahoney and Rood, 1998; Barsoum and Hughes, 1998; Guilloy et al., 2011; Stella and Battles, 2010).  
125 Suitably positioned seedlings on alluvial bars near the main channel generally form more or less elongated  
126 recruitment bands or patches composed of individuals of the same age (a cohort). Such spatial patterns suggest  
127 that a significant proportion of seeds are transported by the river. Following the dispersal and early establishment  
128 phase, subsequent sapling development (first 3 years according to Cooper et al., 1999) is governed by strong  
129 feedback mechanisms between plants and hydro-geomorphological processes (i.e. water flow, sediment transport  
130 and landform construction).

131           *P. nigra* is additionally capable of vegetative reproduction: one individual can produce multiple shoots,  
132 or ramets, which all share the same multilocus genotype (MLG, *sensu* Arnaud-Haond et al., 2007). From here on,  
133 we will use ramets to refer to the different shoots originating from one individual MLG. In highly dynamic rivers,  
134 vegetative reproduction can be a significant alternative mode of recruitment (Barsoum et al., 2004; Francis et al.,  
135 2004) and can complement sexual reproduction in colonizing new areas (Tinschert et al., 2020). However,  
136 vegetative reproduction is reported to be a primarily local strategy, especially in disturbed environments (Arens et  
137 al., 1998; Smulders et al., 2008).

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142 **2.2. Study site**

143 The study site was located along the lower reaches of the Allier River in the Réserve Naturelle Nationale du Val  
144 d'Allier, near Châtel-de-Neuvre (Fig. 1; 46°25'06.5"N, 003°19'43.2"E; 220 m a.s.l.) in central France. Contrarily  
145 to most European rivers, the flow regime along the lower reaches of the Allier River is largely unregulated and  
146 morphodynamics within the Nature Reserve are only moderately impacted by human activities (Garófano-Gómez  
147 et al., 2017). Bank erosion in the outer bends of meanders and recurrent active channel migration promote the  
148 formation of bare gravel bars suitable for *P. nigra* regeneration. The progressive shifting of the river channel  
149 allows for periodic recruitment episodes, resulting in different aged cohorts of *P. nigra* recruits growing in  
150 elongated bands parallel to the main channel (Hortobágyi et al., 2017).

151  
152 **2.3. Sampling strategy**

153 We sampled same-age individuals over very short distances within recruitment bands. Three gravel bars were  
154 selected for this study along a 1.6 km reach of the Allier River. We sampled 21 groups of 15-20 individuals. This  
155 sampling strategy ensured that we incorporated a broad range of distances between individuals: from a few  
156 centimetres to 1.6 km (Fig. 1). The mean distance between individuals from a same patch was 12.33 m. Each  
157 individual was georeferenced in the field using a GPS Trimble GEO7X™ with a decimetric precision. Groups of  
158 individuals were categorised into three age cohorts: 'young' (less than 5 years old), 'middle-aged' (approximately  
159 10 years old) and 'old' (more than 20 years old), based on their appearance in aerial photographs (we used all the  
160 available aerial photographs, i.e. 1992, 1997, 1998, 2002, 2009, 2013 and 2016). During the sampling period in  
161 November and December 2017, we collected cuttings for DNA extraction and tree genotyping since no leaves  
162 were available in winter. We collected cuttings from a total of 28 'young' individuals, 276 'middle-aged'  
163 individuals and 44 'old' individuals (total of 348 individuals sampled). Cuttings were wrapped in wet tissue and  
164 stored in the laboratory at 4°C for 3 weeks prior to DNA extraction.

165  
166 **2.4. DNA extraction and genotyping**

167 Cambium fragments were collected from the cuttings in the laboratory and stored in silica gel to promote rapid  
168 tissue desiccation, thereby avoiding DNA degradation. We used 8 unlinked codominant microsatellites (SSR)  
169 markers to genotype individuals: WPMS13, WPMS22 (Smulders et al., 2002), PMGC2385, PMGC93,  
170 PMGC2578, PMGC14, GCPM2995 and ORPM221 (Chenault et al. 2011; Faivre-Rampant et al. 2016). Markers  
171 were arranged in 2 multiplexes. Multiplex1: ORPM221, GCPM2995, WPMS13, WPMS22; Multiplex2: PMGC93,  
172 PMGC2385, PMGC2578, PMGC14. We used indirect tagging with M13-tailed primer method (Oetting et al.,  
173 1995). In this method, instead of synthesizing one specific fluorescently labelled primer for each SSR marker, only  
174 a dye-labelled M13 primer is needed.

175 The polymerase chain reaction was carried out in a volume of 10 µL, which contained 2.5 µL of DNA (at  
176 10 ng/µL) and 7.5 µL of the following mix: 5 µL Master mix (AmpliTaq Gold™ 360), 0.5 µL primer mix (labelled  
177 with M13 fluorochromes Applied Biosystems™ 6-FAM, NED, PET and VIC), 0.05 µL tagged M13 primer and  
178 1.95 µL H<sub>2</sub>O. The profile of the touchdown phase with 7 cycles was as follow: 95 °C for 30s, 62 °C for 30s and  
179 72 °C for 30s, with a temperature reduction of -1 °C per cycle. The 30 classical PCR cycles were as follow:

180 denaturation at 95 °C for 30s, annealing at 55 °C for 30s and extension at 72 °C for 30s. The M13 tail and  
181 fluorochromes were bound during 8 cycles with the following thermal profile: 95 °C for 30s, 56 °C for 30s and 72  
182 °C for 30s. The final extension step was 72 °C for 5 min. The PCR products were run in an automatic sequencer  
183 (96 capillars 3750xl DNA Analyser Applied Biosystems™). Electropherograms were analysed using  
184 GeneMapper™ v.5. To test reproducibility, 16 randomly chosen individuals were replicated in the genotyping. No  
185 error was detected.

186

## 187 **2.5. Genetic analyses**

188 Two individuals were removed because of missing data for two loci. Loci were tested for null allele frequencies  
189 with Brookfield's methods (Brookfield, 1996) using Microchecker v2.2.3 (Van Oosterhout et al., 2004). The  
190 GCPM2995 marker presented a high null-allele frequency (Table 1) and was therefore removed from the dataset.  
191 To estimate the discrimination power of the dataset, we calculated the probability of sampling two different  
192 genotypes with the same multilocus SSR phenotype (probability of identity) with one to seven markers using  
193 GenAIEx v6.5 (Peakall and Smouse, 2012). This probability decreased from  $9.9^{e10-2}$  to  $4.6^{e10-3}$  with one marker  
194 (depending on the marker), and reached a plateau around  $3^{e-07}$  with five markers suggesting a high discrimination  
195 power of our seven markers combination (see supplementary material Fig S1). Clones were identified using the  
196 "Multilocus matches" procedure from GenALEX.

197

## 198 **2.6. Genetic diversity**

199 As high levels of clonality can have a significant impact on SGS because some multilocus genotypes can be  
200 overrepresented over small distances (Chenault et al., 2011), we kept only one ramet per multilocus genotype when  
201 two or more ramets were present, resulting in a dataset of 280 multilocus genotypes. We estimated allele  
202 frequencies, total and effective number of alleles, expected and observed heterozygosities and inbreeding  
203 coefficient using SPAGeDi 1.5 software (Hardy and Vekemans, 2002). Genetic structure was analysed using the  
204 STRUCTURE 2.3.4 software (Pritchard et al., 2000), with 20 runs, a burnin period of 100 000 iterations followed  
205 by 200 000 additional MCMC iterations, with an admixture model, uncorrelated allele frequencies and recessive  
206 alleles. The best number of clusters (K) was estimated using the  $\Delta K$  method of Evanno et al. (2005) and visually  
207 verifying the highest values of LnP.

208

## 209 **2.7. Spatial genetic structure**

210 SGS was assessed for the whole population and within sampling groups (FSGS) for each age cohort using  
211 SPAGeDi 1.5 software (Hardy and Vekemans, 2002). Loiselle multilocus kinship coefficients,  $R_{ij}$  (Loiselle et al.,  
212 1995), were calculated between each pair of individuals. Because  $R_{ij}$  is a kinship coefficient relative to the  
213 population mean, negative values can result, meaning that two individuals are less related on average than  
214 randomly selected individuals from the population (Hardy, 2003). Kinship coefficient values were averaged within  
215 distance classes ( $d$ ), giving  $F_{(d)}$  and plotted against geographical distances. Ten classes were formed manually.  
216 Significance was tested by running permutations of the spatial position of individuals 10,000 times, yielding a  
217 95% confidence interval for  $F_{(d)}$  for each distance class.



218 Sibship structure was analysed using the software COLONY (Wang, 2004; Wang and Santure, 2009;  
219 Jones and Wang, 2010). For each pair of samples, COLONY computes the likelihood of assignment into half sibs  
220 (one parent in common), full sibs (same mother and father) or clones. We ran COLONY using the dataset  
221 containing one ramet per multilocus genotype (280 samples). In order to find the best assignment with the  
222 maximum likelihood, we chose the “Long run”, “Full likelihood” and “High precision” computing options,  
223 together with no update of allele frequencies, no sibship size prior, dioecious species with male and female  
224 polygamy, and no known parental genotypes or sibships.

225

## 226 **2.8. Clonality**

227 Using the complete dataset, we estimated clonality as:

228

$$1 - \left(\frac{G-1}{N-1}\right),$$

229 with  $N$ , the number of samples and  $G$ , the number of multilocus genotypes (Arnaud-Haond et al., 2007). The  
230 location and elevation (over a range of a few meters) of sampled saplings were plotted on a digital elevation model  
231 (DEM) of the Allier River using QGIS© v2.18.21 (QGIS Geographic Information System. Open Source  
232 Geospatial Foundation Project. <http://qgis.org>)

233 Statistical analyses were performed using R©v3.5.1. (R Development Core Team, 2005). To test for  
234 differences in the spatial elevational and age distributions of replicated genotypes, we conducted non-parametric  
235 test (normality of the data were tested using Shapiro-Wilk). We conducted Wilcoxon test to test for differences in  
236 the distribution of elevation values of replicated and non-replicated genotypes. We conducted Fisher exact tests to  
237 test for differences in proportion of replicated genotypes in relation to their position along recruitment bands;  
238 centre of a band versus its upstream and downstream ends (i.e. the extremities of a band) and their age-class. To  
239 test for a change in density with cohort age, we compared the within-group distance between individuals using a  
240 non-parametric Dunn test of multiple comparisons. Because of unequal sample sizes among the cohorts, the test  
241 was performed on all the groups of young and old individuals and two sets of three randomly chosen groups of  
242 middle-aged individuals.

243

## 244 **3. Results**

### 245 **3.1. Genetic diversity**

246 Genetic diversity was high in our sample population ( $H_e = 0.859$ ) and was comparable among the three age classes  
247 (young,  $H_e = 0.858$ ; middle-aged,  $H_e = 0.859$ ; old  $H_e = 0.848$ ; Table 2). The effective number of alleles,  $NA_e$ , was  
248 10.79 (Table 1). The multilocus inbreeding coefficient was low ( $F_{is} = 0.011$ ). Our  $F_{is}$  value does not show deviation  
249 from Hardy-Weinberg equilibrium (p-value = 0.240) indicating no population sub-structure. This absence of  
250 population subdivision was also confirmed by the STRUCTURE runs indicating a best number of clusters equal  
251 to 1 (data not shown).

252

### 253 **3.2. Clonality**

254 Out of the 348 individuals sampled we identified 280 multilocus genotypes (Table 2), of which 51 were replicated  
255 (i.e. individuals with two or more ramets) and 229 were represented by a single sample. GenALEX and COLONY

256 (BestClone output) identified the same multilocus genotypes. Clonality was 19% for the whole population. We  
257 did not detect ramets of the same multilocus genotype in different gravel bars along the 1.6 km river reach we  
258 sampled. Clonality values were comparable among the three cohorts (Table 2) and the proportion of replicated  
259 genotypes did not differ among age cohorts (Fig. 3a, Fisher test p-value = 0.202). Elevation (i.e. topography) on  
260 the riverbank did not explain the abundance of replicated genotypes (Wilcoxon test p-value = 0.7401). However,  
261 replicated genotypes were more abundant at the upstream and downstream ends of the recruitment bands (Fig. 3b,  
262 Fisher test p-value = 1.032e-5). There were also more replicated genotypes located at the most exposed ends of  
263 the recruitment bands (relative to the water flow in blue in Fig. 4).

264

### 265 **3.3. Spatial Genetic Structure**

266 Along the 1.6 km river reach, no SGS could be detected when considering the whole sample population. However,  
267 we found that individuals in the first distance class (55 m) were significantly more related to one another than on  
268 average in the population (Fig. 2,  $F_{(d)}$  significantly different from 0).

269 Within patches (Fig. 2), we found a significant decline in the pairwise kinship coefficient with linear  
270 distance (p-value = 0.029), for the young individuals, although all  $F_{(d)}$  values were not significantly different from  
271 0 (within the 95 % confidence interval). No FSGS was detected for the middle-aged and old patches. COLONY  
272 identified 15 full-sibs (FS) and 44 half-sibs (HS) pairs within patches, of which 9 and 16, respectively, with a  
273 probability greater than 0.75. In particular, within one patch we found a family group of five FS within distances  
274 of two meters. The mean distance between individuals was significantly smaller in young and middle-aged patches  
275 compared to old patches (Dunn test, p-value < 0.001 for both) but there was no difference between young and  
276 middle-aged patches (Dunn test, p-value = 0.1547).

277

## 278 **4. Discussion**

### 279 **4.1. Genetic diversity and clonality**

280 The high genetic diversity observed in the Allier River population studied (expected heterozygosity,  $He = 0.859$   
281 and effective number of alleles,  $NAe = 10.79$ ) is consistent with literature data on the genetic diversity of trees  
282 and is explained by life history traits promoting the maintenance of high diversity within forest tree species such  
283 as efficient gene flow mechanisms, mostly outcrossing breeding systems and longevity (Austerlitz et al., 2000). It  
284 is also consistent with other studied *P. nigra* populations using SSR markers. At a regional scale (hundreds of km),  
285 the expected heterozygosity of 17 populations originating from 7 catchment systems in Europe was 0.756  
286 (Smulders et al., 2008). A similar value (0.753) was found in Western Europe for 13 sites, although this was over  
287 an even larger spatial scale (several hundreds of km) (DeWoody et al., 2015). At the catchment scale (tens of  
288 kilometres), genetic diversity ranged from 0.73 along the Drôme River (Imbert and Lefevre, 2003) to 0.79 along  
289 the Danube River (Jelić et al., 2015). At the population scale (2-5 km),  $He$  ranged from 0.69 along the Loire River  
290 (Chenault et al., 2011) to 0.82 along the Morava River (Pospišková and Šálková, 2006) and  $NAe$  ranged from 3.83  
291 along the Eder River in Germany (Rathmacher et al., 2010) to 8.53 across the entire Danube catchment system  
292 (Jelić et al., 2015). The lower values reported in a number of these studies can be explained by a correspondingly  
293 high reported degree of population fragmentation, isolation and limited potential for regeneration. However, each  
294 study used different SSR marker sets, which can also explain the observed differences. Indeed, some SSR markers  
295 are more polymorphic than others. For example, in the Allier population, two SRR markers (WPMS22 and

296 PMGC2385) were highly polymorphic. Nevertheless, the high genetic diversity of our population located in a  
297 Nature Reserve where the flow regime is largely unregulated and morphodynamics are only moderately impacted  
298 by human activities (Garófano-Gómez et al., 2017), highlights the importance of this population as an asset in the  
299 conservation of *P. nigra* genetic resources.

300 When considering levels of clonality, our value (19%) was comparable to the levels of clonality (14%)  
301 found in 17 populations in Central Europe (Smulders et al., 2008), mostly sampled along regulated rivers.  
302 Regulated rivers tend to have a reduced surface area of bare alluvial bars. Thus, seed recruitment is highly restricted  
303 and the main reproductive strategy for poplar is to regenerate vegetatively. Ramets are produced by a diversity of  
304 mechanisms each with their own influence on SGS. Root-borne sucker shoots or burial of individuals mostly  
305 produce discrete units of 2-4 ramets growing close to one another (typically only over a few meters; Barsoum et  
306 al., 2004) but longer distances are reported in other *Populus* species (Wiehle et al., 2009). On the other hand,  
307 breakage during floods can spread translocated fragments downstream, transporting them over a wider range, up  
308 to several km (Barsoum, 2002; Barsoum et al., 2004). In France, studies based on microsatellites show levels of  
309 clonality ranging from 1% (Drôme river, Imbert and Lefevre, 2003), to 49 % (lower Loire River, Chenault et al.,  
310 2011). Chenault *et al.* argue that their study site was not suitable for seedling recruitment because of anthropogenic  
311 disturbances and suggest that regeneration occurred *via* layering during flood disturbance events. We detected no  
312 translocated fragments along the Allier River study reach. Over a 30 km Garonne River study reach, Barsoum  
313 (2002) found many translocated fragments, but our Allier River study reach was only 1.6 km long, which reduced  
314 the chances of encountering translocated fragments. Comparing levels of clonality with other studies must however  
315 be undertaken with caution because different sampling strategies were used, some of which were designed to avoid  
316 over-representing the number of ramets from the same MLG.

317

#### 318 **4.2. Dispersal inferred from SGS and FSGS**

319 As our aim was to understand the local pattern of dispersal (by pollen or seeds) on genetic structure, we  
320 kept only one ramet per multilocus genotype in our dataset. No population structure was detected along the 1.6 km  
321 section studied, however, individuals located no further than 55 m from one another (first distance class used in  
322 our global SGS analysis, Fig. 2) were significantly more related than by chance. This suggests that at short  
323 distances (50 m) dispersal is not homogeneous, but that at the 1.6 km scale of the study reach, seeds from different  
324 parent trees are well mixed. In order to test the generality of our results, we reanalysed the data obtained by one  
325 of us (see the reanalysis of the Barsoum et al. (2004) data in supplementary material) in another river catchment  
326 (Garonne, France). The same analyses used for this study were performed (see supplementary material)). This re-  
327 analysis confirmed the absence of population structure over short distances (up to 15 meters). We however found  
328 a significant SGS along the 30-km stretch where individuals were highly related in the first two distance classes  
329 (corresponding to distances of up to 2 km) ( $p$  value < 0.001) (Fig S2). This result indicates that gene flow is  
330 significantly reduced, but only beyond distances of 2 km.

331 Several studies addressed SGS among populations or patches of *P. nigra* over a large range of distances.  
332 Across Europe, isolation by distance (IBD) was reported in the Danube River basin over hundreds of kilometres  
333 (Jelić et al., 2015), along a 50 km stretch of the Drôme River, France (Imbert and Lefevre, 2003) and even over a  
334 2 km stretch of the Eder River, Germany (Rathmacher et al., 2010). SGS was found significant along the Morava  
335 and Eder Rivers, (Pospíšková and Šálková, 2006; Rathmacher et al., 2010) at a spatial scale of tens of kilometres.

336 This was likely due to the high degree of isolation of these populations, which were separated from other  
337 populations by distances of at least 15 km. Many studies have additionally found genetic differentiation within  
338 populations, suggesting barriers to gene flow, or partially isolated populations (Imbert and Lefevre, 2003;  
339 Pospíšková and Šálková, 2006; Rathmacher et al., 2010). Pospíšková and Šálková, (2006) suggested that the  
340 occurrence of genetic spatial patterns is related to pollen dispersion dynamics based on observations of pollen  
341 dispersion ranging from 10 to 230 meters along the Morava River, Czechoslovakia. Braatne et al. (1996) suggested  
342 that most seeds transported by wind are generally deposited within only a few hundred meters of the mother tree.  
343 This suggests that despite the wide distributional range of *P. nigra*, most pollen and seeds disperse over short  
344 distances (Fig. 5).

345 At the fine scale (within patches), we detected a significant FSGS for the young cohorts (around five  
346 years old) both in the Allier (Fig. 2) and in the Garonne (Fig. S2). This indicates that across short dispersal distances  
347 seeds from a single tree are not homogeneously distributed and can become entangled together to form a single  
348 aggregate during transportation by water or other means (e.g. a broken branch carrying open seed pods which fell  
349 into the river). Seeds are released surrounded by poplar fluff that often aggregate into a package while transported  
350 in the air or by water and colonize the area where it lands onshore. When these aggregates disperse, they are mixed  
351 with aggregates from other trees that are transported by the river and reach gravel bars where they germinate.  
352 These processes create dense recruitment bands and could potentially result in the successful fine scale recruitment  
353 of *P. nigra* seedlings originating from a few parental trees. Indeed, both in the Allier and in the Garonne (see  
354 supplementary material), we found several pairs of FS and HS within patches, some of which very close to each  
355 other. In the Allier, we found a group of five FS from the same mother tree at distances of two meters in a young  
356 patch. Similarly, in the Garonne we found significantly more FS in young patches compared to middle-aged and  
357 old patches (See supplementary material Table S2). Thus, at the fine-scale (a few meters), neighbouring seedlings  
358 can be strongly related because the mixing of aggregate of seeds from different mother trees is imperfect.  
359 Conversely, at the scale of a few kilometres, the mixing of seeds could lead to the absence of significant differences  
360 in genetic structure (Fig. 5b).

361

### 362 **4.3. Intra-specific interactions**

363 The occurrence of replicated genotypes mostly at the upstream and downstream ends of the recruitment bands  
364 along the Allier River indicates potential positive intra-specific facilitation interactions at an early stage of gravel  
365 bar colonisation. Facilitation is defined as ‘an interaction in which the presence of one species alters the  
366 environment in a way that enhances the growth, survival and reproduction of a second species’ (Bronstein, 2009,  
367 p 1160), a definition which is also valid when considering different genotypes of the same species (McIntire and  
368 Fajardo, 2014). The upstream and downstream ends of the recruitment bands are more exposed to hydrodynamic  
369 forces than the centre of the recruitment bands because they are located at lower elevations and are directly exposed  
370 to high flow velocities during flooding events. This mechanical stress is known to directly enhance vegetative  
371 regeneration (Francis et al., 2004; Barsoum et al., 2004). At the same study site on the Allier River, Corenblit et  
372 al. (2016a) demonstrated that upstream individuals trap fine sediment, resulting in the formation of a sediment tail  
373 (see also Rodrigues et al. 2006, 2007; Hortobágyi et al., 2018). These sediment tails, composed of fine sediment  
374 and organic matter, create downstream habitats that are favourable for vegetation growth (Francis et al., 2009;  
375 Corenblit et al., 2014). This phenomenon was also observed along the Drôme and Garonne Rivers in France

376 (Barsoum, 2002; Corenblit et al., 2016b) and the Tagliamento River in Italy (Gurnell et al., 2005). Along the Allier  
377 River, poplar recruitment bands rapidly end up in a higher elevation situation compared to the surrounding alluvial  
378 surfaces (Hortobágyi et al., 2018) through sediment accretion. Individuals at the centre of these bands may thus  
379 benefit from habitat improvement and protection from mechanical stress provided by individuals at the upstream  
380 end of these bands. By increasing stem density at the most exposed parts of recruitment bands, clonality increases  
381 sediment trapping, thereby strengthening the capacity of the downstream individuals to survive and grow. At high  
382 densities, individuals at the centre of these bands also benefit from their own local short range mutually protective  
383 effect. In such a situation, a facilitation interaction has been suggested (Barsoum, 2002; Gurnell et al., 2005;  
384 Corenblit et al., 2014, 2016b, 2018).

385         At the recruitment stage, aggregates of related *P. nigra* seeds reach the bare surface of gravel bars and  
386 produce a significant FSGS among young individuals (Fig. 5c). These seedlings can be very abundant (e.g.  
387 > 4000/m<sup>2</sup>; Braatne et al., 1996) and high seedling densities in a disturbed and stressful environment allow  
388 facilitation processes to occur. In these situations, each individual benefits from the presence of others regardless  
389 of their relatedness. In riparian corridors, the habitat changes caused by poplars during the establishment phase  
390 were described as niche construction (Corenblit et al., 2014; Hortobágyi et al., 2018). This results in older poplars  
391 becoming progressively disconnected from the active channel and experiencing less frequent  
392 hydrogeomorphological disturbances such as mechanical and hydric stress (González et al., 2010; Corenblit et al.,  
393 2016a). According to the stress gradient hypothesis (Bertness and Callaway, 1994), different stress levels change  
394 the type of interaction between individuals from positive (facilitation) in stressful environments to negative  
395 (competition) in benign environments. In our case, as observed for the conditionality of mutualistic interactions  
396 (Bronstein, 1994), the interaction shifts during the lifetime of a single individual. As young individuals stabilize  
397 together their habitat during establishment, intra-specific interactions shift from positive to negative (facilitation  
398 to competition) through self-thinning (Yoda et al., 1963; White and Harper, 1970) (Fig. 5c). Self-thinning tends to  
399 significantly reduce the density of trees growing very close to one another, and we showed that the average distance  
400 between individuals increases with cohort age, resulting in a lower density in older cohorts. This reduces short-  
401 distance relatedness by removing some individuals strongly related to their neighbours. During this process, only  
402 a few individuals from the original seed aggregates remain and will eventually reach sexual maturity (Corenblit et  
403 al., 2014, 2018). FSGS is, therefore, gradually lost as stands mature.

404         Studying the spatial genetic structure of populations at different spatial scales (SGS and FSGS) and for  
405 different age cohorts allowed us to reveal intra-specific interactions and how these interactions change through  
406 different life stages. Corenblit et al., (2018) suggest that niche construction processes, in addition to indirect  
407 facilitation interactions, could in particular cases include direct intraspecific interactions such as cooperation or  
408 altruism, which would involve preferential positive interactions among related individuals. Our result showing that  
409 related (but non-clonal) individuals are present in young patches is in line with this hypothesis. The validation of  
410 the hypothesis that niche construction processes involve cooperation or altruism will however require (i) to  
411 describe the FSGS at the very first stages of colonization (first-year seedlings) and (ii) experimental *in situ* or *ex*  
412 *situ* tests of more efficient niche construction by related compared to non-related individuals.

413

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415

416           **5. Concluding remarks**

417           We chose to study a site located in a natural reserve where the flow regime is unregulated and  
418 morphodynamic processes are only moderately impacted by human activities, promoting the formation of bare  
419 gravel bars suitable for *P. nigra* regeneration, i.e. a site where natural processes are largely undisturbed and where  
420 *P. nigra* can thrive naturally. In this site, genetic diversity was high, similar to, or even higher than most data  
421 available for European rivers and we found a significant SGS for the younger cohorts compared to older cohorts  
422 were SGS was not significant.

423           Facilitation is known to occur through niche construction in black poplars at the early stages of  
424 recruitment when seedling density is high, and the environment is disturbed and stressful (Corenblit et al., 2018).  
425 Our results suggest that facilitation could be directed towards individuals that happen to be genetically related  
426 because seeds from a single tree are aggregated in the poplar fluff. This suggests that direct positive interactions  
427 among related individuals, such as cooperation or altruism, could take place in the niche construction process.  
428 Clonality enhances this facilitative effect by increasing stem density at the most exposed parts of recruitment  
429 bands. Once established and stress is reduced, young poplars seem to shift to a competitive interaction.

430           Conservation measures should make sure that suitable sites allowing high densities of seedlings and  
431 saplings are available to ensure establishment of future mature individuals through these facilitation processes.  
432 Finally, our results suggest that facilitation can be directed towards related individuals. This suggests that direct  
433 positive interactions among related individuals, such as cooperation or altruism, could take place in the niche  
434 construction process. Studying these intraspecific interactions could help design better future conservation  
435 strategies.

436  
437

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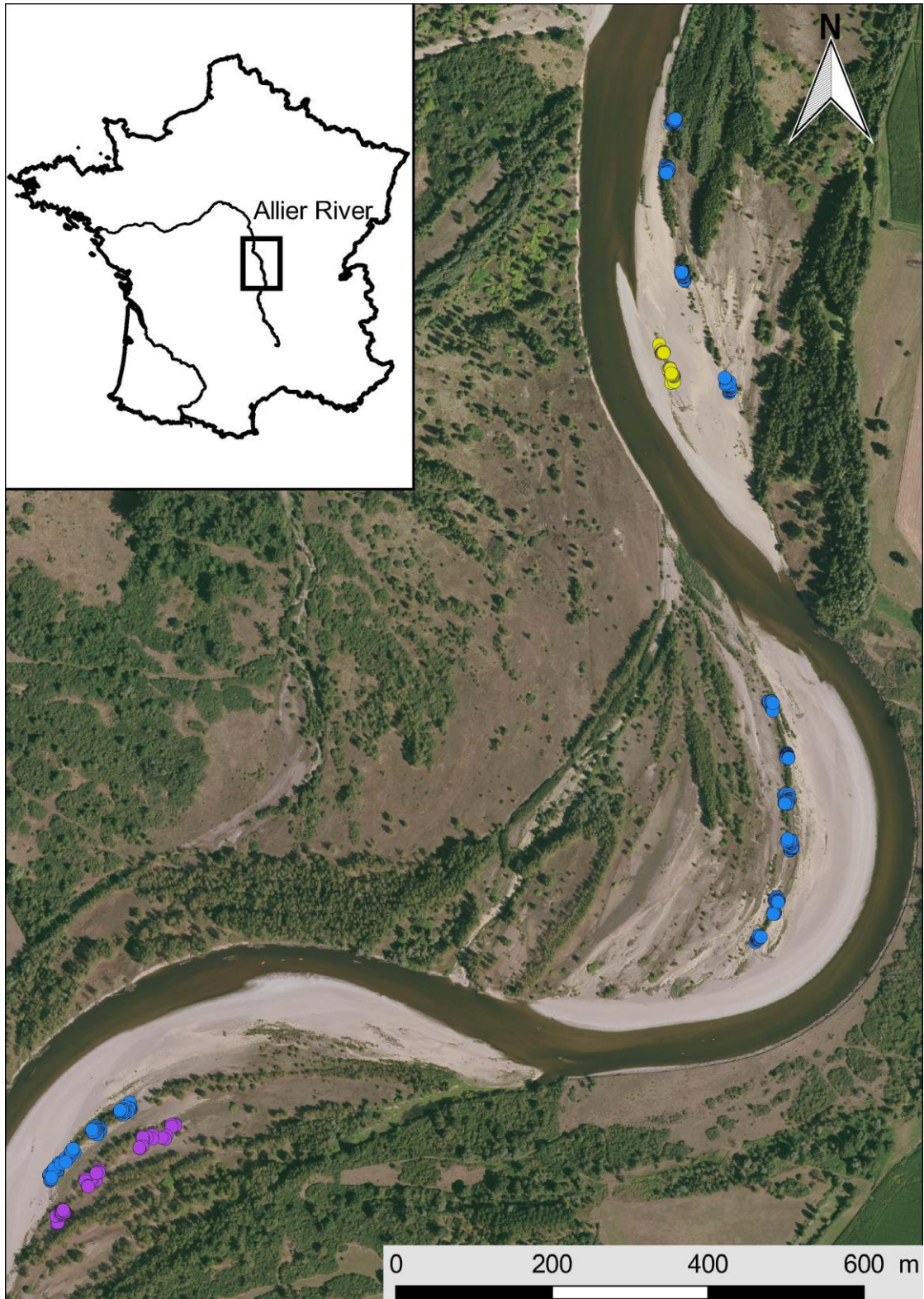
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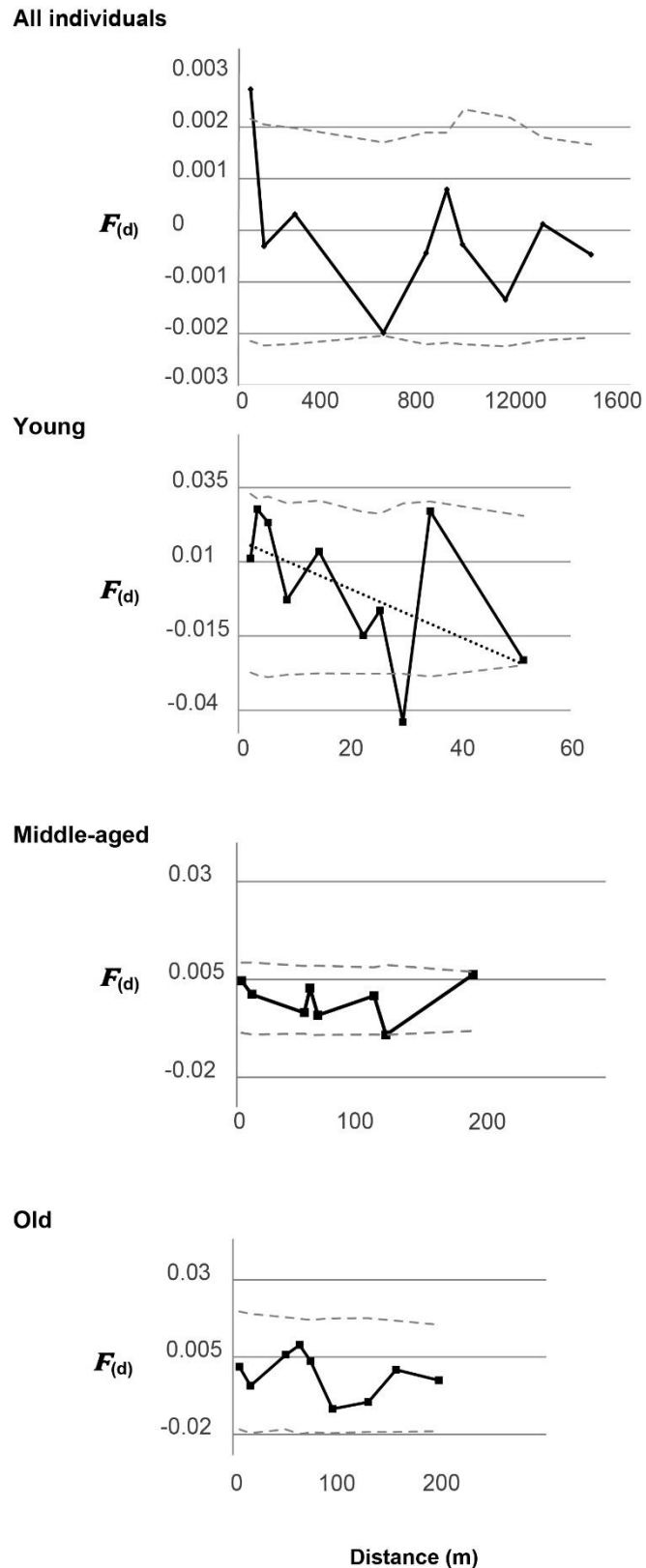
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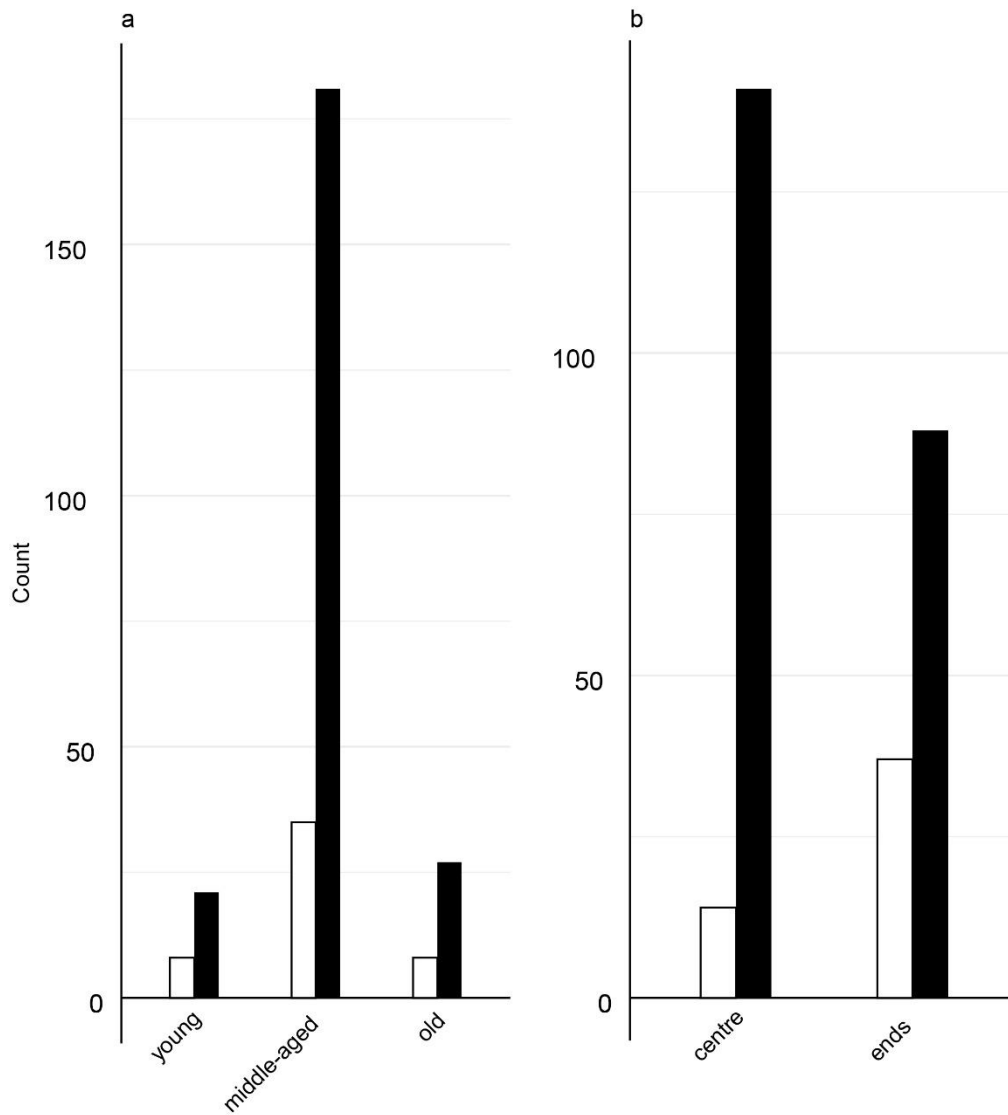


652 **Fig. 1** Aerial photograph of the Allier River study reach, showing the position of the *P. nigra* saplings/trees  
653 sampled along three gravel bars. Colours represent the age of the individuals sampled; purple: old individuals;  
654 blue: middle-aged individuals and yellow: young individuals. River flow direction is from South to North.

655



656 **Fig. 2** Spatial autocorrelation plots for the whole population, and the young, middle-aged and old cohorts  
 657 separately. The average kinship coefficient  $F_{(d)}$  is plotted against geographical distance between individuals.  
 658 Confidence intervals (95%) are indicated by dashed lines. Values within the confidence interval are not  
 659 significantly different from 0 ( $\alpha = 0.05$ ).



660 **Fig. 3** Count of unique genotypes (black bars) and replicated genotypes (white bars) in the Allier population, as a  
 661 function of (a) the age cohort (Young  $\approx$  5 years old; Middle-aged  $\approx$  10 years old; Old  $\approx$  20 years old) and (b)  
 662 position in the recruitment bands (upstream and downstream ends are most exposed to river flow).

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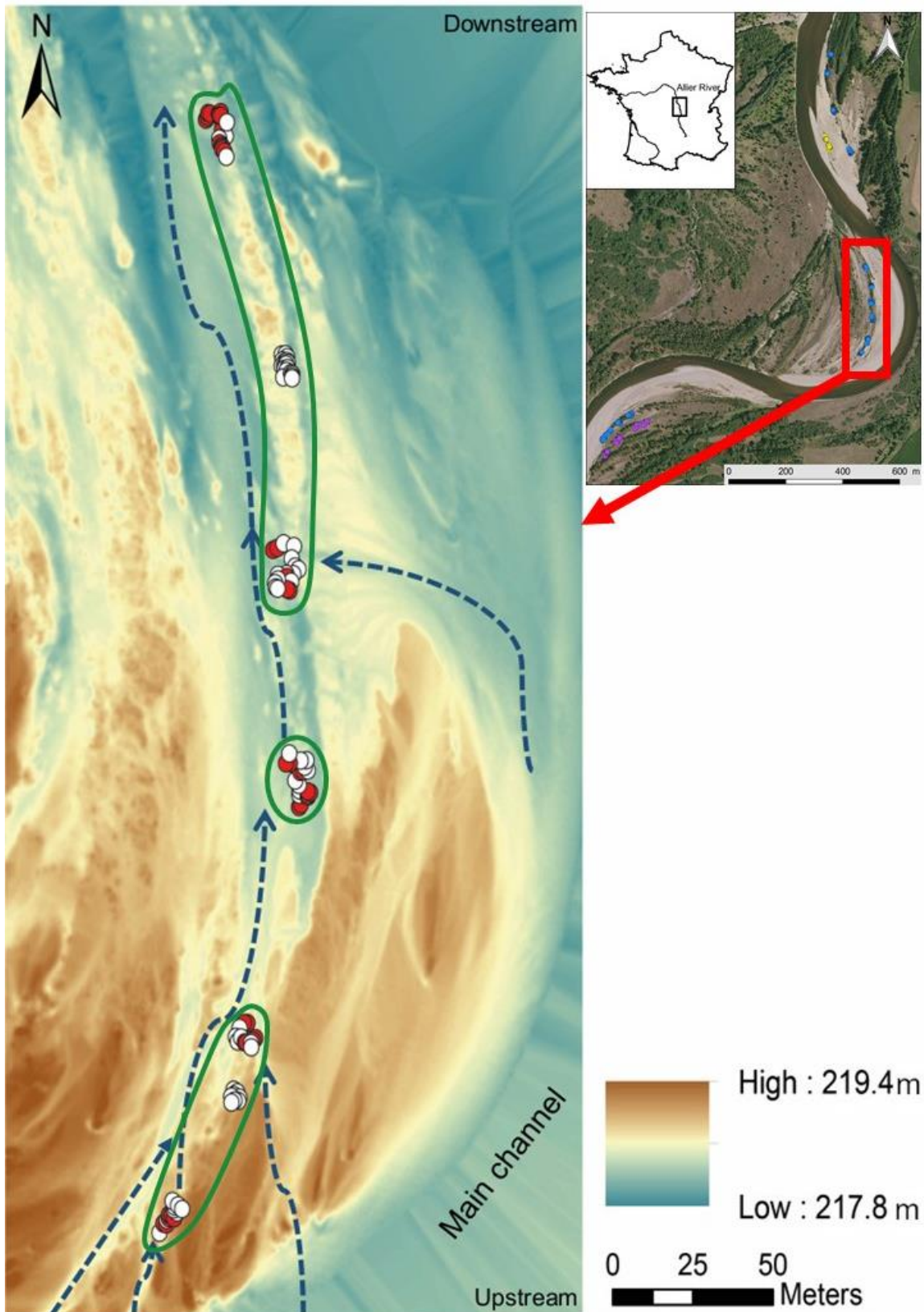
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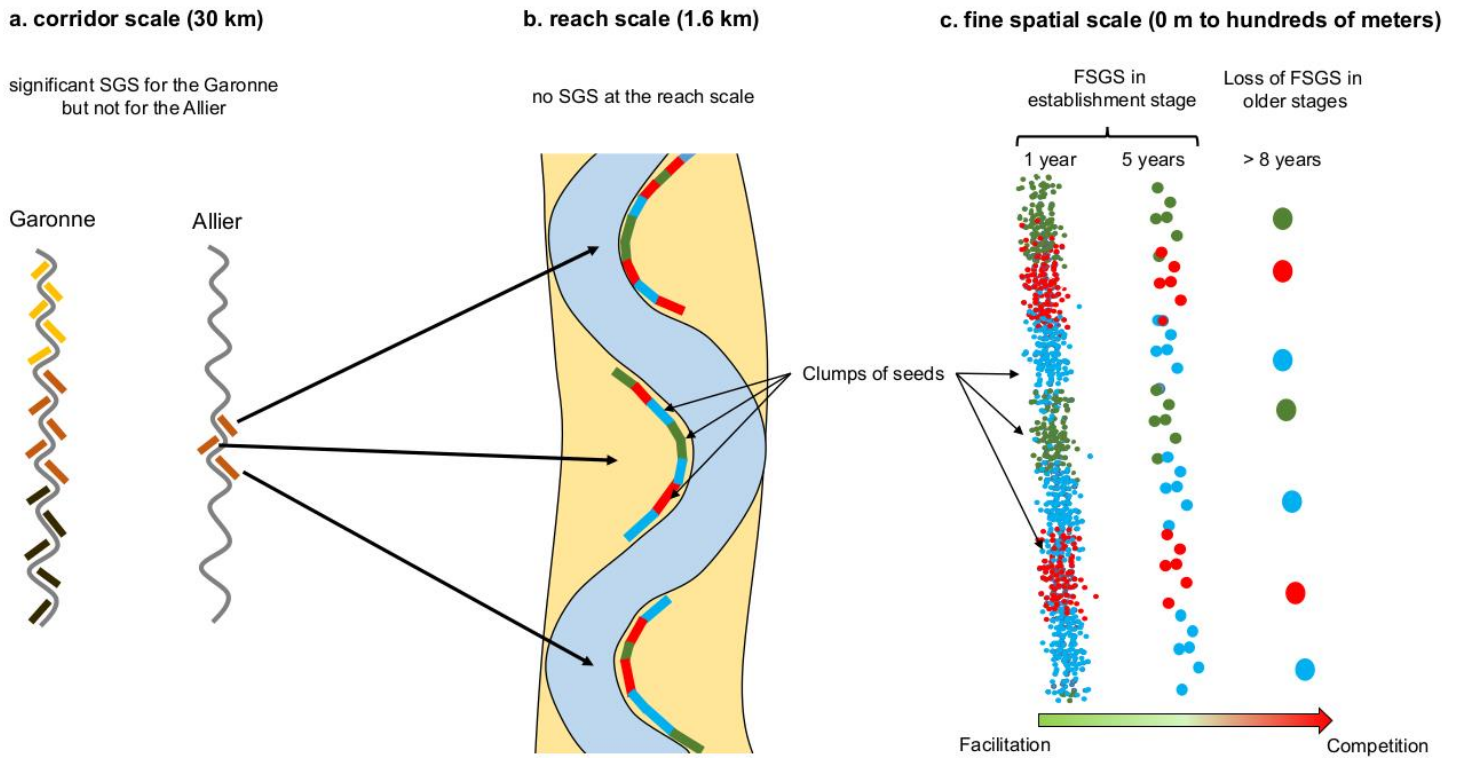
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671 **Fig. 4** Digital elevation model (DEM) of one gravel bar sampled in the Allier. White dots are unique genotypes.  
 672 Red dots are replicated genotypes. The colour scale shows the elevation, from low (blue) to high (brown) values  
 673 of absolute altitudes (m). Blue arrows represent preferential flow paths during high flow. Our field observations  
 674 confirm these preferential flow pathways. The green lines delimit the envelopes of the recruitment bands.



675

676 **Fig. 5** Hypothetical model illustrating SGS at the different scales of our study. At the corridor scale (a), SGS is  
 677 significant because gene flow occurs mostly over a few kilometres and seed dispersal is reduced over longer  
 678 distances. Along the Allier we only sampled a 1.6 km reach and we have no information for the upstream and  
 679 downstream sections of our sampling reach. At the reach (b) and the regeneration band (c) scales, young  
 680 individuals form patches of potentially related individuals (identified through the FSGS analysis); these protect  
 681 one another at the establishment stage (facilitation). When aging, intra-specific competition takes over, leaving  
 682 behind only one or a few individuals from each patch of related individuals. At this stage, FSGS is lost. For (b)  
 683 and (c), the colored dots each represent a different genotype; red = genotype A, green = genotype B and blue =  
 684 genotype C.

685



686 **Tables**

687 **Table 1** Population genetic parameters of the Allier population for each locus and all loci combined. *N* = number  
 688 of individuals; *Na* = total number of alleles; *NAe* = effective number of alleles (Nielsen et al. 2003); *He* and *Ho* =  
 689 expected and observed heterozygosities, respectively; *Fis* = inbreeding coefficient; *P-value* (*Fis* ≠ 0) = the p-value  
 690 of the permutation test. *Na*, *NAe*, *He*, *Ho*, and *Fis* values values were calculated using one sample per multilocus  
 691 genotype (i.e. N = 280).\*except the GCPM2995 marker which was removed from the analysis because of the high  
 692 null allele frequency.

| Locus        | <i>N</i> | Null allele frequency | <i>Na</i> | <i>NAe</i> | <i>He</i> | <i>Ho</i> | <i>Fis</i> | <i>P-value</i> ( <i>Fi</i> ≠ 0) |
|--------------|----------|-----------------------|-----------|------------|-----------|-----------|------------|---------------------------------|
| GCPM2995     | 280      | 0.137                 | 15        | 5.88       | 0.830     | 0.312     | 0.312      | 0.000                           |
| ORPM221      | 280      | -0.010                | 15        | 8.39       | 0.881     | 0.896     | -0.018     | 0.401                           |
| PMGC14       | 280      | 0.004                 | 7         | 3.92       | 0.745     | 0.739     | 0.008      | 0.801                           |
| PMGC93       | 280      | 0.009                 | 6         | 4.18       | 0.761     | 0.741     | 0.026      | 0.409                           |
| PMGC2578     | 280      | -0.007                | 18        | 6.29       | 0.841     | 0.853     | -0.014     | 0.554                           |
| PMGC2385     | 280      | 0.026                 | 32        | 20.81      | 0.952     | 0.896     | 0.059      | 0.000                           |
| WPMS13       | 280      | -0.010                | 15        | 8.04       | 0.876     | 0.893     | -0.020     | 0.372                           |
| WPMS22       | 280      | 0.014                 | 43        | 23.88      | 0.958     | 0.929     | 0.031      | 0.020                           |
| All markers* | 280      | n.a.                  | 19.43     | 10.79      | 0.859     | 0.850     | 0.011      | 0.179                           |

693

694 **Table 2** Population genetic parameters of the different age cohorts sampled along the Allier (all samples of a given  
 695 age cohort are combined). *N*, number of individuals; *G*, number of multilocus genotypes; Clonality; *Na*, total  
 696 number of alleles; *NAe*, effective number of alleles (Nielsen et al. 2003); *He* and *Ho*, expected and observed  
 697 heterozygosities, respectively; *Fi* inbreeding coefficient; *P value* (*Fis* ≠ 0), p value of the permutation test. *Na*,  
 698 *NAe*, *He*, *Ho* and *Fis* values were calculated using one ramet per multilocus genotype.

| Cohort      | Age (years) | <i>N</i> | <i>G</i> | Clonality | <i>Na</i> | <i>NAe</i> | <i>He</i> | <i>Ho</i> | <i>Fis</i> | <i>P value</i> ( <i>Fi</i> ≠ 0) |
|-------------|-------------|----------|----------|-----------|-----------|------------|-----------|-----------|------------|---------------------------------|
| Young       | ≈ 5         | 28       | 23       | 0.19      | 11.57     | 10.08      | 0.858     | 0.894     | -0.043     | 0.159                           |
| Middle-aged | ≈ 10        | 276      | 222      | 0.19      | 19.29     | 10.88      | 0.859     | 0.851     | 0.009      | 0.310                           |
| Old         | ≈ 20        | 44       | 35       | 0.21      | 12.86     | 9.51       | 0.848     | 0.812     | 0.043      | 0.099                           |
| All         |             | 348      | 280      | 0.19      | 19.43     | 10.79      | 0.859     | 0.850     | 0.011      | 0.179                           |

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706 **Supplementary material**

707 **M&M**

708 The data used in (Barsoum et al. 2004) to study clonality along a 30 km reach of the Garonne  
709 river was analysed to study the FSGS. We provide here the general information regarding  
710 sampling and genotyping. For further details see Barsoum et al. (2004).

711 **Sampling strategy along the Garonne River**

712 *P. nigra* stands were sampled in 2004 along a 30 km study reach. Saplings or trees were  
713 categorised using the same three age categories described for the Allier River (i.e. young,  
714 middle-aged, old). For each age category, ten sampling locations (patches) were selected. At  
715 each of these sampling locations 15 saplings or trees were selected by first selecting one  
716 individual and then its 14 downstream nearest neighbours, resulting in a total of 450 individuals  
717 sampled. In order to avoid sampling re-sprouting stems of the same individual, the stem with  
718 the greatest diameter in a patch was sampled. In each sampling location, the first two trees  
719 sampled were used as spatial references and all the other trees were localized using their  
720 distance to these two trees. Using these distances, only the 417 trees that could be mapped  
721 precisely were kept for further analyses.

722 **DNA extraction, genotyping and genetic analysis for the Garonne**

723 Five microsatellite markers WPMS03, WPMS05, WPMS07, WPMS09 and WPMS12  
724 (Smulders et al. 2003) were used in the analyses. To remove the impact of clonality on genetic  
725 structure, further analyses were performed with only one individual of each replicated genotype  
726 per sampling location resulting in a dataset of 360 individuals. We used the same procedures as  
727 in the Allier to study SGS and FSGS.

728

729 **Results**

730 Genetic diversity was high in the Garonne population ( $H_e = 0.812$ ) and clonality was  
731 14% (Table S1). We found a strong SGS along the 30 km section of the Garonne (Fig. S1). For  
732 the analysis over the whole 30 km reach (SGS), individuals were highly related in the first two  
733 distance classes (corresponding to distances of up to 2 km) ( $p$  value  $< 0.001$ ). We found a  
734 significant spatial autocorrelation (FSGS) among the young individuals along the Garonne  
735 River ( $p$ -value = 0.041). No FSGS was detected for the middle-aged and old individuals  
736 sampled (Fig.S1). Within patches, COLONY identified 19 full-sibs and 95 half-sib pairs with  
737 a probability greater than 0.7 and 0.5 respectively. In particular, the highest number of full-sibs  
738 pairs were found in the youngest cohort (Table S2).

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745 **Table S1** Genetic diversity characteristics of the different age cohorts sampled along the  
 746 Garonne Rivers (all samples of a given age cohort are combined). *N*, number of individuals; *G*,  
 747 number of multilocus genotypes;  $1 - \frac{(G-1)}{(N-1)}$ , clonality; *Na*, total number of alleles; *NAe*,  
 748 effective number of alleles (Nielsen et al. 2003); *He* and *Ho*, expected and observed  
 749 heterozygosities, respectively; *Fi* inbreeding coefficient; *P value* (*Fi* ≠ 0), p value of the  
 750 permutation test. *Na*, *NAe*, *He*, *Ho*, *He/Ho* and *Fis* values were calculated using the number of  
 751 multilocus genotypes, *G*.

| Cohort      | Age (years) | <i>N</i> | <i>G</i> | $1 - \frac{(G-1)}{(N-1)}$ | <i>Na</i> | <i>NAe</i> | <i>He</i> | <i>Ho</i> | <i>Fis</i> | <i>P value</i> ( <i>Fi</i> ≠ 0) |
|-------------|-------------|----------|----------|---------------------------|-----------|------------|-----------|-----------|------------|---------------------------------|
| Young       | 5.6 (±1.4)  | 137      | 132      | 0.04                      | 19.4      | 7.01       | 0.822     | 0.815     | 0.009      | 0.563                           |
| Middle-aged | 8 (±1.6)    | 136      | 111      | 0.19                      | 18.0      | 6.70       | 0.809     | 0.771     | 0.047      | 0.006                           |
| Old         | 17.6 (±2.5) | 144      | 123      | 0.15                      | 16.4      | 6.33       | 0.801     | 0.761     | 0.050      | 0.002                           |
| All         |             | 417      | 360      | 0.14                      | 22.6      | 6.65       | 0.812     | 0.783     | 0.037      | 0.000                           |

752

753 **Table S2** Repartition of Full-sibs (FS) and Half-sibs (HF) in the different age cohorts sampled  
 754 along the Garonne river (all samples of a given age cohort are combined). For both FS and HF  
 755 the chi<sup>2</sup> test was significant.

| Age class   | Full-sibs (FS)      |                     |                            | Half-Sibs (HS)      |                     |                            |
|-------------|---------------------|---------------------|----------------------------|---------------------|---------------------|----------------------------|
|             | <i>Nb of FS</i>     | <i>Nb of non-FS</i> | <i>Nb of pair in total</i> | <i>Nb of HS</i>     | <i>Nb of non-HS</i> | <i>Nb of pair in total</i> |
| Young       | 13                  | 914                 | 927                        | 16                  | 911                 | 927                        |
| Middle-aged | 2                   | 730                 | 732                        | 17                  | 715                 | 732                        |
| Old         | 4                   | 844                 | 848                        | 7                   | 841                 | 848                        |
| Total       | 19                  | 2488                | 2507                       | 40                  | 2467                | 2507                       |
|             | FS chi <sup>2</sup> | 108,3 ***           |                            | HS chi <sup>2</sup> | 144,8 ***           |                            |

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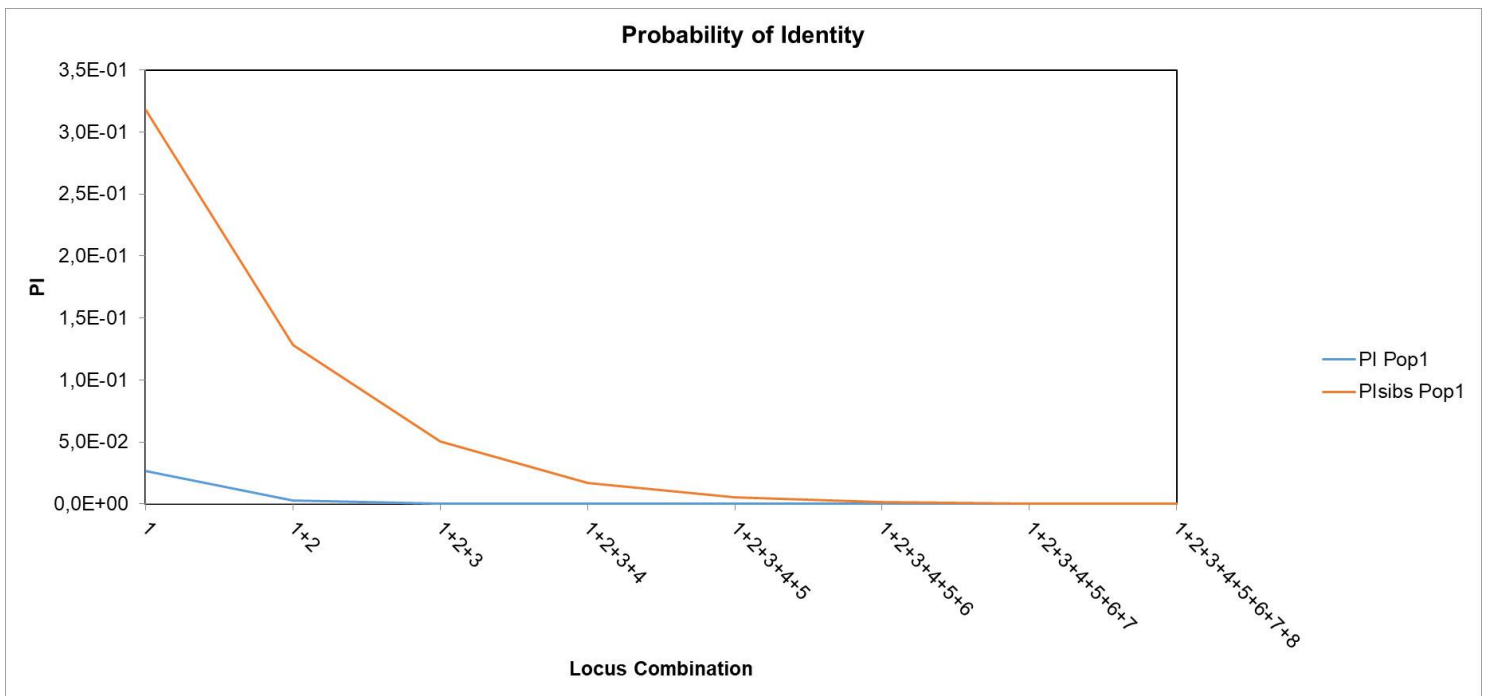
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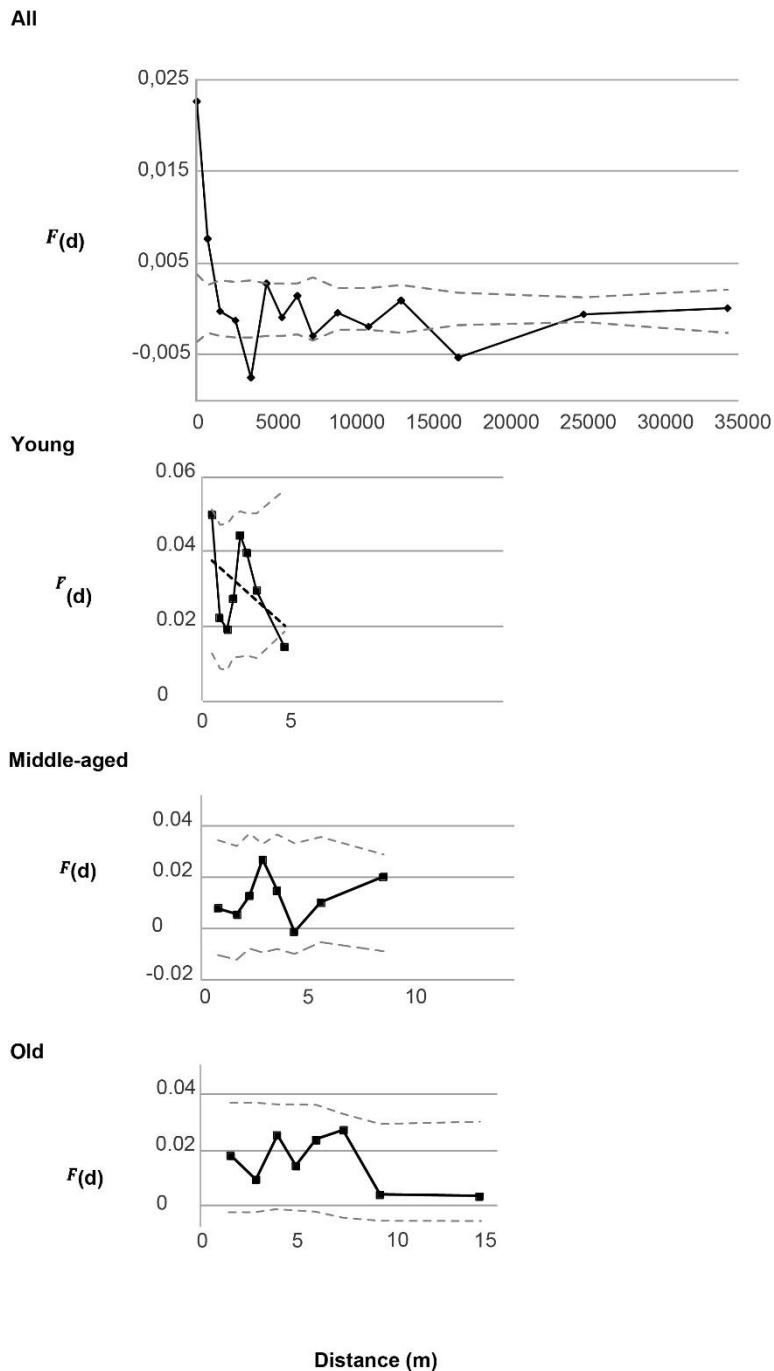
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762 **Figure legend**



763 **Fig.S1** Probability of identity (PI, in blue) and probability of identity for sibs (PISibs, in orange)  
764 in relation to the number of loci considered.

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780 **Fig. S2** Spatial autocorrelation plots for the whole population, the young, middle-aged and old  
 781 cohorts. The average kinship coefficient  $F(d)$  is plotted against geographical distance between  
 782 individuals. Confidence intervals (95%) are indicated by dashed lines. All values within the  
 783 confidence interval are not significantly different from 0 ( $\alpha = 0.05$ ). All values within the  
 784 confidence interval are not significantly different from 0 ( $\alpha = 0.05$ )

785 **Acknowledgements**

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788