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Abstract

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

1. Introduction

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

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

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

2. Material and Methods

2.1. Study Species

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

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

2.2. Study site

The study site was located along the lower reaches of the Allier River in the Réserve Naturelle Nationale du Val 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 to most European rivers, the flow regime along the lower reaches of the Allier River is largely unregulated and morphodynamics within the Nature Reserve are only moderately impacted by human activities (Garófano-Gómez et al., 2017). Bank erosion in the outer bends of meanders and recurrent active channel migration promote the formation of bare gravel bars suitable for *P. nigra* regeneration. The progressive shifting of the river channel allows for periodic recruitment episodes, resulting in different aged cohorts of *P. nigra* recruits growing in elongated bands parallel to the main channel (Hortobágyi et al., 2017).

2.3. Sampling strategy

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

2.4. DNA extraction and genotyping

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

The polymerase chain reaction was carried out in a volume of 10 µL, which contained 2.5 µL of DNA (at 10 ng/µL) and 7.5 µL of the following mix: 5 µL Master mix (AmpliTaq Gold™ 360), 0.5 µL primer mix (labelled with M13 fluorochromes Applied Biosystems™ 6-FAM, NED, PET and VIC), 0.05 µL tagged M13 primer and 1.95 µL H_2O. The profile of the touchdown phase with 7 cycles was as follow: 95 °C for 30s, 62 °C for 30s and 72 °C for 30s, with a temperature reduction of -1 °C per cycle. The 30 classical PCR cycles were as follow:

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

2.5. Genetic analyses

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

2.6. Genetic diversity

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

2.7. Spatial genetic structure

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

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

2.8. Clonality

Using the complete dataset, we estimated clonality as:

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

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

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

3. Results

3.1. Genetic diversity

Genetic diversity was high in our sample population (H_e = 0.859) and was comparable among the three age classes (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 10.79 (Table 1). The multilocus inbreeding coefficient was low (F_{is} = 0.011). Our F_{is} value does not show deviation from Hardy-Weinberg equilibrium (p-value = 0.240) indicating no population sub-structure. This absence of population subdivision was also confirmed by the STRUCTURE runs indicating a best number of clusters equal to 1 (data not shown).

3.2. Clonality

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

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

3.3. Spatial Genetic Structure

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

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

4. Discussion

4.1. Genetic diversity and clonality

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

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

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

4.2. Dispersal inferred from SGS and FSGS

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

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

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

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

4.3. Intra-specific interactions

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

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

At the recruitment stage, aggregates of related *P. nigra* seeds reach the bare surface of gravel bars and produce a significant FSGS among young individuals (Fig. 5c). These seedlings can be very abundant (e.g. > 4000/m²; Braatne et al., 1996) and high seedling densities in a disturbed and stressful environment allow facilitation processes to occur. In these situations, each individual benefits from the presence of others regardless of their relatedness. In riparian corridors, the habitat changes caused by poplars during the establishment phase were described as niche construction (Corenblit et al., 2014; Hortobágyi et al., 2018). This results in older poplars becoming progressively disconnected from the active channel and experiencing less frequent hydrogeomorphological disturbances such as mechanical and hydric stress (González et al., 2010; Corenblit et al., 2016a). According to the stress gradient hypothesis (Bertness and Callaway, 1994), different stress levels change the type of interaction between individuals from positive (facilitation) in stressful environments to negative (competition) in benign environments. In our case, as observed for the conditionality of mutualistic interactions (Bronstein, 1994), the interaction shifts during the lifetime of a single individual. As young individuals stabilize together their habitat during establishment, intra-specific interactions shift from positive to negative (facilitation to competition) through self-thinning (Yoda et al., 1963; White and Harper, 1970) (Fig. 5c). Self-thinning tends to significantly reduce the density of trees growing very close to one another, and we showed that the average distance between individuals increases with cohort age, resulting in a lower density in older cohorts. This reduces short-distance relatedness by removing some individuals strongly related to their neighbours. During this process, only a few individuals from the original seed aggregates remain and will eventually reach sexual maturity (Corenblit et al., 2014, 2018). FSGS is, therefore, gradually lost as stands mature.

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

5. Concluding remarks

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

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

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

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Figure legends

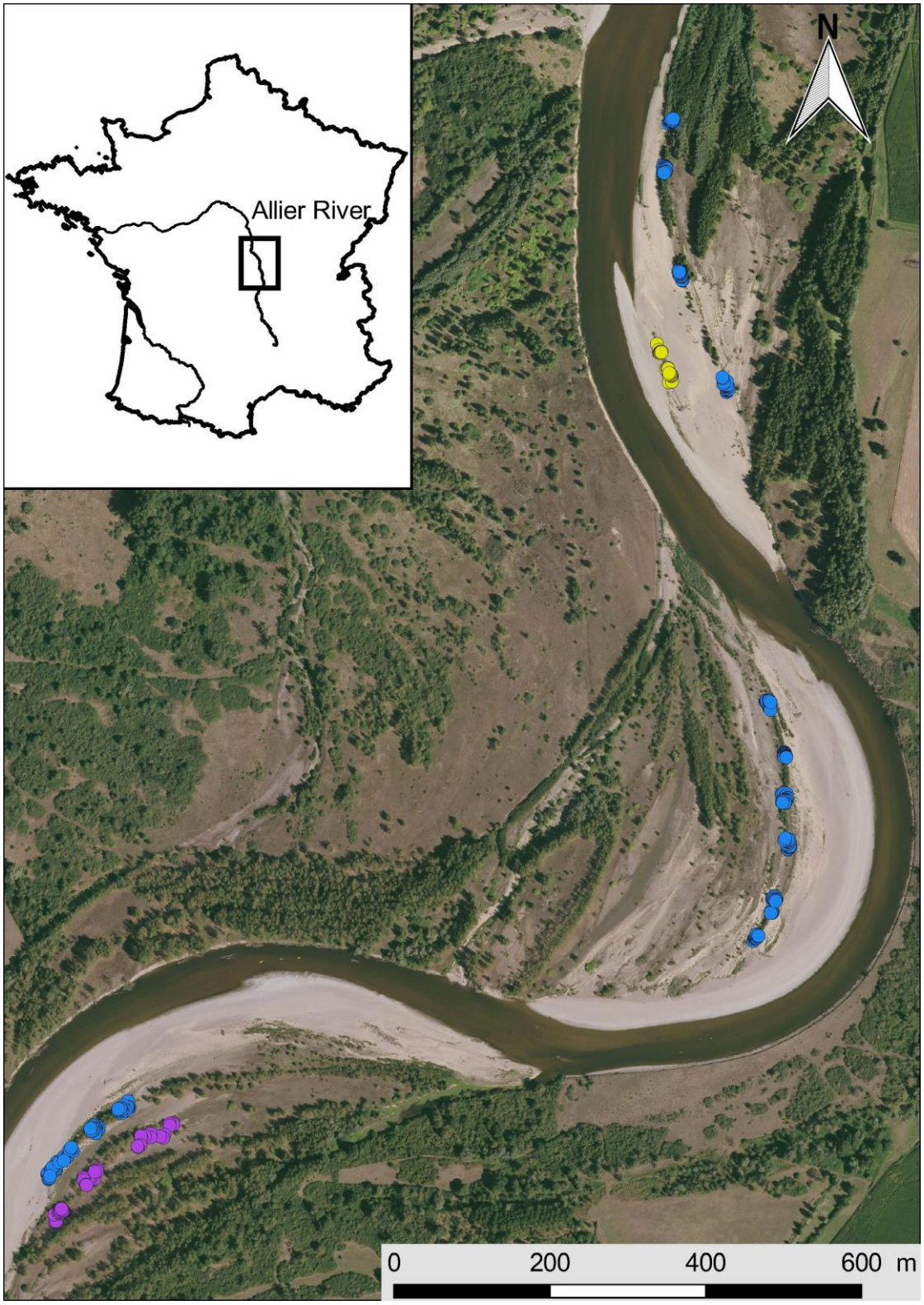


Fig. 1 Aerial photograph of the Allier River study reach, showing the position of the *P. nigra* saplings/trees sampled along three gravel bars. Colours represent the age of the individuals sampled; purple: old individuals; blue: middle-aged individuals and yellow: young individuals. River flow direction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

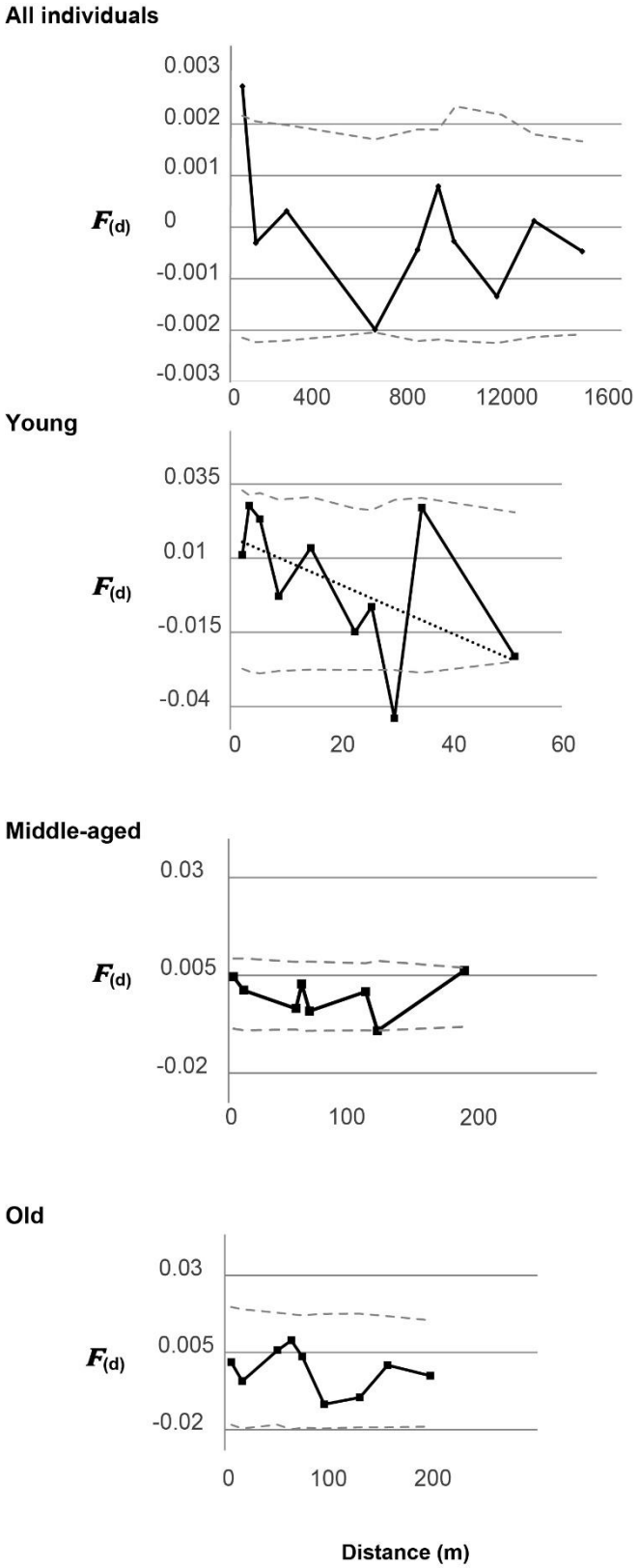
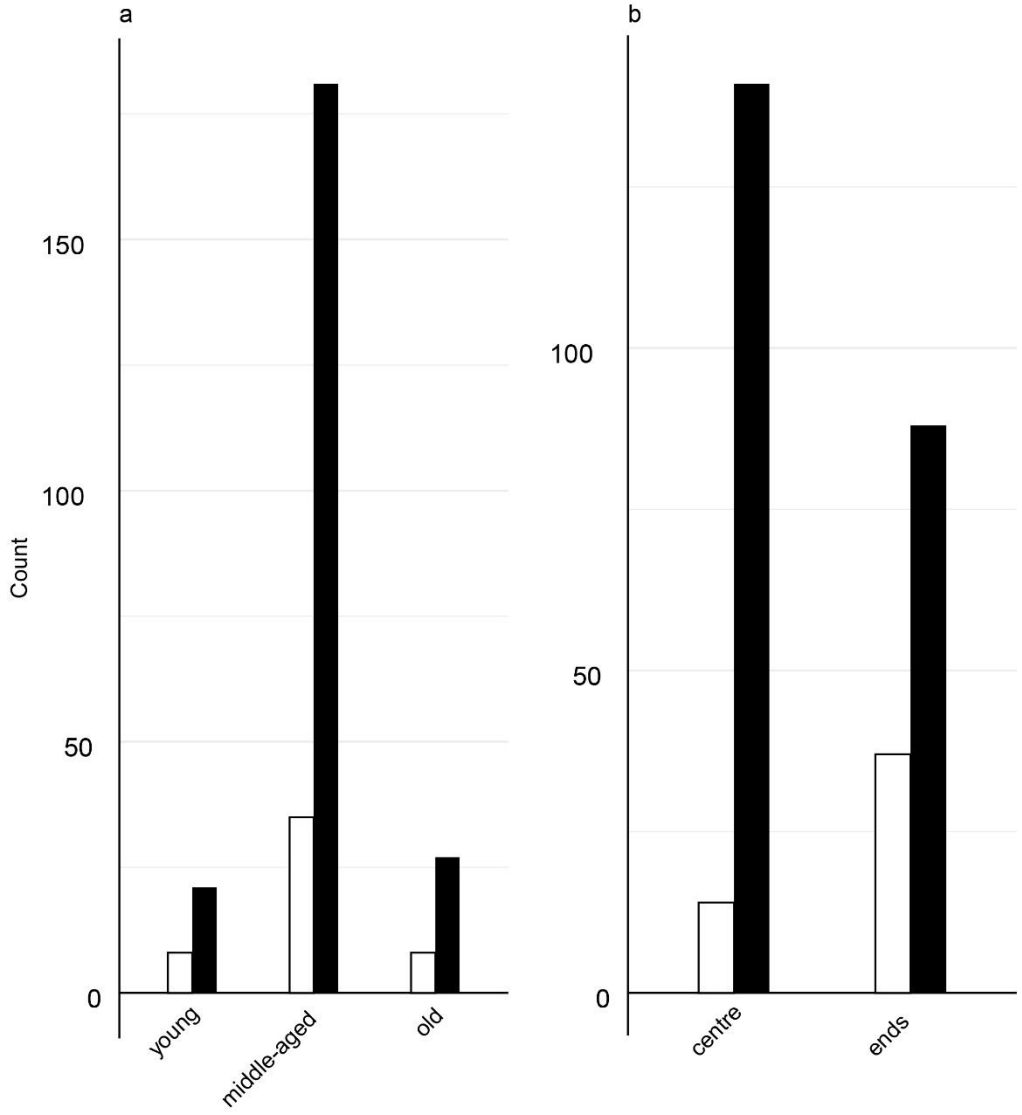


Fig. 2 Spatial autocorrelation plots for the whole population, and the young, middle-aged and old cohorts separately. The average kinship coefficient $F_{(d)}$ is plotted against geographical distance between individuals. Confidence intervals (95%) are indicated by dashed lines. Values within the confidence interval are not 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 ≈ 5 years old; Middle-aged ≈ 10 years old; Old ≈ 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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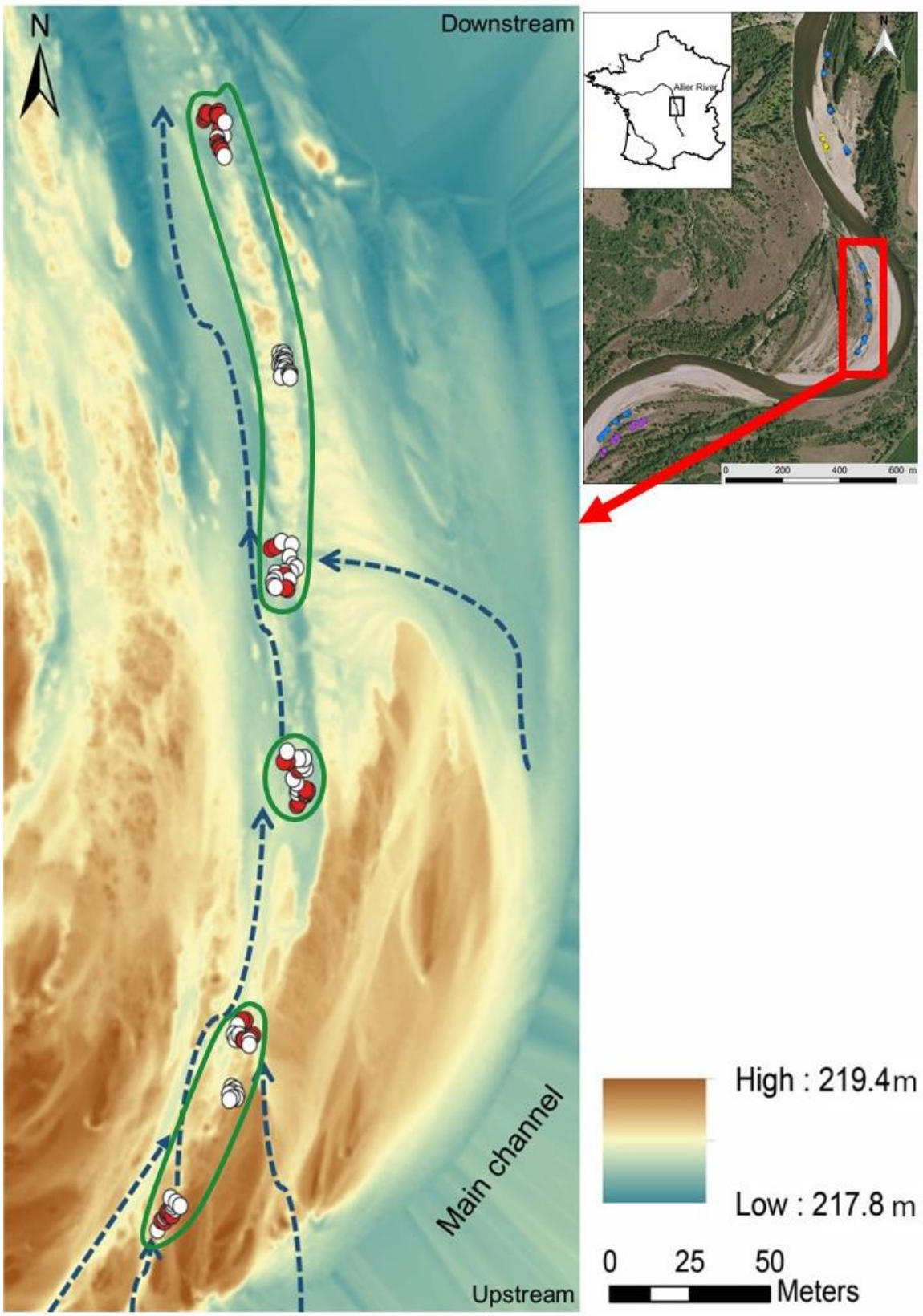
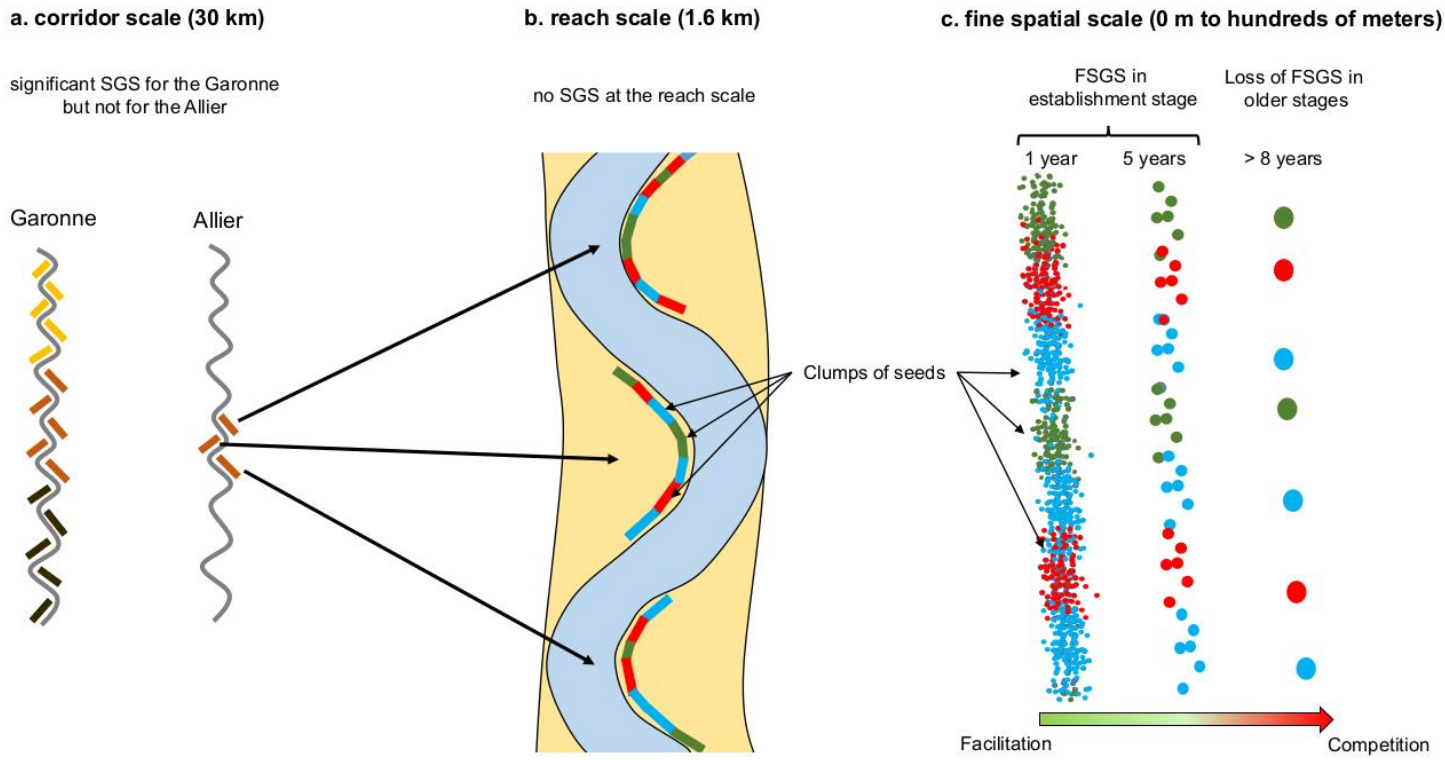


671 **Fig. 4** Digital elevation model (DEM) of one gravel bar sampled in the Allier. White dots are unique genotypes.
672 Red dots are replicated genotypes. The colour scale shows the elevation, from low (blue) to high (brown) values
673 of absolute altitudes (m). Blue arrows represent preferential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



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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	*N*	Null allele frequency	*Na*	*NAe*	*He*	*Ho*	*Fis*	*P-value (Fi ≠ 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)	*N*	*G*	*Clonality*	*Na*	*NAe*	*He*	*Ho*	*Fis*	*P value (Fi ≠ 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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Supplementary material

M&M

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

Sampling strategy along the Garonne River

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

DNA extraction, genotyping and genetic analysis for the Garonne

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

Results

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

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744

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-(*(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* ($Fi \neq 0$), 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)	*N*	*G*	$1-\frac{(G-1)}{(N-1)}$	*Na*	*NAe*	*He*	*Ho*	*Fis*	*P value* ($Fi \neq 0$)
Young	5.6 ($\pm$1.4)	137	132	0.04	19.40	7.01	0.822	0.815	0.009	0.563
Middle-aged	8 ($\pm$1.6)	136	111	0.19	18.00	6.70	0.809	0.771	0.047	0.006
Old	17.6 ($\pm$2.5)	144	123	0.15	16.40	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 chi² test was significant.

	Full-sibs (FS)			Half-Sibs (HS)		
Age class	*Nb of FS*	*Nb of non-FS*	*Nb of pair in total*	*Nb of HS*	*Nb of non-HS*	*Nb of pair in total*
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 chi²	108,3 ***		HS chi²	144,8 ***	

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Figure legend

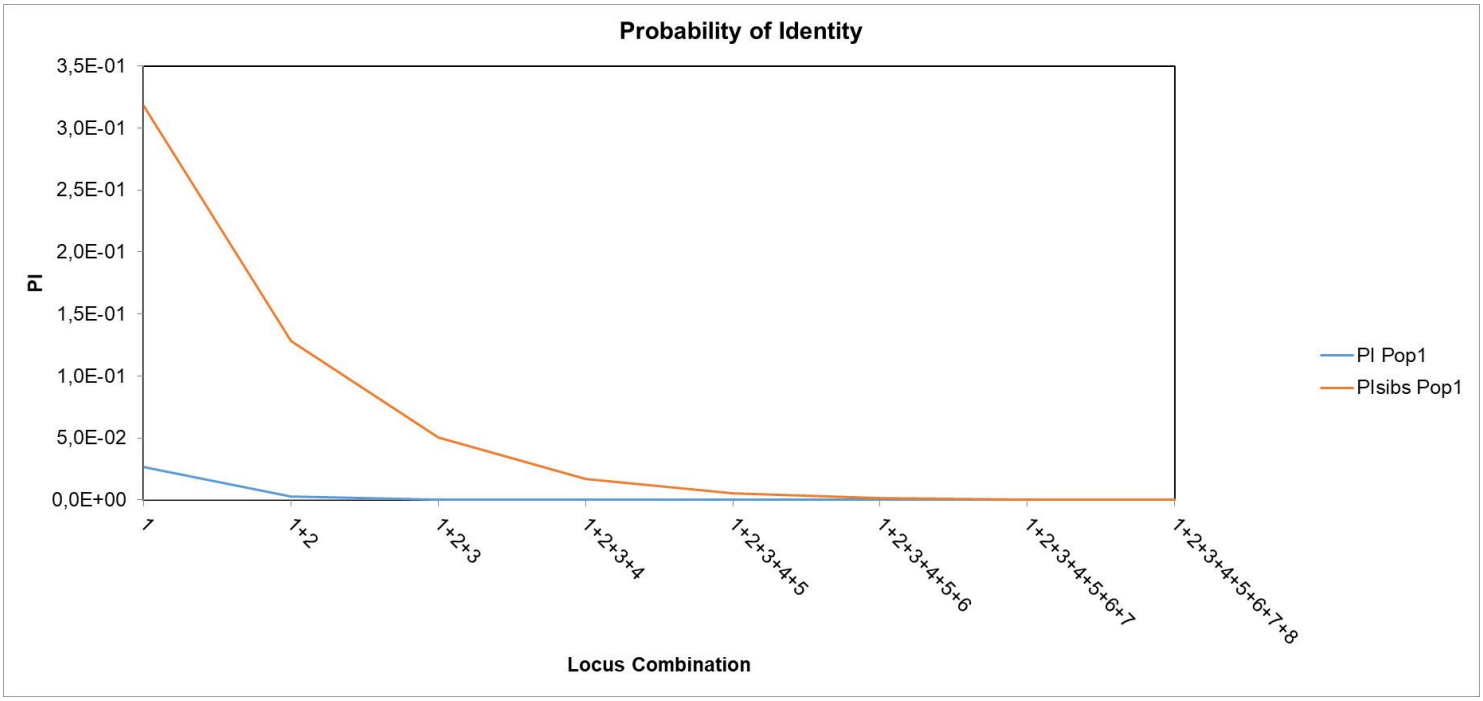
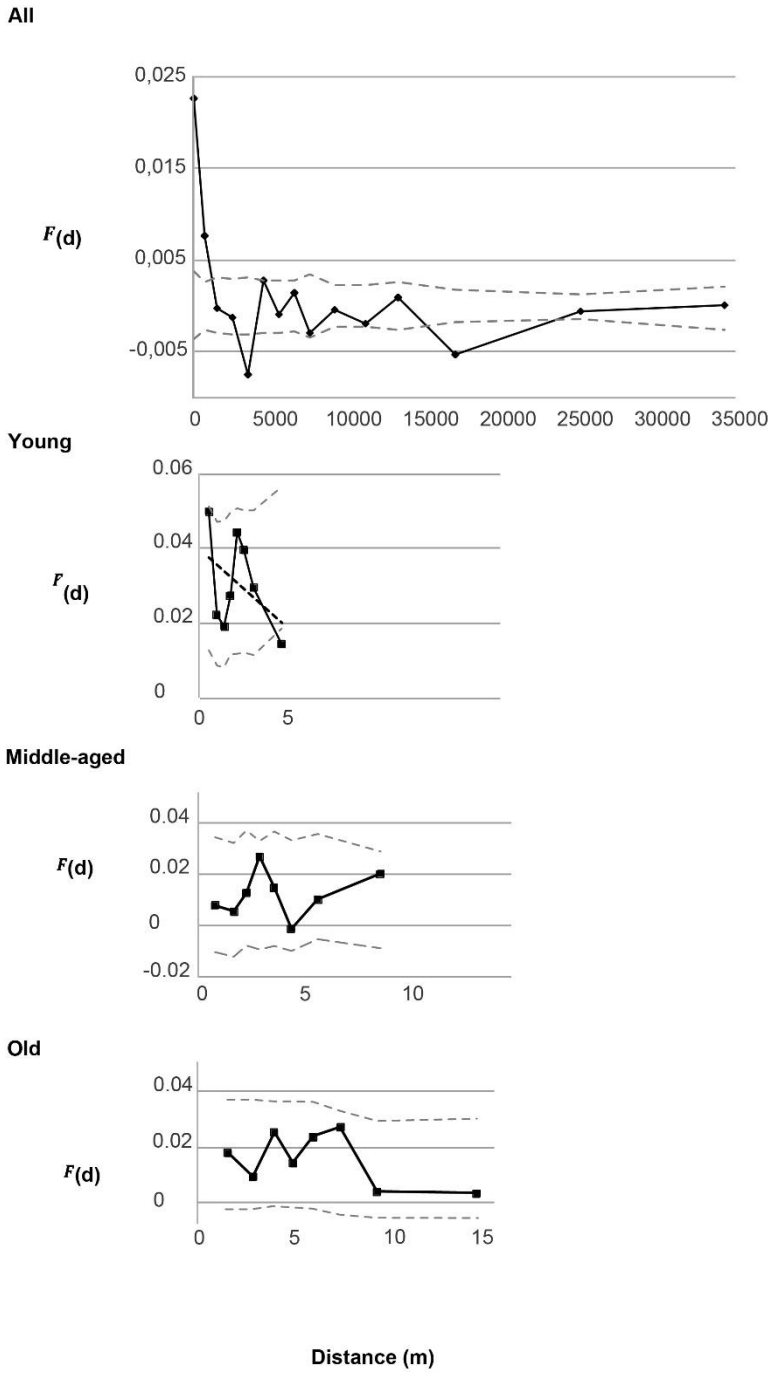


Fig.S1 Probability of identity (PI, in blue) and probability of identity for sibs (PISibs, in orange) in relation to the number of loci considered.



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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787 00031FLOBAR2 (Floodplain Biodiversity and Restoration).

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