
SSR-based analysis of clonality, spatial genetic structure and introgression from the Lombardy poplar into a natural population of *Populus nigra* L. along the Loire River

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Abstract:

A scarcity of favourable habitats and introgression from exotic cultivars are two major threats to black poplars (*Populus nigra* L.) in Europe. Natural vegetative propagation contributes to maintenance of the species in areas where seedling recruitment is limited. Exhaustive sampling of all mature trees in a natural *P. nigra* stand (413 individuals at recorded positions), genotyping at 11 SSR loci, and a standardized analysis framework resulted in a precise description of clonality in terms of (a) frequency, (b) spatial growth form, and (c) impacts on the overall spatial genetic structure (SGS). The high proportion of replicated genotypes detected resulted in a genotypic richness (R) of 0.47. Up to 18 ramets were found per multilocus lineage (MLL), but 95% of MLLs contained fewer than five ramets (Pareto index $\beta = 1.07$). No significant difference in vegetative propagation potential was found between genders. Uneven spatial distribution of ramets, with clustering of clonal ramets (aggregation index $A_c = 0.62$) and near-zero intermingling between MLLs (clonal dominance index $D_c = 0.99$), resulted in a 'phalanx' clonal growth form, explaining most of the SGS observed over short distances (0–20 m, $Sp = 0.0324$). Although they did not exhibit the typical columnar shape of the Lombardy poplar (*P. nigra* var. *italica*), five trees were found to be probable F_1 hybrids of this old and widely distributed cultivar.

Keywords : *Populus nigra* ; Lombardy poplar ; Clonality ; Spatial genetic structure ; Introgression ; Clonal growth form

41 Introduction

42 Various subspecies of black poplar (*Populus nigra* L.) have been proposed on the basis
43 of morphological traits; however, variation may be the result of the species' wide
44 distribution, ranging from the British Isles to Western Asia and from the Mediterranean
45 coast of Africa to Northern Europe, excluding Scandinavia (Dickmann and Kuzovkina
46 2008). This pioneer species is found in the early successional stages of riparian
47 woodlands and is considered an indicator of the health and biodiversity of these
48 ecosystems (Rotach 2004). Although *P. nigra* has little commercial use *per se*, it is
49 considered a key species in numerous European breeding programmes. In 2009, 66% of
50 the poplar cuttings sold by French nurseries were *P. × euramericana* Dode interspecific
51 hybrids (Paillassa 2010), resulting from crossing male black poplars with female
52 American eastern cottonwoods (*P. deltoides* Bartr.).

53 The species is threatened by extinction in several parts of its natural range as a
54 result of agriculture, urbanization and other human activities, which have altered both
55 the area available for colonization and the dynamics of floodplains, thus hindering seed
56 dispersal and germination and favouring latter successional hardwood trees (Lefèvre et
57 al. 1998). Even though *P. nigra* is classified as being of 'Least Concern' in the IUCN
58 red list of threatened species (IUCN 2010), it is thought that there are, for example, only
59 about 7000 trees left in Great Britain, and of these only about 600 are females (Cooper
60 2006). Recent surveys in the North-Western part of its range indicate that the species
61 survives mainly as scattered relicts, most of which were vegetatively propagated and
62 planted by humans (Koskela et al. 2004; Smulders et al. 2008b). National programmes
63 for the conservation of genetic resources have been established in many European
64 countries, under the collaborative EUFORGEN (European Forest Genetic Resources)

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5 65 programme (Frison et al. 1995), making black poplar a model species for *ex-* and *in-situ*
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7 66 conservation genetics (Lefèvre et al. 2001b).

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9 67 Like most poplar species, black poplar is dioecious and anemophilous. The seeds
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11 68 are released in considerable numbers, they have virtually no dormancy and need a
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13 69 substrate that is continuously wet for a four-week period to allow them to settle and
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15 70 establish (Guilloy-Froget et al. 2002). *P. nigra* is also capable of vegetative propagation
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17 71 when biotic (*e.g.*, human, birds) or abiotic (*e.g.*, flood, wind) disturbances lead to the
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19 72 stimulation of dormant primordia in the roots and shoots of either damaged plants or
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21 73 translocated fragments (Barsoum 2002). Levels of clonality ranging from 0 to 97% (*i.e.*,
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23 74 proportions of sampled trees with identical genotypes) have been reported in several
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25 75 natural European *P. nigra* populations (Arens et al. 1998; Barsoum 2002; Barsoum et
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27 76 al. 2004; Cottrell et al. 1997; Koskela et al. 2004; Legionnet 1997; Pospiskova and
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29 77 Bartakova 2004; Pospiskova and Salkova 2006; Rathmacher et al. 2010; Smulders et al.
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31 78 2008b; Storme et al. 2004; Winfield et al. 1998).

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37 79 To facilitate rigorous studies of population and conservation genetics, the
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39 80 frequency, spatio-temporal dynamics, and impacts of clonality must be known. Failing
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41 81 to consider clonality in studied populations can lead to erroneous conclusions,
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43 82 particularly when only a few genotypes predominate or when the sampling schemes
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45 83 used are inappropriate as a result. In addition, both theoretical and empirical studies
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47 84 have highlighted the ecological significance and evolutionary implications of clonality.
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49 85 Because vegetative regeneration is possible even when seedling establishment is
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51 86 impaired or rare, new habitats can be utilized and recovery from disturbances can
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53 87 commence; this has been extensively documented in the American aspen,
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55 88 *P. tremuloides* (Mock et al. 2008). Although no general trend has been established, it
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5 89 has been suggested that clonality affects population genetics parameters such as
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7 90 effective population size, linkage disequilibrium, and heterozygosity (Balloux et al.
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9 91 2003; Yonezawa et al. 2004). At the local scale, uneven spatial distribution of clonal
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11 92 ramets can generate spatial genetic structure (SGS) in established populations (Reusch
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13 et al. 1999). Clonality-driven SGS can have important consequences for reproduction in
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15 93 dioecious or self-incompatible species (Charpentier 2002). SGS can also occur in the
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17 94 absence of clonality as a consequence of limited gene dispersal (Epperson 2007;
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19 95 Vekemans and Hardy 2004) or selection in heterogeneous environments (Epperson
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21 96 1990).

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26 98 Cultivated poplars are considered to represent another threat to *P. nigra* in
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28 99 Europe; there are two reasons for this. First, they have the same water and soil
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30 100 requirements as autochthonous *P. nigra* populations, thus leading to habitat exclusion
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32 101 (Lefèvre et al. 2001a). Second, gene flow from cultivated trees may lead to
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34 102 introgression (also known as “introgressive hybridization”) from exotic species such as
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36 103 *P. deltoides* or *P. trichocarpa* or from allochthonous *P. nigra* gene pools (Cagelli and
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38 104 Lefèvre 1995; Vanden Broeck et al. 2005). *P. nigra* cv. Italica Du Roi (synonymous
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40 105 with *P. pyramidalis* Rozier, *P. italica* (Du Roi) Moench, and *P. fastigiata* Foug.), also
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42 106 known as the Lombardy poplar, is certainly the most ancient poplar cultivar and the one
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44 107 with the widest distribution. Although it was first reported in Lombardy, Italy, at the
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46 108 very beginning of the XVIIIth century, there has been some speculation about its origins
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48 109 (Wood 1994), the two main options being (i) that a mutation in *P. nigra* occurred in
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50 110 Italy and (ii) that it was introduced to Italy from Central Asia. Its timber has been used
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52 111 for building, but its columnar shape also makes it a notable visual element in the
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54 112 landscape. Five cuttings were introduced to France in 1745 and the first plantings were
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4 113 along the Loing canal (~100km from our study site) (Pelée de Saint-Maurice 1762)
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6 114 before Napoleon I promoted its planting across the Empire (Stettler 2009). It was
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8 115 introduced into England in 1758, and into the United States in 1784 (Wood 1994).
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10 116 Nowadays, despite its poor timber quality, the Lombardy poplar is commonly found in
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12 117 rural and urban landscapes across the temperate zone. It is currently unclear whether
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14 118 *P. nigra* cv. *Italica* is a single clone or if it comprises several genotypes that all exhibit
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16 119 the distinctive columnar habit. Although most *Italica*-like trees are males, a few female
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18 120 columnar *P. nigra* trees or cultivars have been reported, one of them being *P. nigra* L.
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20 121 cv. *Thevestina* (Dode) Bean. The reference cultivar found in the International Poplar
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22 122 Commission database (<http://www.populus.it/>) is a male and is referred to as “San
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24 123 Giorgio”. The Lombardy poplar is densely branched and is often planted as windbreaks
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26 124 or as single trees; thus it is supposed to be a major pollen producer. Moreover, since the
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28 125 cultivar is part of the *P. nigra* species, barriers against introgression into autochthonous
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30 126 wild *P. nigra* populations could be assumed to be low. A few previous studies have,
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32 127 however, reported introgression levels (*i.e.*, the proportion of potential F₁ siblings
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34 128 originating from *Italica*) of only 0 – 1.6% (Imbert and Lefèvre 2003, Tabbener and
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36 129 Cottrell 2003, Vanden Broeck et al. 2004).

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44 130 Here, we report on an exhaustive sampling strategy involving accurate
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46 131 geopositioning and SSR genotyping in a natural population belonging to a European
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48 132 Intensive Study Site (EVOLTREE ISS Loire – Zone 4) and representative of numerous
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50 133 *P. nigra* populations in France (*i.e.*, mature populations with significant levels of
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52 134 anthropogenic disturbance). Beyond the usual genetic diversity estimates, major outputs
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54 135 from this study include: (i) the quantification and spatial description of clonality using a
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56 136 recently defined standardized analysis framework; (ii) an evaluation of the proportion of
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4 137 SGS that is attributable to the clonal growth form; and (iii) the identification of
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7 138 Lombardy poplar introgression events with high confidence levels.
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10 11 140 **Materials and methods**

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15 16 142 Study site and plant material

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18 143 The study site (7 ha, 915 m long) is located alongside the Loire River near the city of
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20 144 Saint-Ay, France (47°51'N / 1°45'E) (Fig. 1). Part of it belongs to the Saint-Mesmin
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22 145 French National Natural Reserve. Aircraft laser altimetry (data from Direction
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24 146 Régionale de l'Environnement de l'Aménagement et du Logement, Service Loire et
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26 147 Bassin Loire Bretagne, Orléans, France, 2002) revealed a curvilinear depression
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28 148 suggestive of a past meander of the river (Electronic Supplementary material 1). We,
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30 149 therefore, hypothesize that most of the study site originates from a sandy island that
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32 150 once merged with the riverbank. This is a common phenomenon on this dynamic river
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34 151 system (Gautier and Grivel 2006). Aerial pictures from 1949 onwards (public domain
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36 152 data from Institut Géographique National, Paris, France) reveal that: (i) the merging
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38 153 occurred before 1949; (ii) mature *P. nigra* trees, although at lower densities, have been
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40 154 present since 1949; and (iii) the study area has not been cultivated during that period.
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42 155 Anthropogenic disturbance, however, is highly probable in this suburban area. It may
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44 156 have taken several forms, such as grazing, cutting fodder or fuel wood, dumping garden
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46 157 waste, and path clearing. Clearing is particularly obvious in the north-eastern extremity
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48 158 of the study site. The land adjacent to the river floods frequently, but most of the study
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50 159 site (north of the path) is located above usual flood level. Capillarity, however, can lead
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52 160 to temporary water accumulation in the lowest points of the depression during very
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5 161 severe flood events (Saint-Mesmin French National Reserve Administrator, pers.
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7 162 comm.). Black poplars represent at least 75% of the trees in the study area (amounting
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9 163 to 60 trees / ha). They are not restricted to this area as mature trees can be found on both
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11 164 sides of the river and also on most of the islands located nearby. Willows (*Salix alba* L.)
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13 165 compete with black poplars on the bank of the river. Other pioneer - and interestingly
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15 166 alien - tree species are found as scattered individuals (*Juglans regia* L., *Acer negundo*
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17 167 L.) or groups of trees (*Robinia pseudoacacia* L., *Prunus mahaleb* L.). Although
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19 168 considered to be post-pioneer species, the other trees that are present (*Quercus robur* L.,
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21 169 *Acer platanoides* L., *Acer pseudoplatanus* L., *Acer campestre* L., *Fraxinus excelsior* L.)
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23 170 are also indicative of an open-habitat. As expected in such an open space with
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25 171 heterogeneous soil conditions, more than 40 herbs, grasses and shrubs have been
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27 172 identified (Saint-Mesmin French National Reserve Administrator, pers. comm.). A
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29 173 significant part of the ground flora is indicative of high nitrogen availability (*Urtica*
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31 174 *dioica* L., *Lamium maculatum* L., *Galium aparine* L.). Hygrophilous species such as
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33 175 *Iris pseudacorus* L., *Glechoma hederacea* L. and *Agrostis stolonifera* L. are restricted to
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35 176 the flood-prone areas (south of the path), since water availability declines sharply with
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37 177 elevation.
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45 178 Except for a few seedlings immediately adjacent to the river, juvenile trees were
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47 179 absent. All sexually mature trees were inventoried (Fig. 1) and their location determined
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49 180 by triangulation using a DT610 electronic digital theodolite (Sokkia Topcon, Mâcon,
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51 181 France). When this technique could not be applied because of topographical constraints,
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53 182 a S500 centimeter precision surveying system was used instead (Leica Geosystems, Le
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55 183 Pecq, France). Sex was determined by looking at the flowers at various dates (>1
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57 184 observation date per individual).
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5 185 Height, using a Forestor Vertex dendrometer (Haglöf Sweden AB, Långsele,
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7 186 Sweden), and girth at breast height were recorded for all studied trees. In the case of
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9 187 multi-stemmed trees (*i.e.*, forking below breast height, or clumped trees with several
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11 188 trunks sprouting from a common base), the girth of each stem was measured and the
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13 189 maximum value recorded. Both parameters exhibited relatively Gaussian distributions.
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15 190 Height and girth ranged from 5.2 to 31.7 m and from 25 to 409 cm, respectively, and the
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17 191 two parameters were highly correlated (Electronic Supplementary Material 2).
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21 192 Tree ages were assessed for a sample of 20 single-stemmed individuals covering
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23 193 most of the observed range of variation in girth (individuals exceeding 250 cm girth
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25 194 could not be evaluated due to technical constraints). Increment core samples were
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27 195 collected at breast height. After drying, transverse longitudinal sections were cut from
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29 196 each core. Because core analysis of black poplar wood is very difficult, two assessors
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31 197 counted tree rings in a double-blind manner using 6x magnifying lenses. Microscopic
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33 198 analysis did not improve reliability since false-rings were even more likely to be
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35 199 mistaken for true rings. The mean divergence between operators was 20%, and the
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37 200 resulting tree ages (averages of the two estimates) varied between 9.5 and 52.5 years
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39 201 (Electronic Supplementary Material 3). The overall correlation with girth was
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41 202 sufficiently strong ($r_{\text{Spearman}} = 0.82$, Electronic Supplementary Material 3) to consider
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43 203 girth ranking as a good predictor of age ranking, at least for single-stemmed individuals.
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49 204 Many Lombardy poplars have been identified on both sides of the Loire River,
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51 205 in the urban area surrounding the study site, and within the study site itself. All of them
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53 206 are clearly planted ornamental trees. Thirteen large individuals close to the study site
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55 207 were selected for genotyping (Fig. 1). Eleven of these were located on a campsite south-
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57 208 west of the study area ($153 \leq \text{girth} \leq 255$ cm). Core-analysis was conducted on one of
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4 209 these (girth = 212 cm), and the resulting age estimate was 34.5 years. The two other
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7 210 individuals studied were growing very close to each other on the northern edge of the
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9 211 study site (girth = 130 and 134 cm).
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11 212 Young fresh leaf material was collected from the thirteen Lombardy poplars and
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13 213 the 413 *P. nigra* trees in the inventory and stored at -80 °C whilst awaiting DNA
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15 214 extraction. Each stem of clumped trees was sampled to verify that they represented a
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17 215 single genotype.
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23 217 DNA extraction and SSR analysis
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28 219 DNA was extracted from single leaves using a DNeasy 96 Plant Extraction kit (Qiagen,
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30 220 Courtaboeuf, France) according to the manufacturer's instructions.
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33 221 Genotyping was based on the following 11 unlinked nuclear SSRs (with their
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35 222 corresponding linkage group): PMGC2852 (I), PMGC667 (II), PMGC486 (III),
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37 223 PMGC2235 (IV), PMGC2838 (V), PMGC2578 (VI), PMGC61 (VIII), PMGC333 (XI),
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39 224 PMGC14 (XIII), PMGC433 (XVI) (<http://poplar2.cfr.washington.edu>), and WPMS05
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41 225 (XII) (Smulders et al. 2001; Van der Schoot et al. 2000).
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45 226 The Polymerase Chain Reaction was carried out in a volume of 10 µL, which
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47 227 contained 1 µL template DNA and 9 µL of the following mix: 1 × PCR buffer, 1.5 mM
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49 228 MgCl₂, 62.5 µM dNTPs mix (all from Invitrogen, Cergy-Pontoise, France), 0.2 µM
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51 229 primers (Eurofins MWG Operon, Ebersberg, Germany), 0.02 µM fluorescently labelled
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53 230 forward primer with either 6-FAM, HEX (Eurofins MWG Operon) or NED (Applied
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55 231 Biosystems, Courtaboeuf, France) fluorescent dyes, and 0.25 U *Taq* polymerase
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57 232 (Invitrogen). Amplification was conducted in a GenAmp 9700 thermocycler (Applied
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233 Biosystems) for 30 cycles, each with the following profile: a 30 s DNA denaturation
 234 step at 94 °C, a 30 s annealing step at 50 or 55 °C depending on primers, and a 60 s
 235 extension step at 72 °C. The final extension step was extended to 6 min.

236 As the last denaturation step, a mix containing 2 µL amplified DNA, 7 µL
 237 Formamide and 0.25 µL 400HD-Rox size marker (Applied Biosystems) was maintained
 238 at 95 °C for 3 min. The fragment separation was then performed in an ABI Prism 3100
 239 Genetic Analyser (Applied Biosystems). The software GENOTYPER 3.7 (Applied
 240 Biosystems) was used to score the SSR data.

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242 Clonality detection and description

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244 Identification of multilocus genotypes (MLG) and multilocus lineages (MLL) was
 245 based on procedures implemented in GENCLONE 2.0 (Arnaud-Haond and Belkhir 2007)
 246 and followed the standardized method proposed by Arnaud-Haond et al. (2007).

247 The genotypic resolution associated with each possible combination of analysed
 248 loci was computed as the resulting number of distinct MLGs (Arnaud-Haond et al.
 249 2005).

250 Keeping only one ramet per identified MLG, and taking into account departures
 251 from Hardy-Weinberg equilibrium as measured by Wright's inbreeding coefficient (F_{is}),
 252 the probability (p_{gen}) of occurrence of each observed genotype was estimated according
 253 to Young et al. (2002):

$$254 \quad p_{gen}(F_{is}) = \prod_{i=1}^l [(f_i g_i)(1 + z_i F_{is(i)})] 2^h$$

255 where l is the number of loci, h is the number of heterozygous loci, f and g are ‘round-
 256 robin’ allelic frequency estimates of the observed alleles f and g at the i^{th} locus, and
 257 $z_i = 1$ (or -1) if the i^{th} locus is homozygous (or heterozygous).

258 When n ramets with a genotype identical to a previously encountered MLG are
 259 detected in a sample population (N), the probability (p_{sex}) of these being derived from
 260 distinct reproductive events can be estimated following Parks and Werth (1993):

$$261 \quad p_{sex}(F_{is}) = \sum_{i=n}^N \frac{N!}{i!(N-i)!} [p_{gen}(F_{is})]^i [1 - p_{gen}(F_{is})]^{N-i}$$

262 The significance of p_{sex} was considered from the first re-encounter ($n = 1$).

263 To ascertain the uniqueness of MLGs with missing data (*i.e.*, unamplified loci),
 264 such MLGs were examined on a case-by-case basis after removing the missing loci
 265 from the entire dataset. Based on the recalculated p_{sex} estimates, these MLGs were either
 266 designated as being unique or were pooled with another MLG into a MLL. Although
 267 somatic mutations can be hypothesized, a similar approach was used to group MLGs
 268 that differed at only one locus into MLLs, in order to account for possible scoring
 269 errors.

270 The genotypic richness (R) of the population was computed as $R = (G - 1) / (N -$
 271 $1)$ where G is the number of MLLs, and N the number of sampled trees (Dorken and
 272 Eckert 2001).

273 For subsequent analyses at the MLL level, $MLL_{\geq 3}$ (with three or more ramets)
 274 were reduced to their dominant genotype while $MLL_{=2}$ (with two ramets) were
 275 assigned either (i) the heterozygous genotype at the mismatching locus if the other
 276 genotype was homozygous (*i.e.*, accepting the miscoded homozygote hypothesis) or (ii)
 277 the genotype with the most frequent allele at the locus that differed (*i.e.*, accepting the
 278 somaclonal mutation hypothesis).

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4 279 In order to characterize the MLL size (N_R , number of ramets) frequency
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6 280 distribution, a cumulative function of the Pareto distribution was fitted to the data as
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9 281 proposed by Arnaud-Haond et al. (2007). This function takes the form $F_{\geq X} = const. \cdot X^{-\beta}$
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11 282 where $F_{\geq X}$ is the frequency of ramets belonging to a MLL $_{\geq X}$ (with X or more ramets).
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14 283 The shape parameter (β), also called the patchiness exponent, measures the relative
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16 284 importance of large vs. small MLLs. β increases exponentially with increasing evenness
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19 285 of distribution. A graphical representation of $\log(F_{\geq X})$ vs. $\log(X)$ and its associated
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21 286 coefficient of determination r^2 were generated to check the quality of the Pareto
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24 287 approximation.

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26 288 Two spatial descriptors were computed for each MLL: (i) d_{max} , the maximum
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29 289 distance between ramets, and (ii) $\bar{d}_{neighb.}$, the average distance between nearest
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32 290 neighbours. The relationships between N_R and these two parameters were investigated.

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34 291 The aggregation index (A_c) proposed by Arnaud-Haond et al. (2007) was
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36 292 calculated using GENCLONE 2.0 as:

$$37 \quad 293 \quad A_c = (p_{sg} - p_{sp}) / p_{sp}$$

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40 294 where p_{sg} is the average probability of clonal identity of all sample unit pairs and p_{sp} is
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43 295 the average probability of clonal identity among pairwise nearest neighbours. The
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46 296 significance of A_c was assessed by a 10000-permutation test.

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48 297 In order to quantify the degree of intermingling between MLLs, the clonal
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51 298 dominance index (D_c) was calculated following Ohsako (2010) for each MLL $_{\geq 3}$ as:

$$52 \quad 299 \quad D_c = (N_R - 1) / (N_T - 1)$$

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55 300 where N_R is the MLL size (number of ramets) and N_T is the total number of trees present
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58 301 within the minimal convex envelope containing all ramets of the MLL.

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5 303 Detection of introgression from the Lombardy poplar
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9 305 CERVUS 3.0.3 (Marshall et al. 1998) was used to detect potential F₁ hybrids of the
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11 306 Lombardy poplar in the identified MLLs. The multilocus profile of each Lombardy
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13 307 poplar tree examined was tested for parentage assignment by simple exclusion (Jones
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15 and Ardren 2003). The following two criteria were applied for each pairwise
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17 308 comparison: (i) a minimum of eight typed loci in common, and (ii) a maximum of one
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19 309 mismatch corresponding to putative false homozygote coding. Individual probabilities
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21 310 of non-exclusion ($p_{non-excl.}$) with both parents unknown were calculated using CERVUS
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23 311 3.0.3 according to Jamieson and Taylor (1997).
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30 314 Genetic diversity
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35 316 Deviations from a 1 : 1 sex-ratio were assessed at tree and MLL levels using chi-square
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37 317 tests.

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40 318 ARLEQUIN 3.5 (Excoffier et al. 2005) was used to compute neutral genetic
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42 319 diversity parameters at the MLL level: observed (H_o) and expected (H_e) heterozygosities
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44 320 (Nei 1978), number of alleles per locus (A), effective number of alleles per locus (A_e)
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46 321 (Hartl and Clark 1997), and Wright's inbreeding coefficient (F_{is}) per locus and sample
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48 322 (Weir and Cockerham 1984). Departures from Hardy-Weinberg equilibrium were
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50 323 revealed by bilateral exact tests on F_{is} .
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55 325 Spatial genetic structure
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5 327 SGS was explored by spatial autocorrelation analysis using GENCLONE 2.0. Multilocus
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7 328 kinship coefficients (F_{ij}) according to Loiselle et al. (1995) were computed for all pairs
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9 329 of sampling units (*i.e.*, trees or MLLs). F_{ij} values were averaged within a given distance
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11 330 class d to produce $\overline{F}_{(d)}$ values. In an isotropic bi-dimensional space, the pairwise
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14 331 genetic relationships between sample units are expected to vary linearly with the natural
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16 332 logarithm of the geographic distance. The S_p statistic defined by Vekemans and Hardy
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18 333 (2004), which enables comparisons among species independent of the sampling scheme,
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20 334 was calculated as $-\hat{b}_F / (1 - \overline{F}_{(1)})$; where \hat{b}_F is the slope of the linear regression of $\overline{F}_{(d)}$
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22 335 on the natural logarithm of the geographic distance, and $\overline{F}_{(1)}$ is the mean F_{ij} over the
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24 336 first distance class. In this formula, the first distance class is supposed to contain all
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26 337 (nearest) neighbour pairs. Since 98% of neighbour pairs of trees were in the 0 – 20 m
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28 338 distance class, the distance limits were set to 20, 30, 40, 50, 100, 200, 300, 400, 500,
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30 339 and 1000 m.
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37 340 To assess the potential impact of clonal growth form on SGS, a preliminary
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39 341 analysis was performed at the tree level, in which all ramets within a MLL were
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41 342 assigned the same genotype; a second analysis was then performed at the MLL level. In
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43 343 the first analysis, the significance of $\overline{F}_{(d)}$ and \hat{b}_F were assessed by 10000-permutation
44
45 344 tests based on the geographic locations of trees. In the second analysis, a 10000-
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47 345 resampling approach was used, in which one ramet was randomly selected from each
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49 346 MLL at each resampling step (Alberto et al. 2005). This yielded a 95% confidence
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51 347 interval for $\overline{F}_{(d)}$ for each distance class. The significance of \hat{b}_F was assessed as above.
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59 349 **Results**
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5 3506
7 351 Clonality8
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11 353 The genotypic resolution followed an asymptotic trend (Fig. 2), where the gain from
12 354 using additional markers increased sharply between one and four loci and appeared to
13 355 stabilize at very low values when there were more than six loci (*i.e.*, less than 5%
14 356 additional MLGs identified per additional locus).

15
16 357 Among the 413 trees, we were able to genotype 379 fully at the 11 SSR loci and
17 358 these clustered into 222 MLGs. All ramets within a MLG were associated with a p_{sex}
18 359 value below 10^{-7} . The 34 remaining trees had one (22), two (8), three (3) or four (1)
19 360 loci missing. By sequentially removing the missing loci before re-analysing the data, it
20 361 was possible to assign 22 of these trees to previously identified MLGs ($p_{sex} < 10^{-5}$). By
21 362 sequentially removing the mismatched loci for MLGs differing at only one locus, these
22 363 MLGs could be clustered into 37 MLLs ($p_{sex} < 10^{-5}$). A total of 194 distinct MLLs were
23 364 therefore identified, of which 79 $MLL_{\geq 2}$. The resulting genotypic richness (R) was
24 365 0.47. Sex data were consistent with this grouping since all ramets within a MLL were of
25 366 the same gender.

26
27 367 MLL size (N_R) ranged from one to 18 ramets, but 95 % of MLLs contained
28 368 fewer than five ramets (Fig. 3). The logarithm of the cumulative distribution of ramets
29 369 among MLLs was significantly linearly related to the logarithm of N_R (Fig. 3), thus
30 370 supporting the Pareto distribution hypothesis. The associated patchiness exponent
31 371 estimate was $\beta = 1.07$.

32
33 372 Clonality appeared to be evenly distributed through the study site since
34 373 differential plotting of individuals belonging to unreplicated genotypes, to small MLLs

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4 374 and to large MLLs did not reveal any structured geographical pattern (Fig. 4). MLL
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6 375 geographic size, as measured by the maximum distance between two ramets (d_{max}),
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8 376 ranged from 0.9 to 30.3 m. The intra-MLL average distance between nearest neighbours
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11 377 (\bar{d}_{neighb}) ranged from 0.9 to 18.6 m. A significant linear relationship was found between
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14 378 N_R and d_{max} (Fig. 5). Although resulting in a non significant linear correlation
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17 379 coefficient, a triangular relationship was found between N_R and \bar{d}_{neighb} : MLLs with few
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20 380 ramets were associated with a large range of \bar{d}_{neighb} values while low \bar{d}_{neighb} values
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23 381 (≤ 5 m) were consistently found in MLLs containing six or more ramets (Fig. 5). A
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25 382 similar relationship was found between N_R and mean or individual tree girth (single-
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27 383 stemmed individuals only): high ramet numbers were associated with low girths while
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30 384 MLL sizes ranging from one to five were associated with a large range of girth values
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32 385 (Fig. 6).

34 386 The estimated aggregation index (A_c) was 0.62 ($P < 0.001$), indicating
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36 387 significant spatial clustering of clonal ramets compared to the whole population. The
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39 388 mean clonal dominance index (\bar{D}_c) was 0.99, indicating that the spatial range of a MLL
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42 389 was almost exclusively occupied by ramets belonging to that MLL. This parameter
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44 390 differed from 1 in only two MLL $_{\geq 3}$ ($D_c = 0.67$ and 0.71).

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49 392 Introgression from the Lombardy poplar

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53 394 Of the thirteen Lombardy poplars sampled, eleven were similar to the San Giorgio
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55 395 reference genotype at all studied loci. Although belonging to the main group of eleven
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57 396 trees forming a row on a campsite nearby, the two others differed from the San Giorgio
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60 397 genotype at one and two loci, respectively. These differences always corresponded to

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4 398 one-repeat-unit changes and were restricted to one allele per differing locus. Genotyping
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7 399 newly collected leaves led to the same results, suggesting somatic mutations had
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9 400 occurred before planting.

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11 401 Five $MLL_{=1}$ (two males and three females, girth = 41, 159, 164, 189, and 208 cm) were
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13 402 identified as possible F_1 hybrids of the San Giorgio genotype. Some alleles from this
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15 403 genotype were found at very low frequencies in the MLLs (*e.g.*, $f = 0.008$ at locus
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18 404 PMGC2852), thus resulting in low probabilities of false paternity assignment
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21 405 ($7.7 \times 10^{-5} \leq P_{non-excl.} \leq 2.7 \times 10^{-3}$). None of the identified introgressed hybrids
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23 406 exhibited the typical columnar shape of the Lombardy poplar. Core-analysis of three of
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25
26 407 them revealed ages of 12.5, 53, and 45.5 years (girth = 41, 159, and 208 cm,
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28 408 respectively). All five individuals were removed from subsequent analyses. No potential
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31 409 F_1 progeny from any of the two identified somaclonal mutants of San Giorgio was
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33 410 found.

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37 412 Genetic diversity

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42 414 The sex-ratio was 1 : 0.92 on an individual tree basis, which was not significantly
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44 415 different from a 1 : 1 ratio (Table 1). As no significant difference was found between
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46 416 males and females for both the number of $MLL_{\geq 2}$ (40 and 39, respectively) and the
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48
49 417 mean number of ramets per $MLL_{\geq 2}$ (i.e., $N_R = 4.1$ and 3.4, respectively, $P = 0.50$), the
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51 418 sex-ratio was also balanced at the MLL level (Table 1).

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54 419 The level of polymorphism was highly variable among the 11 studied loci,
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56 420 ranging from four (PMGC333) to 22 alleles (PMGC667). High rates of rare alleles led
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59 421 to a two-fold difference between mean observed and effective allele numbers, \bar{A} and
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5 422 \bar{A}_e (Table 2). Compared to the nine other loci, PMGC433 and PMGC2838 combined
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7 423 low polymorphism, high rates of rare alleles, and (possibly as a consequence) lower
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10 424 observed and expected heterozygosities, H_o and H_e . Mean \bar{H}_o and \bar{H}_e values were
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12 425 very close, leading to a non-significant overall F_{is} (Table 2). Two loci (PMGC2852 and
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14 426 PMGC333) exhibited significant heterozygote excess and three (PMGC667,
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16 427 PMGC2838 and WPMS05) significant deficit (Table 2).
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429 Spatial genetic structure

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26 431 At the tree level, the regression of F_{ij} over the natural logarithm of the
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28 432 geographic distance produced a significantly negative regression slope ($\hat{b}_F = -0.0263$,
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30 433 $P < 0.001$), indicating higher genetic similarity among trees that were closer together. A
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32 434 significant positive mean kinship coefficient was found in the first distance class only
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34 435 ($d_I = 0 - 20$ m, $\bar{F}_{(1)} = 0.1870$; Fig. 7). At the MLL level, the kinship - distance
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36 436 regression slope was much shallower, but still significant ($\hat{b}_F = -0.0045$, $P = 0.001$;
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38 437 Fig. 7). The S_p statistic was seven-fold smaller at the MLL level than at the tree level,
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40 438 decreasing from 0.0324 to 0.0046, while $\bar{F}_{(1)}$ decreased to 0.0230.
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440 **Discussion**

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54 442 SSRs have proved efficient in poplars for fingerprinting and for detecting
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56 443 introgression from different species (Fossati et al. 2003; Liesebach et al. 2010;
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58 444 Smulders et al. 2008a). Despite the fact that it belongs to the *P. nigra* species, the
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4 445 Lombardy poplar (*i.e.*, the San Giorgio reference genotype) carried some alleles that
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6 446 were comparatively rare in the studied *P. nigra* population. This allowed us to consider
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8 447 2.6% of MLLs being probable F₁ hybrids of this cultivar with low probabilities of false
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10 448 paternity assignment. Of course, these probabilities are based on the hypothesis that the
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12 449 allelic frequencies observed within the studied population are representative of the
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14 450 population's parental gene pool. However, poplar seeds are dispersed by water over
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16 451 long distances, and Lombardy poplars are very frequent in rural and urban landscapes of
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18 452 the Loire Valley. It is thus expected that introgression events, if any, would most
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20 453 probably originate from crosses upstream of the study site. This idea is supported by age
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22 454 inconsistencies between the studied Lombardy poplar trees and most of the probable
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24 455 introgressed F₁ individuals, and also by the fact that the two Lombardy poplar
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26 456 somaclonal mutants found at close vicinity of the study site were not found to be
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28 457 potential parents of any studied tree. When trying to identify introgression events from
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30 458 the Lombardy poplar in natural *P. nigra* stands, Imbert and Lefèvre (2003) also reported
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32 459 rare alleles at one SSR locus but only mentioned a rough estimate of a few percent
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34 460 introgressed genotypes. Other studies have reported introgression levels between 0%
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36 461 (Tabbener and Cottrell 2003), and 1.6% (Vanden Broeck et al. 2004). Both these studies
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38 462 concluded that there was a negligible threat to local black poplar populations because of
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40 463 the early flowering of the Lombardy poplar, and a consequent lack of synchronism with
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42 464 *P. nigra* females of northern origin. We do not share this optimistic point of view for
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44 465 two main reasons, namely (i) an underestimation of introgression rates due to the fact
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46 466 that advanced-generation intraspecific hybrids cannot be detected with high levels of
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48 467 confidence and (ii) weak support for the asynchronism hypothesis in a species with a
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50 468 wide distribution area, especially in the context of a changing climate. Moreover, the
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4 469 five probable identified introgressed individuals were not recognizable based on their
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7 470 phenotype with respect to branching. We thus suspect that genotyping existing *ex-situ*
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9 471 collections of *P. nigra* to check for possible introgression from *Italica* would produce
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11 472 surprising results.
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14 473 In previous studies, SSR analysis of commercial cultivars from different taxa
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16 474 (Fossati et al. 2003; Liesebach et al. 2010) and natural *P. nigra* stands (Barsoum et al.
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18 475 2004; Pospiskova and Bartakova 2004; Pospiskova and Salkova 2006; Rathmacher et
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20 476 al. 2010; Smulders et al. 2008b) allowed detection of replicated genotypes. When
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22 477 considering the evolution of marginal gain in terms of additional differentiated MLGs
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24 478 per additional locus, the 11 SSRs used in the present study allowed a genotypic
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26 479 resolution close to optimum. Indeed, although ‘clonality is merely a genotype resolution
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28 480 phenomenon dependent upon the resolution power of molecular markers culminating
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30 481 with direct sequencing of DNA’ (Lushai and Loxdale 2002), increasing the number of
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32 482 markers not only allows the detection of rare somatic mutation events but also increases
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34 483 the chance of scoring errors occurring. Somatic mutations are expected to occur at
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36 484 significant rates for SSRs, for which high mutation rates ranging from 10^{-7} to 10^{-3} per
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38 485 locus per generation have been reported in eukaryotes (Buschiazzo and Gemmell 2006).
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40 486 As an illustration, two somatic mutants were identified among the thirteen Lombardy
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42 487 poplars analysed here. However, using the standardized procedure proposed by Arnaud-
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44 488 Haond et al. (2007), MLGs differing at only one locus were grouped into MLLs despite
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46 489 their somatic-mutant *vs.* scoring-error status. Reviewing the data, it appears that there
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48 490 are only four circumstances out of 45 for which a mutational event corresponding to the
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50 491 Stepwise Mutation Model could be hypothesized (*i.e.*, both MLGs heterozygous with a
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52 492 one-repeat allelic difference). Somatic mutations may be useful tools, acting as a
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4 493 molecular clock in many clonal cells or organisms including poplars (Ally et al. 2008;
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6 494 Mock et al. 2008). Nevertheless, there are many pitfalls in their analysis including (i) a
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8 495 lack of knowledge about mutation rates during mitosis, (ii) a complex heterogeneity of
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10 496 mutational events at allele, locus, individual and/or taxon levels, and, again, (iii) the
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12 497 difficulty in distinguishing between true somatic mutations and scoring errors (Heinze
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14 498 and Fussi 2008).

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18 499 The population studied exhibited substantial asexual recruitment. If one ramet
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20 500 per MLL represented a potential founder, then 53% of the population originated from
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22 501 vegetative propagation. The genotypic richness ($R = 0.47$) was intermediate within the
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24 502 range of values found in other *P. nigra* studies (or computed from them when not
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26 503 originally expressed as $G - 1 / N - 1$). Considering clumped trees to be clonal ramets, as
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28 504 suggested by Barsoum et al. (2004), would lead to even lower genotypic richness
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30 505 values. R values across all studied European *P. nigra* stands found in the literature
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32 506 appear to follow a distribution skewed towards higher values, with fifteen values out of
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34 507 nineteen falling between 0.8 and 1 and only three occurrences below 0.2 (Arens et al.
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36 508 1998; Barsoum et al. 2004; Legionnet 1997; Pospiskova and Bartakova 2004;
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38 509 Pospiskova and Salkova 2006; Rathmacher et al. 2010; Smulders et al. 2008b). Very
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40 510 low values of 0.01 and 0.04 have also been reported in mature stands in Great Britain
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42 511 (Smulders et al. 2008b) and in the Netherlands (Arens et al. 1998), respectively,
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44 512 although both sampling schemes were designed to avoid collecting clonal individuals.
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46 513 In contrast, despite a nearest neighbour sampling strategy, Barsoum et al. (2004) found
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48 514 high R values (> 0.8) in three age cohorts, with a significantly higher number of clonal
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50 515 ramets in the 'middle-aged' stands (8 years old) than in both the 'young' (5.6 years old)
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52 516 and 'old' (17.6 years old) stands. Sampling in this previous study covered islands and
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4 517 gravel bars, each of them having certainly been more favourable (spatially and
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7 518 temporally) for seedling recruitment and also less affected by anthropogenic disturbance
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9 519 than our study site. Tree densities were consistently higher on the islands and gravel
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11 520 bars than those recorded in Saint-Ay (0.2 trees.m⁻² in ‘old’ stands vs. 0.006 trees.m⁻² in
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13 521 the current study), and the existence of more dynamic sites certainly explained why the
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15 522 ‘old’ cohorts encountered by Barsoum et al. (2004) were much younger than most
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17 523 individuals examined by us. Vegetative propagation in Saint-Ay certainly benefited
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19 524 from the availability of open space, although the sites available for colonization were
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21 525 generally unfavourable (spatially and temporally) for seedling recruitment.
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26 526 The MLL size (N_R) distribution was skewed towards smaller values, ranging
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28 527 from one to 18 ramets and exhibiting exponential decay. In previous studies, only small
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30 528 clones of two to four ramets have been observed (Barsoum et al. 2004; Legionnet 1997;
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32 529 Rathmacher et al. 2010) while Arens et al. (1998) and Smulders et al. (2008b) found
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34 530 larger clones of up to 22 and 32 ramets, respectively. A clone size of 70 ramets was
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36 531 recently reported in a British population, but this was probably planted (Smulders et al.
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38 532 2008b). The β Pareto index associated with the partitioning of ramets among MLL size
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40 533 classes should allow reliable comparisons between studies. The present study provides a
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42 534 first estimate of β in *P. nigra*. The calculated value (1.07) was moderate in comparison
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44 535 with those presented in a literature review pertaining to several clonal species (Arnaud-
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46 536 Haond et al. 2007). These authors reported extreme values of 0.06 (*Posidonia oceanica*)
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48 537 and 2.96 (*Sinularia flexibilis*), indicating dominance of some large clonal patches and
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50 538 high evenness, respectively. They also provided the only reference available for a tree
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52 539 species, namely *Prunus ssiiori* ($\beta = 0.88$).
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4 540 Considering within-clone, between-clone, and between-species contacts, Lovett
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7 541 Doust (1981) recognized a spectrum of growth forms in clonal plants, with the two
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9 542 extremes referred to as ‘phalanx’ and ‘guerrilla’ forms. The high aggregation
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11 543 ($A_c = 0.62$) and clonal dominance ($D_c = 0.99$) indexes computed in the present study
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13 544 allow us to conclude that *P. nigra* exhibits a typical ‘phalanx’ growth form, where
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15 545 ramets of the same MLL are aggregated and do not share their space with ramets of any
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17 546 other MLL. Despite being less explicit, all published data on *P. nigra* clonal growth are
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19 547 also indicative of a ‘phalanx’ growth form with zero or near-zero intermingling of
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21 548 clones (Barsoum et al. 2004; Legionnet 1997). Although only possible with non-
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23 549 exhaustive sampling strategies, larger study areas have allowed the identification of
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25 550 long distance dispersal events up to 19 km (Barsoum et al. 2004), while the maximum
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27 551 distance found in the current study was $d_{max} = 30.3$ m. The significant positive
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29 552 correlation between N_R and d_{max} and the absence of a significant correlation between N_R
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31 553 and $\bar{d}_{neighb.}$ may indicate that clonal growth in this open habitat is an expansion process
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33 554 rather than one that leads to a densification of clonal patches. The triangular
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35 555 relationships found between N_R and both $\bar{d}_{neighb.}$ and girth need further examination,
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37 556 however. The fact that the MLLs with high ramet numbers comprised small trees
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39 557 growing close together could be the result of either poor-quality, stressful, micro-habitat
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41 558 conditions promoting vegetative propagation, or a possible genotypic trade-off between
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43 559 the number and size of ramets, as found in other clonal species (Stuefer et al. 2002),
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45 560 Although the whole study site appeared to be favourable for clonal propagation, and
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47 561 although a significant correlation was found between girth and age, the first hypothesis
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49 562 cannot be rejected. More precise tree ages and thus, more detail pertaining to intra-MLL
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51 563 age structure, would facilitate investigations and interpretations. Core analysis is,
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4 564 however, very difficult in *P. nigra* wood, as experienced here, and root age would
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7 565 certainly be more informative than stem age when studying clonal growth. It has been
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9 566 hypothesized that flood training is a key mechanism of asexual regeneration in *P. nigra*
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11 567 (Barsoum et al. 2004), but we did not observe the linear ramet distributions associated
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13 568 with this type of sprouting frequently at the study site. Although no excavation was
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15 569 conducted, root suckering seems the most probable type of vegetative spread on this
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17 570 site. The aggregated pattern could thus result from the emergence of new shoots from
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19 571 the parental root system and be maintained by the selective advantage of permanent or
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21 572 at least transient physiological integration (*i.e.*, physical links between ramets) over
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23 573 fragmentation, as expected in habitats with restricted favourable patches compared to
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25 574 unfavourable ones (Oborny and Kun 2002). However, inferring the temporal dynamics
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27 575 of clonal growth from spatial structure at a single time point can be problematic for
28
29 576 three reasons: (i) the difficulty of disentangling the timing of the colonization process
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31 577 from density-dependent events (*e.g.*, both recent colonization in an empty space and
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33 578 competitive exclusion can result in a segregated distribution); (ii) there are possible
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35 579 trade-offs between clonal growth forms of a given species under different
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37 580 environmental conditions (Ye et al. 2006); and (iii) community-level analysis, including
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39 581 among-species interactions, is required (Gough et al. 2002).
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47 582 SSR-based observed and expected heterozygosities found in the literature for
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49 583 *P. nigra* vary within the ranges 0.67 – 0.93 and 0.65 – 0.90, respectively (Fossati et al.
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51 584 2003; Imbert and Lefèvre 2003; Pospiskova and Bartakova 2004; Pospiskova and
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53 585 Salkova 2006; Rathmacher et al. 2010; Smulders et al. 2008b; Storme et al. 2004; Van
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55 586 Dam and Bordacs 2002). The values reported here ($\overline{H}_o = 0.68$, $\overline{H}_e = 0.69$) are very
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57 587 close to the lower limits. Overall, the value of F_{is} (0.008, n.s.) did not indicate any
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4 588 significant deviation from Hardy-Weinberg equilibrium. Since no significant difference
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7 589 was found between male and female vegetative propagation potentials, the sex-ratios
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10 590 were equally balanced at both the tree and MLL levels. We are not aware of any
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12 591 previously published data on the relative vegetative propagation potential of the two
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14 592 genders of *P. nigra*.

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16 593 Clonality was the main driver of SGS in the studied population. In total, 90% of
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18 594 the identified $MLL_{\geq 2}$ exhibited a d_{max} falling within the distance range of significant
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21 595 kinship coefficients (F_{ij}) found at the tree level (0 – 20 m). Both the slope (\hat{b}_F) of the
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23 596 linear regression of F_{ij} over the natural logarithm of geographic distance and the
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25 597 associated Sp statistic sharply decreased at the MLL level. The presence of significant
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28 598 residual SGS at the MLL level is consistent with two recent reports relating to *P. nigra*
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30 599 (Pospiskova and Salkova 2006; Rathmacher et al. 2010). Although both studies
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32 600 excluded clonal ramets from the analysis, they reported higher values for both Sp
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34 601 (0.0166 and 0.0146 vs. 0.0046 in the present study) and \hat{b}_F (-0.0158 and -0.0136 vs. -
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36 602 0.0045). The scale of these previous studies was, however, much larger (5 km and
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38 603 2.5 km, respectively), possibly leading to a sub-structuring of populations as suggested
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40 604 by significant positive overall F_{is} values (*i.e.*, the Wahlund effect). When calculating Sp
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42 605 statistics for 47 plant species, Vekemans and Hardy (2004) found values ranging from
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44 606 0.0003 to 0.2632. They pointed out that the breeding system, life form (*i.e.*, herbaceous,
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46 607 small trees or trees), and population density were statistically linked to patterns of SGS.
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48 608 When considered in isolation, pollen and seed-dispersal modes were not found to be
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50 609 good predictors. Epperson (2007) expected unbalanced seeds vs. pollen dispersal
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52 610 patterns to generate SGS, but experimental and theoretical data do not fully support
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54 611 such a general trend (Ng et al. 2006; Sagnard et al. 2010). Results found in the literature

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4 612 from paternity (pollen) and parent-pair (seeds) assignments in *P. nigra* are scarce and
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6 613 highly variable. Pospiskova and Salkova (2006) reported maximum distances for pollen
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8 614 and seed dispersal of 230 and 370 m, respectively. Rathmacher et al. (2010) found that
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10 615 50% of the pollen and 30% of the seeds of the species travelled more than 500 m.
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14 616 *In-* and *ex-situ* conservation can both benefit from a better description of
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16 617 clonality. Past samplings in natural *P. nigra* stands across Europe for *ex-situ*
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18 618 conservation have yielded a gene bank collection with 26% duplicated accessions
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20 619 (Storme et al. 2004). Although some of the populations studied were probably
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22 620 composed of vegetative copies propagated by humans and distributed over large areas
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24 621 through cuttings, inappropriate sampling schemes certainly also contributed to this
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26 622 result. Most studies on black poplar, including this one, reported a ‘phalanx’ growth
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28 623 form usually with small numbers of ramets per clone. Consequently, duplications could
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30 624 be minimized by using an appropriate sampling mesh in combination with sex
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32 625 determination whenever possible. However, the deployment of high-throughput
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34 626 molecular techniques would allow efficient detection of clones at limited cost: as few as
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36 627 six SSR markers proved sufficient to identify 95% of the MLGs in the present study.
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38 628 With respect to *in-situ* conservation, clone size (N_R) distribution may be a critical factor
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40 629 to take into account since the larger the number of ramets, the longer the clone may
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42 630 survive under a gap disturbance regime, as has been simulated for *P. tremuloides*
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44 631 (Namroud et al. 2006). However, these simulations were not spatially explicit. At the
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46 632 species – rather than genotype – level, clonal growth form has been shown to affect
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48 633 competitiveness in a plant community, with ramet aggregation reducing the competitive
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50 634 ability of a clonal species in an open environment (Lenssen et al. 2005). Clustering can
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52 635 also affect mating patterns in dioecious species (Charpentier 2002). In addition, possible
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4 636 trade-offs between sexual and asexual fecundities may occur, as documented for other
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7 637 species (Sun et al. 2001) with different implications at the tree, clone or population
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9 638 levels.

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14 640 **Acknowledgments**

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27
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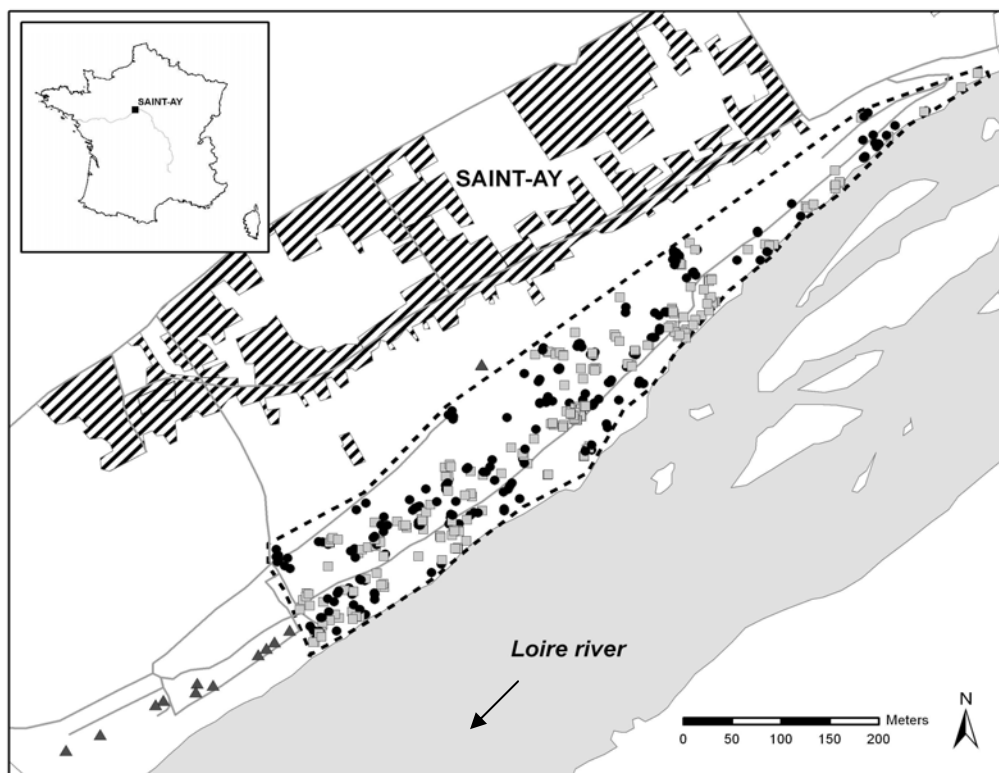


Fig. 1 – Study site (dotted line). Exhaustive inventory of adult trees within this 7 ha area revealed 199 female (black circles) and 214 male (grey squares) wild *P. nigra* trees. Triangles refer to the 13 sampled Lombardy poplars (non exhaustive inventory) used for genotyping and paternity analysis.

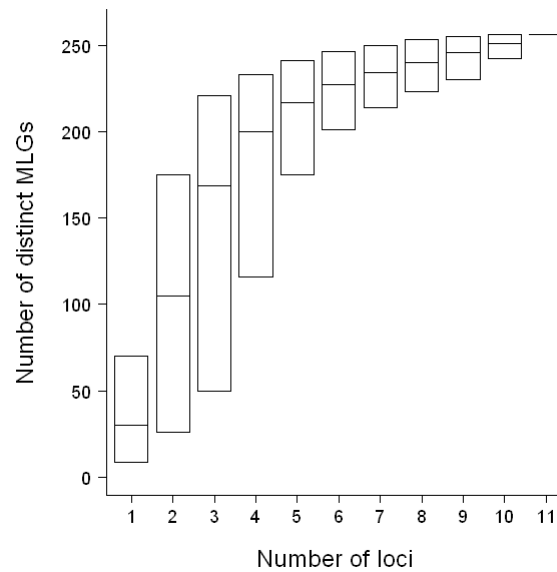


Fig. 2 – Genotypic resolution associated with each possible SSR combination. The boxes are bounded by the most and least informative combinations of loci. The inner line represents the mean value.

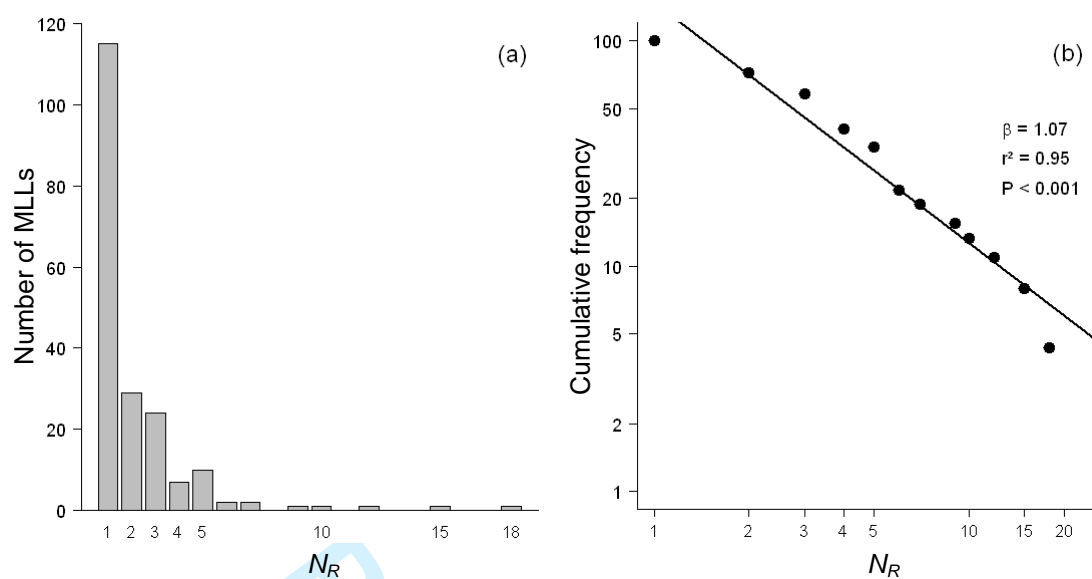


Fig. 3 – (a) Distribution of MLL size classes (N_R , number of ramets) and (b) associated log-log reverse cumulative frequency distribution.

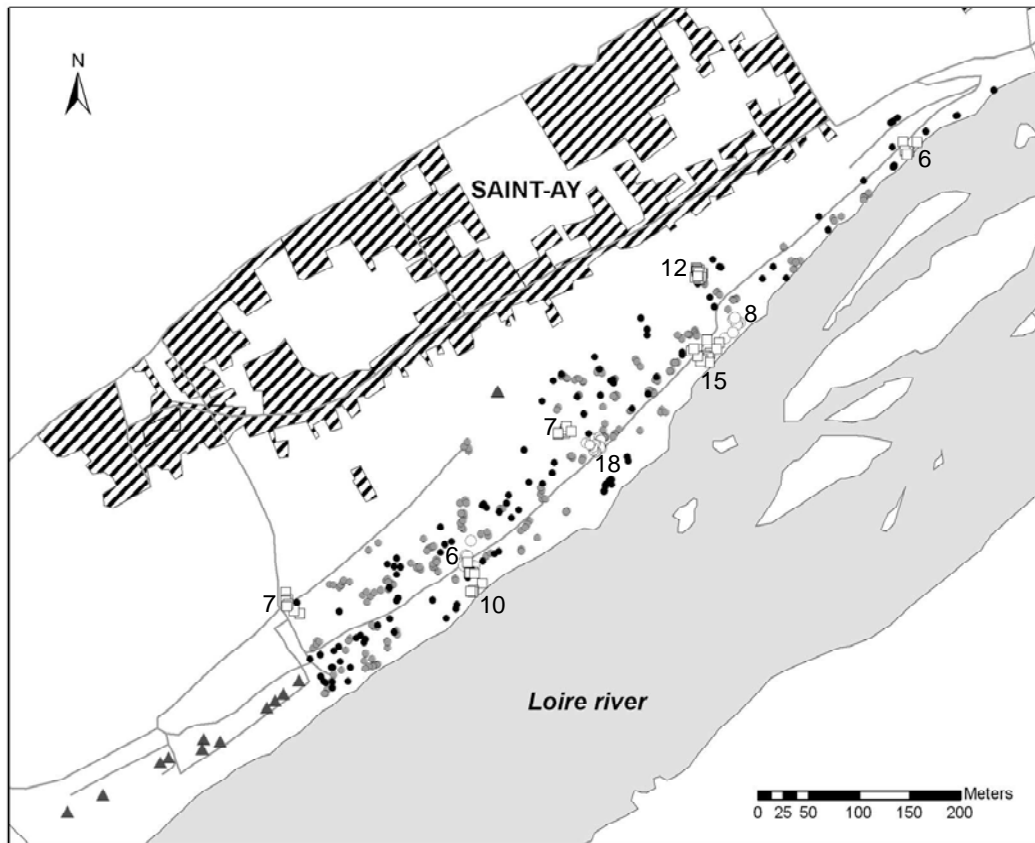


Fig. 4 – Differential plotting of studied individuals belonging to an $MLL_{=1}$ (*i.e.*, unreplicated individuals ; black dots), an $MLL_{2 \leq N_R \leq 5}$ (grey dots), or an $MLL_{\geq 6}$ (white dots and white squares with numbers indicating the corresponding ramet numbers). Triangles refer to the 13 sampled Lombardy poplars.

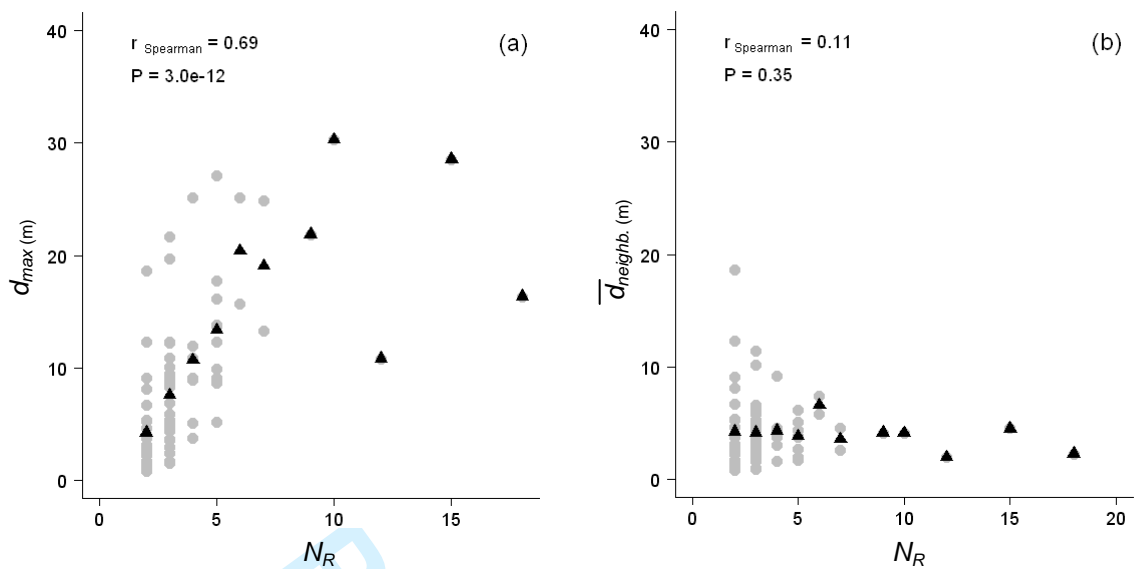


Fig. 5 – Relationships between MLL size (N_R , number of ramets) and (a) the maximum distance between two ramets (d_{max}), (b) the mean distance between closest neighbours ($\bar{d}_{neighb.}$). Mean values (black triangles) were computed for each N_R class. Spearman's correlation coefficients were computed at the MLL level.

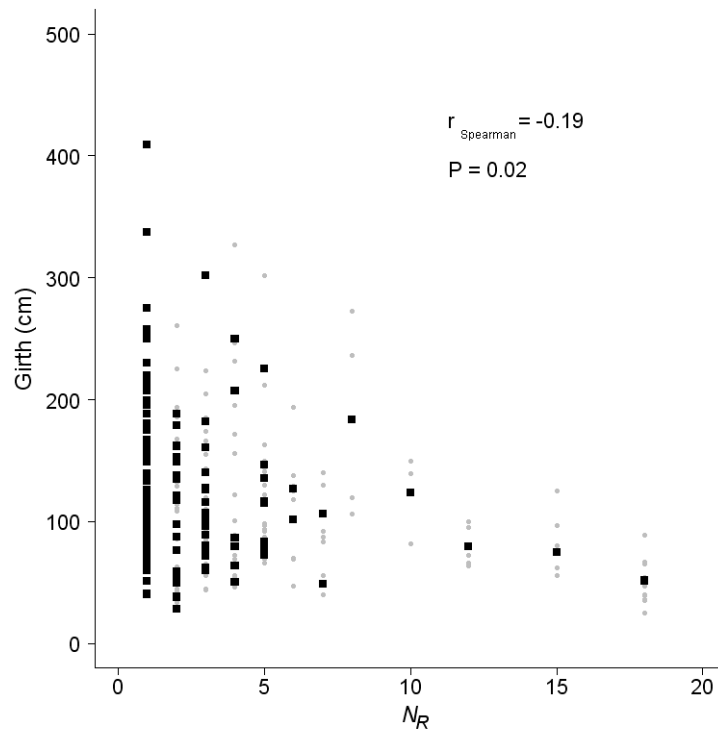


Fig. 6 – Relationships between MLL size (N_R , number of ramets) and girth at breast height at the individual ramet level (grey dots) and at the MLL mean level (black squares). Analysis was restricted to single-stemmed individuals. Spearman's correlation coefficient was computed for the MLL mean level.

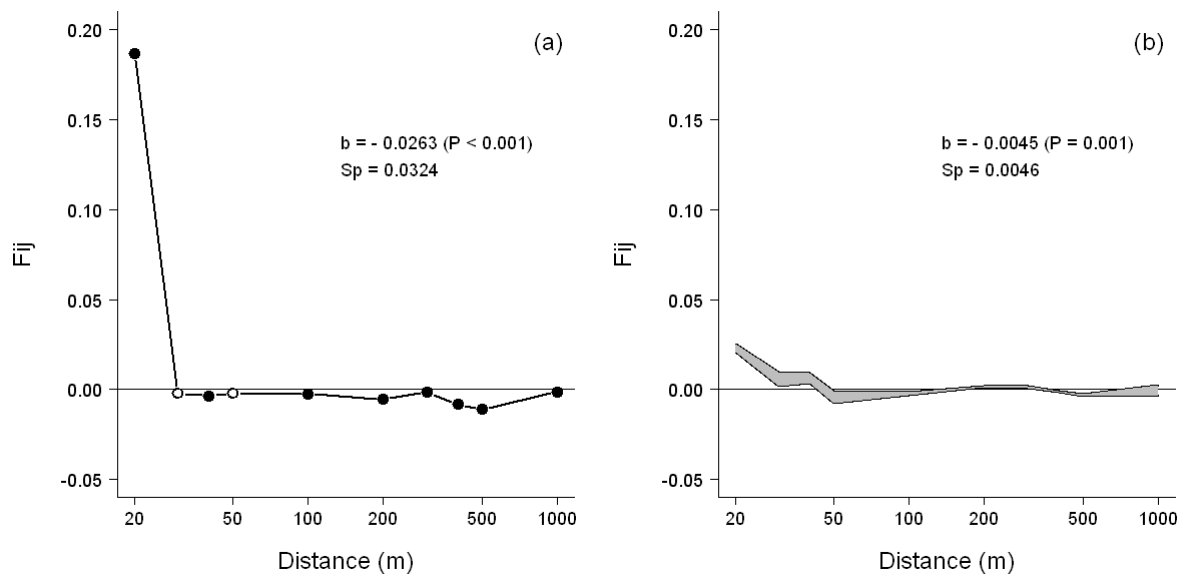


Fig. 7 – Spatial genetic structure analysis at (a) tree and (b) MLL levels. Both correlograms show the evolution of mean kinship coefficients (F_{ij}) between pairs of sampling units over ten geographic distance classes. At the tree level, significant ($P < 0.05$) and non-significant mean F_{ij} values are represented by black and white circles, respectively. At the MLL level, the envelope (95% CI) is the result of a 10000-resampling procedure (a single ramet selected in each MLL at each resampling step). The five trees identified as probable F_1 siblings originating from the Lombardy poplar were removed from the analysis.

Table 1 – Sex-ratio at tree and MLL levels with a distinction between mono-ramet ($MLL_{=1}$) and multi-ramet ($MLL_{\geq 2}$) MLLs. The five trees identified as probable F_1 siblings originating from the Lombardy poplar were removed from the analysis.

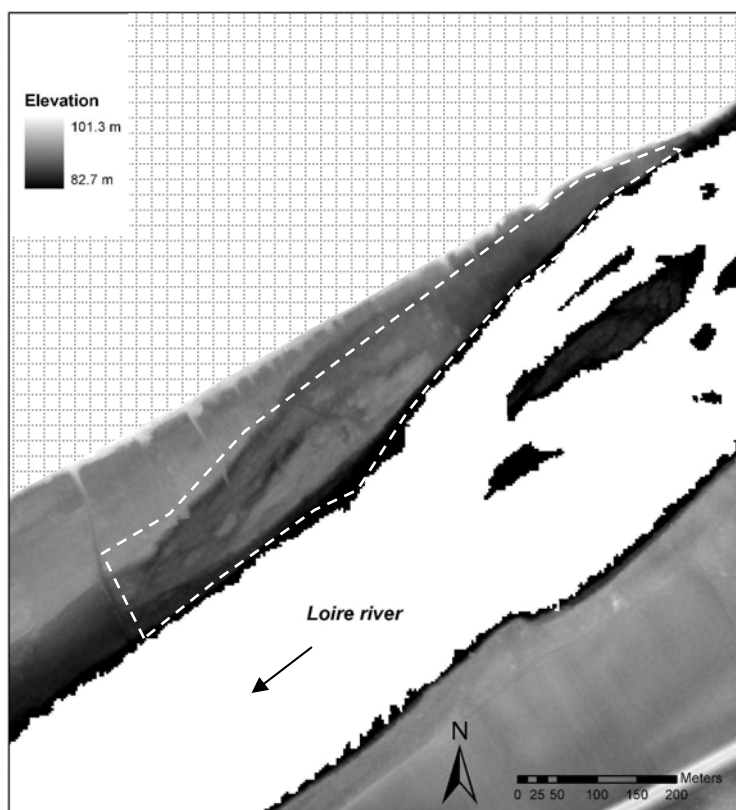
	Number of trees	Number of MLLs		
		$MLL_{=1}$	$MLL_{\geq 2}$	Total
Males	212	48	40	88
Females	196	62	39	101
Total	408	110	79	189
Sex-ratio	1 : 0.92	1 : 1.29	1 : 0.98	1 : 1.15
$P(>\chi^2_{1:1})$	0.43	0.18	0.91	0.34

Table 2 – Genetic diversity at the MLL level. The five trees identified as probable F_1 siblings originating from the Lombardy poplar were removed from the analysis.

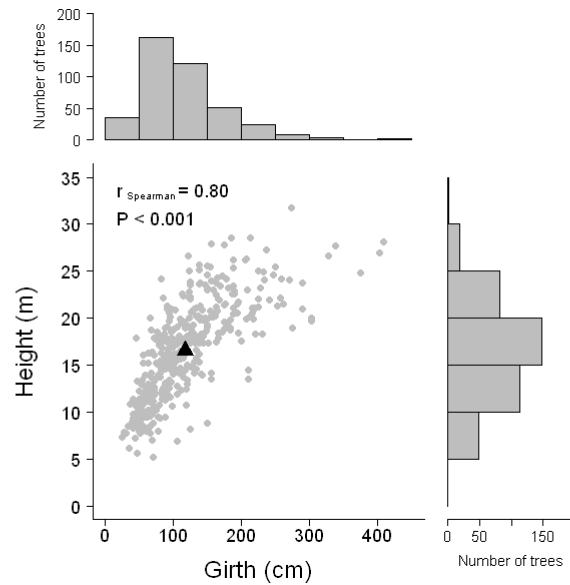
Locus	LG	Motif	A	A_e	H_o	H_e	F_{is}^a
PMGC2852	I	(GA) _n	13	5.4	0.91	0.82	- 0.12***
PMGC667	II	(GA) _n	22	9.1	0.73	0.89	0.19***
PMGC486	III	(GA) _n	10	5.3	0.85	0.81	- 0.05
PMGC2235	IV	(GA) _n	13	3.9	0.73	0.75	0.02
PMGC2838	V	(GA) _n	5	1.6	0.37	0.38	0.03**
PMGC2578	VI	(GA) _n	15	4.3	0.74	0.77	0.04
PMGC61	VIII	(CTT) _n	7	4.3	0.74	0.77	0.04
PMGC333	XI	(CTT) _n	4	2.7	0.72	0.64	- 0.14**
WPMS05	XII	(GT) _n	14	6.8	0.83	0.86	0.03*
PMGC14	XIII	(GA) _n	7	3.9	0.76	0.75	- 0.01
PMGC433	XVI	(GA) _n	6	1.2	0.15	0.16	0.08
Overall ^b			10.5	4.4	0.68	0.69	0.008
± SD			± 5.4	± 2.3	± 0.23	± 0.22	

^a Significant deviation from Hardy-Weinberg equilibrium : * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

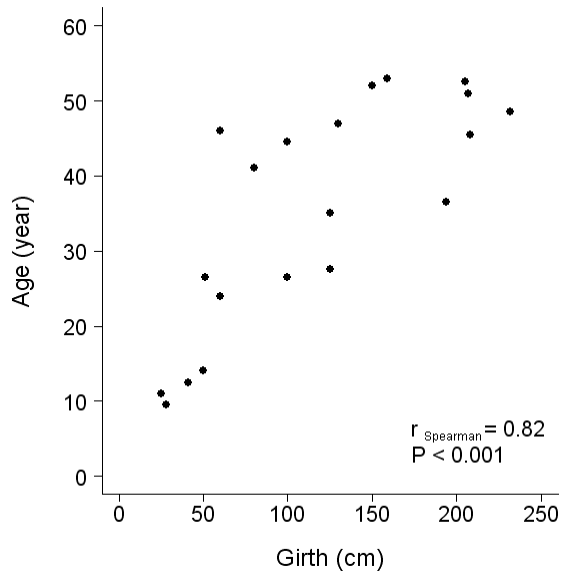
^b Mean value (A , A_e , H_o , and H_e) or global sample estimate (F_{is})



Supplementary Material 1 – Study site (dotted line) aircraft laser altimetry (data from Direction Régionale de l’Environnement de l’Aménagement et du Logement, Service Loire et Bassin Loire Bretagne, Orléans, France, 2002).



Supplementary Material 2 – Relationship between tree height and girth at breast height and relative distributions of both traits within the studied wild *P. nigra* population (413 trees). The triangle refers to the mean point. In the case of clumped or forked trees, the girth of each stem was measured and the largest value was recorded.



Supplementary Material 3 – Relationship between girth at breast height and tree age estimates (core analysis) on a subset (20 single-stemmed trees) of the studied *P. nigra* population. Increment core samples could not be collected from trees with a girth greater than 250 cm.