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Factors determining fine-scale spatial genetic structure within coexisting populations of common beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), and sessile oak (*Q. petraea* (Matt.) Liebl.)

Elżbieta Sandurska[1] , Bartosz Ulaszewski[1] , Katarzyna Meyza[1] , Ewa Sztupecka[1] and Jarosław Burczyk[1*]

Abstract

Key message Naturally regenerating populations of common beech, pedunculate, and sessile oaks develop strong spatial genetic structures at adult and seedling stages. Significant genetic relationship occurs between individuals growing up to 60 m apart. This indicates the minimum distance separating trees from which seeds used for reforestation should be harvested to avoid the adverse effects of excessive relatedness among offspring.

Context Spatial genetic structure is an inherent characteristic of naturally regenerating plant populations and has practical implications in forests for the management of genetic resources.

Aims We investigated the extent of spatial genetic structure in three broad-leaved forest tree species (common beech—*Fagus sylvatica* L.; pedunculate oak—*Quercus robur* L.; and sessile oak—*Q. petraea* (Matt.) Liebl.) coexisting in the same nature reserve, explored its variation among species and different life stages (adults/offspring), and tested its possible determinants.

Methods We explored patterns of spatial distribution of individuals, and using microsatellites, we estimated parameters of spatial genetic structure based on kinship relationships, considering possible sources of variation.

Results In adults, the strongest spatial genetic structure was found for *Q. petraea* ($Sp = 0.0187$), followed by *F. sylvatica* ($Sp = 0.0133$), and the weakest in *Q. robur* ($Sp = 0.0080$). It was uniform across different age classes in pedunculate oak but decreased with age in sessile oak. No apparent relationship between age and spatial genetic structure was found in beech. Offspring exhibited significant spatial genetic structure (ranging from 0.0122 in beech to 0.0188 in sessile oak). The cohorts of seedlings having both parents present within the study site had stronger spatial genetic structures than cohorts of seedlings with only one local parent.

Conclusion Spatial genetic structure is strong in naturally regenerating populations of heavy-seeded forest trees. Pollen immigration from outside of a local forest stand can significantly decrease the extent of spatial genetic structure in offspring generations.

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*Correspondence:
Jarosław Burczyk
burczyk@ukw.edu.pl
Full list of author information is available at the end of the article

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1 Introduction

Natural regeneration in plants, coupled with limited seed and pollen dispersal, likely results in a fine-scale spatial genetic structure (SGS) within populations (Epperson 1992; Vekemans and Hardy 2004), which is defined as a nonrandom distribution of genes and genotypes in two-dimensional space where a population resides. A strong positive SGS may increase the probability of mating among related individuals, which in turn is likely to increase inbreeding in consecutive generations (Epperson 2003). Biparental inbreeding can reduce the fitness of offspring due to inbreeding depression. Therefore, the information on the extent of SGS and its determinants is extremely valuable for managing semi-natural populations. In forest trees, such results can be used in designing appropriate strategies for managing and conserving genetic resources, as well as for seed collection procedures aimed at maximizing the genetic diversity of harvested seed lots (Gapare and Aitken 2005).

SGS is closely linked to the mating system, including pollen and seed dispersal modes, life form, and population density (Vekemans and Hardy 2004). Hence, assessing SGS may be an indirect proxy for estimation of the extent of gene dispersal (Rousset 2000; Vekemans and Hardy 2004; Oddou-Muratorio et al. 2010) or even to predict the connectivity between local breeding units (Smouse and Peakall 1999). Conversely, the presence of SGS is often considered indirect evidence of the natural establishment of a population (Vekemans and Hardy 2004; Chung et al. 2005; Rajendra et al. 2014). However, the development of SGS is a complex process. In addition to the mode of propagule dispersal, it depends on other factors such as colonization history, availability of suitable habitats, or post-dispersal mortality, which may be governed by local population density or competition with other species (Hampe et al. 2010).

Recent studies have attempted to investigate which factors influence the extent of SGS and to what degree. Vekemans and Hardy (2004) showed that population density influences the strength of local genetic drift and is therefore one of the major determinants of SGS. Theoretical predictions (Doligez et al. 1998) have suggested that spatial aggregation of individuals may promote SGS. Spatial aggregation was found to be linked with SGS in *Silene ciliata* (Lara-Romero et al. 2016), but it remains unclear if the variation of the spatial distribution of individuals affects the emergence of SGS in long-lived forest trees. However, clumping may result from different factors like environmental heterogeneity or intraspecies competition, and not only from spatial heterogeneity of seed and pollen dispersal, often considered a major driver of SGS (Epperson 2005).

Variation of SGS across different age classes has been widely studied, although the results obtained are inconsistent. In some investigations, stronger genetic structure was shown in older cohorts (Kalisz et al. 2001; Jensen et al. 2003; Jacquemyn et al. 2006; Jones and Hubbell 2006; Troupin et al. 2006), while in others, the genotypes of mature populations were nearly randomly distributed (Chung et al. 2003; Fuchs and Hamrick 2010). Considering the successional stage of populations Chung et al. (2007) indicated that SGS may decline as populations become older. However, relevant studies on forest trees are particularly difficult due to the longevity of the target populations.

The effects of pollen and seed dispersal on the extent of SGS in forest trees have already been investigated by examining maternal and paternal alleles in the offspring (Nakanishi et al. 2009; Hampe et al. 2010; Sebbenn et al. 2011). Positive kinship coefficients were detected in the short-distance classes for both maternal and paternal alleles, with a rapid decrease as the distance increased. This indicates that limited seed dispersal was responsible for strong spatial genetic structure and limited pollen dispersal had an intensifying effect on SGS. On the other hand, the impact of immigration of external seeds and pollen on the development of local SGS is still deficient.

Forest management can also affect SGS, but not necessarily the levels of genetic diversity (Buiteveld et al. 2007; Piotti et al. 2013; Rajendra et al. 2014; Sjölund and Jump 2015; Gonzalez-Diaz et al. 2017). Nevertheless, most of those studies compared quite different populations, and any differences observed could result from uncontrolled factors. However, one of the main questions considering SGS is whether it is homogeneous within a population or if it is heterogeneous and structured based on different but identifiable factors affecting the formation of SGS.

Common beech (*Fagus sylvatica* L.) and oaks, both pedunculate oak (*Quercus robur* L.) and sessile oak (*Q. petraea* (Matt.) Liebl.), are among the most prominent broadleaved tree species in Central and Western Europe. The tree species are monoecious, wind-pollinated, and highly outcrossing, with seeds dispersed mainly by gravity and animals (Packham et al. 2012;

Eaton et al. 2016), which promotes the formation of SGS in naturally established populations (Vekemans and Hardy 2004). However, patterns and determinants of SGS in these species have only been explored to a limited degree. In early studies of common beech based on isozyme markers, SGS was found to be low (Merzeau et al. 1994; Leonardi and Menozzi 1996). However, later surveys based on microsatellite markers (Vornam et al. 2004; Jump and Peñuelas 2007; Chybicki et al. 2009) detected significant SGS with positive autocorrelation patterns extending up to approximately 30–40 m, which seems typical for wind-pollinated trees with limited seed dispersal (Vekemans and Hardy 2004). Similarly, early studies on *Quercus robur* and *Q. petraea* conducted using isozymes and microsatellites, indicated low or insignificant spatial genetic autocorrelation (Bacilieri et al. 1994; Streiff et al. 1998; Vekemans and Hardy 2004). However, later works on *Q. robur* and *Q. petraea* (Cottrell et al. 2003; Jensen et al. 2003; Valbuena-Carabaña et al. 2007; Hampe et al. 2010; Vranckx et al. 2014; Curtu et al. 2015), as well as other oak species (Valbuena-Carabaña et al. 2007; Curtu et al. 2015; Lind-Riehl and Gailing 2015), generally confirmed the relatively low SGS in oak populations.

Despite similar pollen and seed dispersal in beech and oaks, the currently available data suggest that SGS is usually more pronounced in beech than in oaks. Unfortunately, only a few studies have been conducted over larger and comparable study areas, usually with a low number of genetic markers. Nevertheless, because SGS may vary widely among different populations within the species (Jump et al. 2012), comparable sampling designs, preferably in a similar environmental location, are needed to explore similarities or differences between beech and oak's SGS.

SGS analyses in forest trees should ideally be conducted in natural populations (e.g., national parks or nature reserves), where natural regeneration is the primary mode of reproduction. It is expected that ongoing evolutionary processes in such populations secure the maintenance of genetic diversity, satisfying the long-term sustainability requirements of forest tree populations in nature reserves (Grumbine 1994; Bengtsson et al. 2003). Such results are important for managing their genetic resources, but they should also serve as a reference for comparisons with populations artificially created and managed by humans.

In this study, we investigated the fine-scale SGS in adult and offspring generations of common beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), and sessile oak (*Q. petraea* (Matt.) Liebl), coexisting in the same forest stand not exposed to management practices (nature reserve). These populations have already been subjected

to spatial distribution (oaks only) and genetic analyses, and despite the high genetic diversity observed in all species, the oaks exhibited some symptoms of population decline (Sandurska et al. 2017, 2019). In this study, besides SGS analyses, we also performed spatial distribution analyses for beech. Our working *null* hypotheses were as follows: (i) adult and offspring individuals of the studied species are randomly distributed over the study site, (ii) the intensity of SGS is similar among the studied species, (iii) the intensity of SGS is similar across different size/age groups of adults within species, (iv) adults and offspring of the same species represent similar SGS pattern, and (v) offspring resulting from fertilization by pollen immigrating from background pollen sources exhibit similar SGS as offspring sired within the study site. We anticipate that the knowledge gathered in this study will be useful for managing genetic resources in naturally regenerating populations of temperate forest tree species of similar biology, such as beech and oaks.

2 Material and methods

2.1 Study populations and sampling

The study was performed in a forest stand of the Jamy Nature Reserve, established in 1968 in the Jamy Forest District in North-Central Poland. It functions as a forest reserve, and although the dominant species is hornbeam (*Tilio-Carpinetum*), its main target of conservation is beech forest, consisting of *Galio odorati-Fagetum* and *Luzulo pilosae-Fagetum*. The central point of the round-shaped study plot of approximately 5.5 ha has coordinates φ18° 56′ 6.07′′ E, λ53° 35′ 9.67′′ N and is divided by a railway siding unused since 1950 (Fig. 1). The upper canopy of trees is dominated mostly by oaks (*Quercus robur* L, *Q. petraea* (Matt.) Liebl.), several of them over the age of 215 years with a diameter at breast height (DBH) often exceeding 100 cm, but there is also a considerable fraction of beech (*Fagus sylvatica* L.) of similar age and size, together with some linden (*Tilia cordata* Mill.) and pine (*Pinus sylvestris* L.) trees. On the lower canopy, there is mainly beech aged of 40–80 years (DBH: 30–60 cm) with an admixture of hornbeam and linden. Oaks of an intermediate age class do not occur at the stand. Finally, the layer of undergrowth consists mainly of beech seedlings. For this study, plant material was sampled from three cohorts, namely *adults* (trees with DBH > 25 cm (all individuals sampled)), *seedlings* (< 30 cm tall (a sample of about 640 individuals within each species)), and *saplings* (trees with DBH < 20 cm (a sample of 300 individuals in beech only)). A sample of leaves was collected in August of 2013 and 2014. The numbers of individuals sampled and genotyped in each cohort and species are presented in Table 1, and the distribution of sampled individuals is shown in Fig. 4 (Appendix). Adults and saplings were
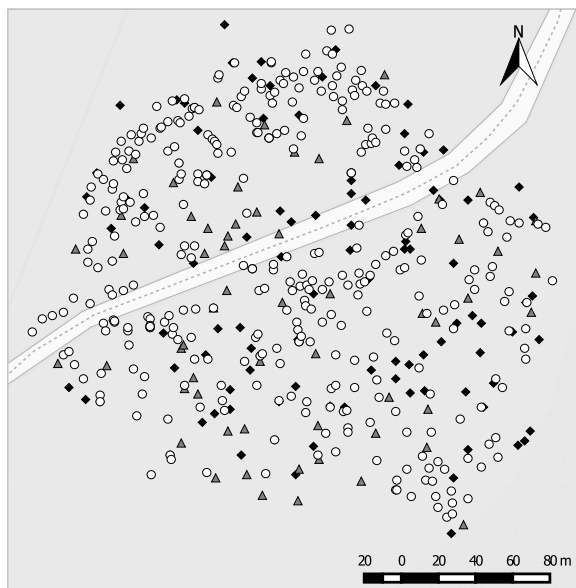
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Fig. 1 Locations of adult trees of *Fagus sylvatica* (white circles), *Quercus robur* (black diamonds), and *Q. petraea* (gray triangles) on the study area. The line running across the map is an unused railway siding. Oaks are assigned to one of the two species based on the highest assignment probability as determined by the STRUCTURE software

georeferenced using Trimble® GPS Pathfinder® system; the precise positions of seedlings were determined relative to the nearest adult trees or locally defined transects.

We used the genotypic data presented in Sandurska et al. (2017, 2019). All individuals were genotyped with large sets of nuclear microsatellites (Sandurska et al. 2023): 20 nuclear microsatellite markers for beech (*FcC00468, FcC00730, FcC00927, FcC03095, csolfagus_05, csolfagus_06, csolfagus_19, csolfagus_29, csolfagus_31, concat14_A_0, DE576_A_0, DUKCT_A_0, DZ447_A_0, EEU75_A_0, EJV8T_A_0, EMILY_A_0, ERHBI_A_0, sfc_0036, fc_1143, FS1_15*) and 19 microsatellite markers for oaks (*PIE-20, PIE-102, PIE-215, PIE-223, PIE-242, PIE-243, PIE-267, ssrQrZAG 7, ssrQrZAG 11, ssrQrZAG 20, ssrQrZAG 96, ssrQpZAG 15, ssrQpZAG 110, ssrQrZAG 30, ssrQrZAG 65, ssrQrZAG 87, ssrQrZAG 101, ssrQrZAG 112, ssrQpZAG 9*). Details on laboratory protocols are presented in Sandurska et al. (2017).

2.2 Genetic identification of the species status in oaks

We performed a genetic analysis to identify species of adult and seedling oaks belonging to one of the two species, either *Quercus robur* or *Q. petraea*. This was possible due to the use of microsatellite loci that help to differentiate the oak species (Guichoux et al. 2011). This was of particular importance in the case of seedlings, for which precise species identification is difficult. For this purpose,

STRUCTURE v.2.3.4 software (Pritchard et al. 2000) was used. The analyses were carried out jointly for adults and seedlings, assuming the existence of two groups ($K=2$), using 100 000 burn-in periods, 500,000 MCMC repetitions, and an admixture model with correlated allele frequencies. This allowed us to precisely determine the probability of assigning each individual to a particular species. Ultimately, each individual was assigned to one group (species) based on the highest probability of assignment. The association of genetic clusters with the appropriate species was carried out based on leaf morphological traits of a group of adults.

2.3 Spatial distribution

Firstly, we investigated if adult and offspring individuals of each species are randomly distributed in the studied plot. For that purpose, univariate O-ring statistics O(r) (Wiegand and Moloney 2004) was used with a ring of a constant width of 5 m. To avoid estimation bias due to edge effects, the maximal ring was set at 130 m or less than half of the plot width. The significance of the O(r) function was tested with a common null model of complete spatial randomness (CSR), and a 95% confidence interval was calculated using the 25th and 975th of a total 1000 randomizations (999 replicates by Monte Carlo simulation) of the function O(r). O(r) values above and below this envelope indicate significant spatial clustering or repulsion (hyper-dispersion), respectively, at radius *r*. Results of the analyses of adults and seedlings of *Q. robur* and *Q. petraea* originate from our earlier work (Sandurska et al. 2019).

To characterize the small-scale interaction structure between beech and oaks, we used bivariate analysis with the same parameters as for univariate analyses (ring width = 5 m, maximal ring = 130 m). As a null model, we chose the toroidal shift where pattern 1, in our case beech, is shifted as a whole random vector against pattern 2 (both oaks together), which is fixed (Wiegand and Moloney 2014). This choice was made after prior observations of species locations, analysis of their DBH and genetic variation (Sandurska et al. 2017, 2019), which were suggesting that oaks had settled down in this area earlier than beech. Moreover, to investigate if the spatial distribution of DBH of the species was random, we conducted the bivariate analysis with one quantitative mark. As a function, we chose spatial Moran *I* correlogram (Legendre and Legendre 2012), and as a null model, randomization of only the DBH of beech, while the DBH of oaks was fixed. Because the DBH distributions in the pedunculate and sessile oaks were similar (Sandurska et al. 2019), we decided to analyze the spatial distribution of DBH together for both oaks. The basic interest in analyzing patterns of this type is to explore

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Table 1 Parameters of the spatial genetic structure for *Fagus sylvatica*, *Quercus robur*, and *Q. petraea*

	Population/cohort	N	$b_{logO(r)}$	DBH (SD)	Sp	b_{log} (SD)	F(1)	Dc	Nb	σ
Fagus sylvatica	Adults (all)	347	**-0.00108**	41.36 (14.61)	0.0133	**-0.0128 (0.0015)**	0.0395	0.0069	75.199	29.44
	DBH < 32.3 cm	116		28.69 (2.25)	0.0127	**-0.0122 (0.0018)**	0.0386	0.0025	78.563	50.19
	32.3 cm ≤ DBH < 43.7 cm	116		37.68 (3.07)	0.0108	**-0.0104 (0.0016)**	0.0374	0.0029	92.322	50.71
	DBH ≥ 43.7 cm	115		57.85 (13.48)	0.0135	**-0.0131 (0.0028)**	0.0350	0.0025	73.884	48.56
	Seedlings (all)	644	**-0.00578**	–	0.0122	**-0.0119 (0.0009)**	0.0301	0.0136	81.828	21.89
	with 1 local parent	343		–	0.0110	**-0.0107 (0.0014)**	0.0309	0.0076	90.769	30.73
	with 2 local parents	272		–	0.0160	**-0.0154 (0.0015)**	0.0340	0.0059	62.643	29.06
	Saplings (all)	300	**-0.00359**	–	0.0157	**-0.0150 (0.0016)**	0.0436	0.0075	63.817	25.97
	with 1 local parent	217		–	0.0190	**-0.0181 (0.0019)**	0.0500	0.0055	52.531	27.66
	with 2 local parents	48		–	0.0171	**-0.0165 (0.0041)**	0.0378	0.0015	58.346	55.93
Quercus robur	Adults (all)	105	-0.00001	78.40 (18.19)	0.0080	**-0.0080 (0.0014)**	0.0158	0.0021	124.913	68.94
	DBH < 71.4 cm	35		60.19 (10.90)	0.0092	**-0.0092 (0.0048)**	0.0326	0.0008	107.646	102.70
	71.4 cm ≤ DBH < 83.5 cm	35		76.78 (3.50)	0.0092	**-0.0090 (0.0034)**	0.0434	0.0012	108.297	85.07
	DBH ≥ 83.5 cm	35		97.82 (10.45)	0.0087	**-0.0089 (0.0030)**	0.0177	0.0009	115.435	98.81
	Seedlings (all)	191	**-0.00203**	–	0.0175	**-0.0173 (0.0014)**	0.0646	0.0045	56.986	31.88
	with 1 local parent	115		–	0.0158	**-0.0157 (0.0012)**	0.0557	0.0027	63.328	42.91
	with 2 local parents	70		–	0.0238	**-0.0232 (0.0032)**	0.0926	0.0018	42.054	42.72
Quercus petraea	Adults (all)	60	0.00001	76.07 (15.11)	0.0187	**-0.0185 (0.0020)**	0.0359	0.0013	53.612	56.24
	DBH < 68 cm	20		59.49 (9.01)	0.0230	**-0.0228 (0.0074)**	0.0376	0.0007	43.394	71.33
	68 cm ≤ DBH < 82 cm	20		76.42 (4.44)	0.0103	-0.0102 (0.0059)	-0.0146	0.0008	96.650	97.34
	DBH ≥ 82 cm	20		92.89 (9.42)	0.0096	**-0.0098 (0.0042)**	0.0251	0.0005	103.864	127.05
	Seedlings (all)	456	**-0.02209**	–	0.0188	**-0.0185 (0.0011)**	0.0447	0.0110	53.319	19.68
	with 1 local parent	158		–	0.0166	**-0.0165 (0.0013)**	0.0449	0.0041	60.098	34.17
	with 2 local parents	291		–	0.0223	**-0.0221 (0.0014)**	0.0467	0.0084	44.931	20.68

N number of individuals, *DBH(SD)* mean DBH of trees with standard deviation [cm], *Sp* coefficient of spatial genetic structure, $b_{log}(SD)$ the regression slope of F_{ij} on the natural logarithm of the distance *r*, between individuals, with standard deviation, *F(1)*, the mean F_{ij} among individuals of the first distance class (0–20 m); *Dc*, density per m²; *Nb*, neighborhood size; *σ*, gene dispersal distance. Statistically significant ($p < 0.05$) values of $b_{logO(r)}$ and b_{log} according to the permutation test are in bold

the impact of proximity (and mark) of the first pattern (in our case, beech) on the marking of the second pattern (in our case, oaks) (Wiegand and Moloney 2014). To test if a given null model fits the summary statistic of the observed data over a particular distance interval, we used a Goodness-of-Fit test (GoF test) (Loosmore and Ford 2006; Wiegand and Moloney 2014). All these calculations and simulations were performed using the software PROGRAMITA (Wiegand 2003). Finally, we calculated $b_{logO(r)}$ as the slope of the regression of O(r) function on the natural logarithm of distance to compare the overall strength of aggregation among populations (Lara-Romero et al. 2016).

2.4 Spatial genetic structure

First, the SGS analyses were performed for all adults and all offspring of *F. sylvatica*, *Q. robur*, and *Q. petraea*. Then, in order to investigate the variation of SGS across different size/age classes, the adult trees within each species were divided into three equally frequent size groups based on their DBH, and the SGS was determined within each group separately (Table 1). Finally, we assessed the effect of long-distance pollen dispersal (immigration

from outside of a local stand) on the SGS of offspring cohorts. Using parentage methods, in each species we classified offspring into three categories, namely (i) with both parents present within the local stand, (ii) with just a single local parent (presumably mother fertilized with immigrant pollen), and (iii) with no compatible parent (seed immigrant) and performed SGS analyses within the first two groups. Parentage analyses were performed using NMπ software (Chybicki 2018).

SGS was assessed using a spatial autocorrelation analysis. Kinship coefficient F_{ij} (Loiselle et al. 1995) was obtained in 9 distance classes which were selected to provide comparable numbers of pairs in each class. Up to 120 m, the intervals increased with 20 m (0–20 m, 20–40 m, 40–60 m, 60–80 m, 80–100 m, 100–120 m), and then the size of distance classes increased (120–150 m, 150–200 m, above 200 m). Autocorrelation analysis was carried out under a null hypothesis of the absence of spatial autocorrelation. For this purpose, a permutational test was applied, and 95% confidence intervals obtained by permuting individual locations 10,000 times. Additionally, for each calculated average kinship coefficient for a given distance class, the confidence intervals

were obtained using standard errors computed through jackknifing over loci (Sokal and Rohlf 1995). All these analyses were performed with SPAGeDi v.1.5 (Hardy and Vekemans 2002).

To compare the intensity of SGS among cohorts, *Sp* statistics of Vekemans and Hardy (2004) were calculated. The *Sp* statistics quantify SGS by the ratio: $-b_{\log}/(1-F(1))$, where $b_{\log}$ is the regression slope of F_{ij} on the natural logarithm of the distance r, between individuals i and j, and $F(1)$ is the mean F_{ij} among individuals within the first distance class (0–20 m), including all pairs of neighbors. Additionally, using the relation that *Sp* is inversely proportional to the effective size of Wright's neighborhood (*Nb*) (Wright 1946), the second parameter was also calculated. Using SPAGeDi, we also estimated gene dispersal distance σ (σ^2 is the mean-squared parent–offspring distance) and the effective size of the neighborhood (*Nb*) (Vekemans and Hardy 2004).

3 Results

3.1 Genetic identification of the species status in oaks

The procedure implemented in the STRUCTURE program detected a clear division of oaks into two species. Phenotypic analysis of adults' leaves indicated that cluster 1 represents pedunculate oaks, while cluster 2 corresponds to sessile oaks (Appendix Fig. 5). Four adult trees (2.42%) and seven seedlings (1.08%) were recognized as hybrids (with an assignment probability 0.5 ± 0.125), but for the purpose of SGS analyses, they were assigned to one of the species based on the highest assignment probability. Finally, 105 adult individuals were classified as *Q. robur*, and 60 as *Q. petraea*, with high levels of probability assignment (0.986 ± 0.047 and 0.965 ± 0.079, respectively). Seedlings were assigned to species based only on genetic analysis, according to the highest assignment probability. Thus, 191 seedlings were identified as *Q. robur*, and the remaining 456 as *Q. petraea*. The mean probabilities of assignment to individual species were very high and amounted to 0.980 ± 0.059 and 0.962 ± 0.080 for the pedunculate and sessile oaks, respectively. It is noteworthy that in both adults and seedlings, the mean probability of assignment was higher for *Q. robur*.

3.2 Spatial distribution

O-ring analyses demonstrated that the beech population had a clear tendency to spatial aggregation of individuals. The O(r) values were above the confidence envelope for distances between 0 and 50 m and for 90 to 130 m (Appendix Fig. 6). The $b_{\log O(r)}$ statistics (slope of the regression of the O-ring function on ln of distance) was significant and equaled -0.00108 ($p < 0.0001$). In the case of the combined oak groups, the O(r) function pointed to a more random distribution of individuals. There was significant

spatial clustering only at $r = 20$–30 m, but for the remaining distances, the O(r) function was within the confidence interval suggesting spatial randomness. O-ring analyses performed for both oak species separately indicated a random distribution of individuals within species, which was also confirmed by the non-significant Goodness-of-Fit (GoF) test (Appendix Fig. 6). The $b_{\log O(r)}$ statistics were not significant for adults of either oak species (Table 1).

The pair correlation function of bivariate analysis for beech and combined oak species was completely within the simulation envelopes. However, its distribution was closer to the upper limit of the confidence interval, which resulted in a significant GoF test statistic over the 0–100 m distance interval ($p = 0.022$) (Appendix Fig. 7), indicating a slight grouping of beeches against the oaks. There was no deviation of Moran's *I* function from the *null* distribution of DBH (Appendix Fig. 7).

The strong clustering of seedlings/saplings of all species could be clearly observed by inspecting the distribution of individuals in the study plot (Appendix Fig. 4). Indeed, all offspring cohorts exhibited significant spatial aggregation of individuals (Appendix Fig. 8), that was strong and significant for beech seedlings until approximately 110 m, with $b_{\log O(r)} = -0.00578$ ($p < 0.0001$). However, this was less pronounced in beech saplings $b_{\log O(r)} = -0.00359$ ($p < 0.0001$). Among seedlings, the lowest tendency for spatial aggregation was found for *Q. robur* ($b_{\log O(r)} = -0.00203$; $p < 0.0001$), with O(r) statistics being above the null CI envelope at low distances 0–12 m. In contrast, *Q. petraea* seedlings appeared to be highly clustered ($b_{\log O(r)} = -0.02209$; $p < 0.0001$), with O(r) statistics at the lowest distances being several times higher than that for beech and especially *Q. robur* offspring. In all cases, GoF test appeared to be significant.

3.3 Spatial genetic structure

Adult beech trees exhibited a strong spatial genetic structure with $Sp = 0.0133$ (Table 1). Pair-wise kinship coefficients (F_{ij}) reached the mean value of 0.04 at the first distance class and decreased quickly, significant only up to about 60 m (Fig. 2). Autocorrelograms and parameter estimates of SGS for pedunculate oak ($Sp = 0.0080$) suggested markedly different spatial structure patterns as compared to beech (Table 1). Here, adult trees presented a monotonous and gentle (although significant; $b_{\log} = -0.008$; $p < 0.001$) decrease of F_{ij} with distance across the entire range, with positive significant values up to 70 m (Fig. 2). For the sessile oak, the values of SGS parameters ($Sp = 0.0187$ and $b_{\log} = -0.0185$) were more than twice as high as for the pedunculate oak. The differences are also visible in the shape of autocorrelograms (Fig. 2). While the curve for *Q. robur* has a smooth, uniformly decreasing shape, the curve for *Q. petraea* is very
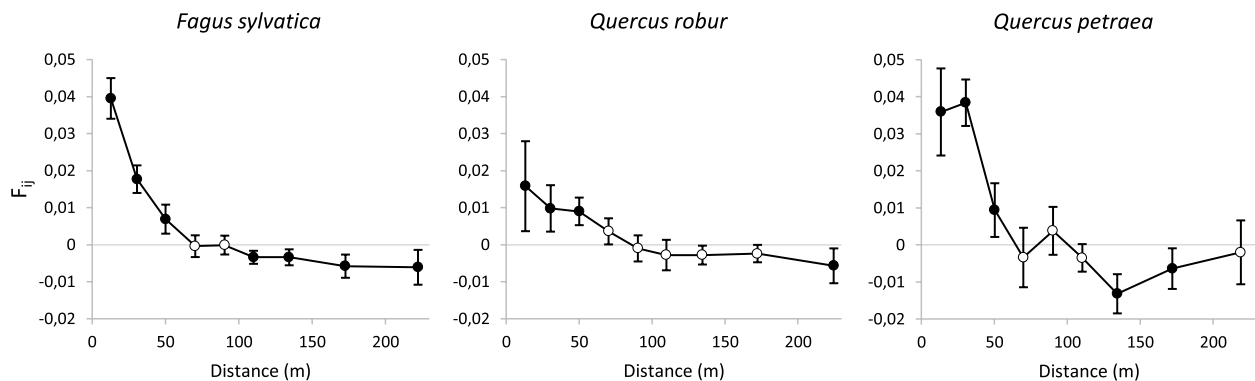
Fig. 2 Spatial genetic structure present in adults for *Fagus sylvatica*, *Quercus robur*, and *Q. petraea*. Error bars represent standard errors assessed by jackknifing data for each locus. Values significantly ($p < 0.05$) different from the expectation under a random distribution of genotypes in permutational test are indicated by filled symbols

steep in the first distance classes, showing a significant genetic structure up to approximately 70 m.

The three adult groups of pedunculate oak, defined based on DBH, were homogeneous (no statistical differences) and showed similar SGS patterns as the total population (Table 1), and the *Sp* parameters within each group were statistically significant. In sessile oak, the b_{log} parameter was significant for the groups of the smallest and the largest individuals, despite the small sample size (20 individuals). However, this parameter was not significant (likely due to low sample size) for the group of individuals with intermediate DBH (Table 1). Sessile oaks demonstrated a significant (as revealed by b_{log} estimates) heterogeneity of SGS among the groups, The *Sp* and b_{log} parameters were over two times higher for the group of smaller trees (DBH < 68 cm) than for large ones (DBH ≥ 82 cm) (Table 1). The three size groups of beech exhibited significant levels of SGS, being significantly heterogeneous among the groups, with the lowest *Sp* parameter observed for intermediate size class.

The analyses of the seedling and sapling SGS were carried out in three groups, namely (i) all individuals, (ii) individuals with only one local parent, and (iii) individuals with two identified local parents. Beech seedlings demonstrated a similar pattern of spatial genetic structure to the adult trees (*Sp* = 0.0122; Table 1, Fig. 3). However, 29 seedlings (4.5%) had no parent within the study plot (representative of seed immigration), 343 (53.3%) seedlings had only one parent, and 272 (42.2%) seedlings had both parents identified, suggesting the pollen immigration level at 55.8%. There was a significant difference in SGS for seedlings with both parents (*Sp* = 0.0160 and b_{log} = −0.0154) compared to those with only one parent (*Sp* = 0.0110 and b_{log} = −0.0107). This indicates that pollen immigration decreased the *Sp* parameter of seedlings resulting from local pollination by approximately 31.3%, as compared to the seedlings resulting from background pollination. Furthermore, local pollen dispersion was not random, but also had a significant impact on the pattern of the SGS of seedlings. Beech saplings exhibited relatively strong SGS (*Sp* = 0.015), which appeared to be similar for different groups of saplings. There was a considerable proportion of saplings with no local parent (35 saplings, 11.7%), which explains the lower *Sp* estimate that was obtained for all saplings.

Pedunculate oak seedlings exhibited a strong and significant pattern of SGS, which was much stronger than for adults (Table 1) and also stronger than beech seedlings. The *Sp* and b_{log} parameters for the group of all seedlings (*Sp* = 0.0175 and b_{log} = −0.0173) were more than twice as high as in the adult population (Table 1, Fig. 3). Parentage analyses in pedunculate oak seedlings revealed that only 6 (3.1%) of them had no local parent, suggesting a low level of seed immigration. Both parents were identified for 70 (36.6%) seedlings, while 115 (60.2%) seedlings had just one local parent, indicating that the proportion of pollen immigration was relatively high (62.16%). The SGS for the group of seedlings with two parents present in the local stand was estimated to be *Sp* = 0.0238 and b_{log} = −0.0232 (Table 1). However, for seedlings with one local parent, the estimates were lower (*Sp* = 0.0158 and b_{log} = −0.0157). These parameters were approximately 33% lower than those obtained for seedlings with both local parents and highlights that pollen dispersal within the local stand is an important factor shaping the SGS of seedlings of pedunculate oak (Fig. 3).

Sessile oak seedlings presented a strong SGS (*Sp* = 0.0188, b_{log} = -0.0185; Fig. 3), which was found to be similar to that observed in adults, unlike in pedunculate oak. Only seven seedlings had no parent inside the study area, suggesting a low level of seed immigration (1.5%). Moreover, both parents were found inside the study area for 291 (63.8%) seedlings, while only one parent was
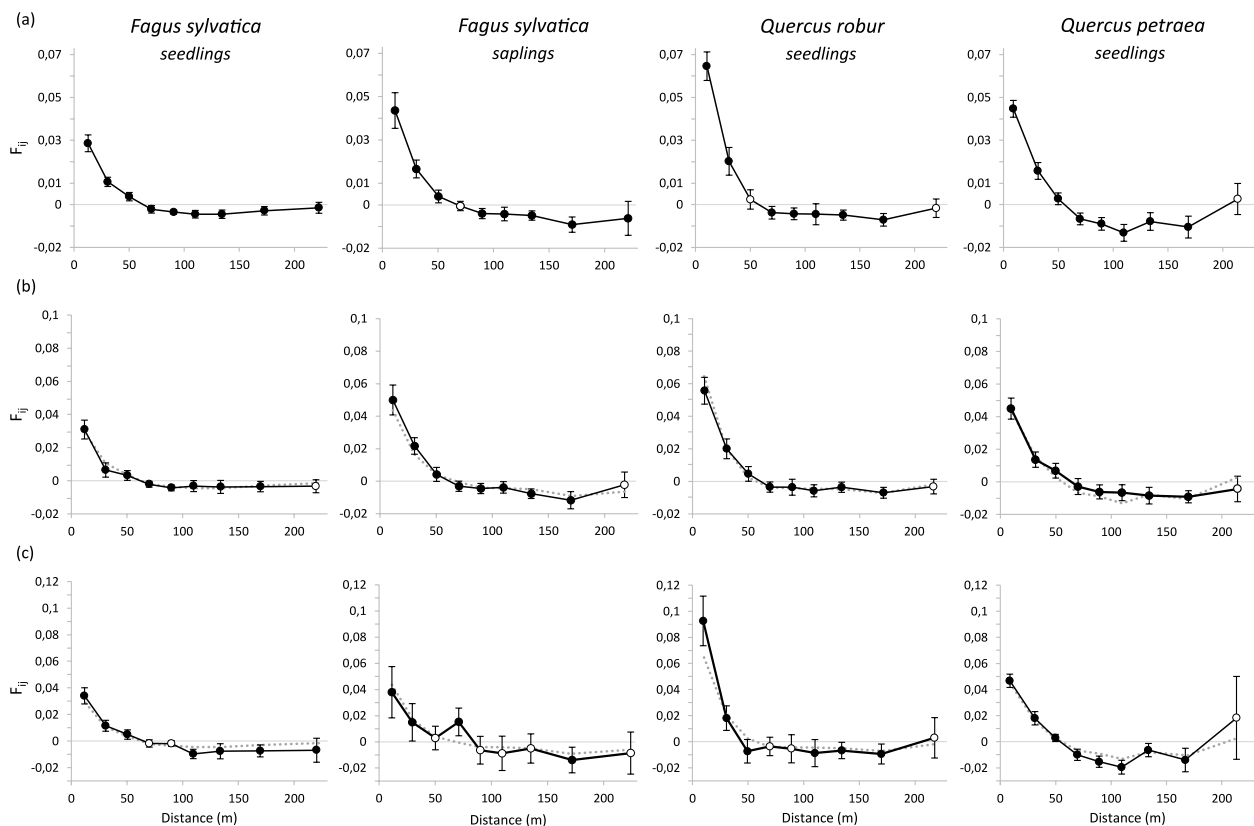
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Fig. 3 Spatial genetic structure present in seedlings and saplings for *Fagus sylvatica* and seedlings for *Quercus robur* and *Q. petraea*. Results were calculated considering **a** all pairs of individuals, **b** only pairs of individuals with assigned exactly one local parent, and **c** only pairs of individuals with assigned two local parents. Numbers of individuals within each groups are presented in Table 1. Error bars represent standard errors assessed by jackknifing data for each locus. Values significantly ($p < 0.05$) different from the expectation under a random distribution of genotypes in permutational test are indicated by filled symbols. Dotted lines in figure **b** and **c** indicate SGS for all pairs of seedlings or saplings

found for 158 (34.6%) seedlings, suggesting a low level of pollen immigration (35.2%). The SGS parameters estimated for seedlings with one local parent ($Sp = 0.0223$, $b_{\log} = -0.0221$) was lower by approximately 25% than those estimated for the seedlings with both parents identified within the local stand ($Sp = 0.0166$, $b_{\log} = -0.0165$). It is worth noting that the shapes of autocorrelograms for seedlings of both oak species were similar (Fig. 3).

4 Discussion

Our earlier study (Sandurska et al. 2017, 2019) demonstrated that the three species investigated herein, growing in the same forest stand, exhibited high levels of genetic diversity. However, age structure and differences between adult and seedlings generations indicated some symptoms of oak population decline. The fine-scale SGS revealed in this study allows for more profound insights into the history and possible future changes of the stand. Unfortunately, the establishment records for this stand remain unclear, as no relevant forestry documentation is available before 1945. The group of the

largest and presumably oldest oaks (both pedunculate and sessile) showed a weak but significant SGS, which suggests that they could have been established from naturally widely dispersed seeds (Hampe et al. 2010) or were partly artificially planted (or both). For pedunculate oak the SGS structure was also weak for smaller and presumably younger trees, while younger sessile oak trees exhibited a relatively strong SGS pattern implying their natural establishment. Notably, the distribution of adult oaks showed no patterns of spatial aggregation. On the other hand, adult beech trees exhibited a significant clustering. The specific age structure of the adult cohort in beech, namely a few largest trees followed by a considerable group of the middle-aged cohort (Sandurska et al. 2017, 2019), emphasizes the role of beech as a colonizing species in the study site.

The SGS patterns differed significantly among the studied species with the strongest SGS observed in sessile oak, followed by beech and then pedunculate oak (hypothesis *(ii)*). The kinship coefficients of *F. sylvatica* adults remained significant up to 60 m, while in most previous studies,

significant positive spatial autocorrelation for beech was noted only up to approximately 30–40 m (Merzeau et al. 1994; Leonardi and Menozzi 1996; Vornam et al. 2004; Jump and Peñuelas 2007; Chybicki et al. 2009). The SGS parameters observed in our study for adult beech trees ($Sp = 0.0133$) do not show any particular deviation from SGS observed in monospecific natural stands as reported in other studies (0.0112–0.0186, Jump and Peñuelas 2007; 0.0067–0.0072, Chybicki et al. 2009; 0.0069–0.0268, Piotti et al. 2013), although the natural status of some stands could be questioned to some degree. Jump and Peñuelas (2007) suggested that genetic structure in wind-pollinated trees may have been underestimated previously due to low numbers of marker loci used. Higher distances of significant kinship coefficients detected in our study may be due to greater accuracy of the estimates, especially with the large sample sizes (about 300 adult trees and saplings, and above 600 seedlings) and a relatively large battery of genetic markers used (Cavers et al. 2005). We genotyped 20 SSR loci, which is not usually encountered for beech. Similar estimates were found in the French population ($SGSmax = 60$ m, $Sp = 0.0114$) by Sjölund and Jump (2015), based on 11 SSR markers, but the result was specific to the studied population (long-lasting coppice management).

The correlogram of sessile oak adults was steeper in the first distance interval than for pedunculate oak and is a different pattern than that observed in other studies on *Q. petraea*. Previous studies on sessile oaks in mixed stands with, for instance, *Quercus pyrenaica* (Valbuena-Carabaña et al. 2007) or mixed deciduous forest (Curtu et al. 2015) found a more uniform distribution of the kinship curve and thus a weaker SGS pattern than observed in this study. Indeed, the Sp statistics calculated for sessile oak adults in this study was 0.0187, much higher than the estimates reported by other authors ($Sp = 0.002$–0.015, Valbuena-Carabaña et al. 2007; $Sp = 0.0033$, Curtu et al. 2015). It is worth mentioning that the density of sessile oak in those studies ranged from 7 to 22 ind/ha, depending on the study site, which was similar to the density of individuals in our study (13 ind/ha). The reason for a weaker SGS in *Q. robur* as compared to *Q. petraea* remains unclear, although it is known that *Q. robur* has a greater ability for pollen and seed dispersal (Petit et al. 2003; Lagache et al. 2014). Earlier studies also found a weaker SGS in *Q. robur* than *Q. petraea* (Streiff et al. 1998; Cottrell et al. 2003; Jensen et al. 2003).

Analysis of SGS across different size/age groups of adults within species (hypothesis *iii*) demonstrated significant heterogeneity in beech and sessile oak. However, for the sessile oak there was a clear trend of enhancing SGS with decreasing age. Tero et al. (2005) compared two young with two old subpopulations of *Silene tatarica* (L.) Pers. and found a stronger SGS for the older individuals. Similar observations were reported by Jones and Hubbell (2006)

for *Jacaranda copaia* (Aubl.) D. Don. In their study, one population was divided into four equal age groups based on DBH, as in our study. However, a stronger SGS was found for the oldest cohort, and Sp statistics were increasing with age, which is exactly the opposite to our findings in sessile oak. It seems that the greatest changes in SGS parameters occur in the initial stages of life when seedling mortality results from the density effect (Hampe et al. 2010). Therefore, it should be expected that for natural, balanced populations, the intensity of SGS should decrease with the age of individuals. The situation observed in the sessile oak population does not suggest a natural mode of regeneration of the oldest sessile oaks (e.g., human activity) or a relatively recent invasion of this species into the studied stand. Indeed, it seems that the suggested invasion of *Q. petraea* into *Q. robur* stands through pollen swamping (Petit et al. 2003) may prevent the development of a strong SGS, at least at the early stages of invasion.

Considering hypothesis (*iv*), for beech and sessile oak, we found similar SGS between adults and seedlings, although this differed markedly (two-fold difference) between young and old trees for pedunculate oaks (Table 1). Moreover, for *Q. robur* adults, F_{ij} autocorrelogram was linear and gently decreasing, for seedlings, and it was steep at short distances but stabilized at larger distances. A similar shape of kinship curves, with a high peak in the first distance classes for the offspring, then being less prominent for adults, was previously noticed at the monospecific or predominant stands of *Q. robur* (Hampe et al. 2010; Vranckx et al. 2014). Hampe et al. (2010) highlighted that marked genetic structure in the first distance interval for the offspring may result directly from a fraction of maternal half-sibs growing in a neighborhood close to their mother tree, likely from gravity-dispersed seeds. Moreover, our Sp estimates (adults, 0.008; seedlings, 0.0175) were similar to those found by Hampe et al. (2010) (adults, 0.005; seedlings, 0.015), as well as in many other studies.

In general, seedlings exhibited evident SGS in all three species (Table 1). The presence of SGS in naturally established seedling generations has often been reported in beech and oaks (Vornam et al. 2004; Jump and Peñuelas 2007; Chybicki et al. 2009; Hampe et al. 2010; Vranckx et al. 2014; Curtu et al. 2015). However, in our study, SGS was more strongly pronounced in oaks than in beech. With the aid of parentage assignment of seedlings (one or both local parents identified), we were able to assess to what degree SGS depends on seed or pollen dispersal (hypothesis *v*). We observed similar seed and pollen immigration rates in this study compared to other investigations conducted in continuous pure-species stands of beech and oaks (Burczyk et al. 2006; Chybicki and Burczyk 2010; Oddou-Muratorio et al. 2010; Milleron et al. 2012; Bontemps et al. 2013; Vranckx et al. 2014; Gerber et al. 2014). In our case, gene

immigration was slightly lower for oaks (seed immigration *Q. robur* 3.1%, *Q. petraea* 1.5%, pollen immigration *Q. robur* 62.16%, *Q. petraea* 35.2%) than for beech (seed immigration 4.5%; pollen immigration 55.8%). But the differences could be attributed not only to the variable weight of seeds but also to a variable proportion of oak and beech forests in the near vicinity of the studied stand, with the latter species being more common or the location of sampled seedlings relative to the study plot edges.

However, we noticed a significant SGS for seedlings with only one parent (presumably mother) identified within the local population. In such a case, contributing pollen gametes are assumed to represent a random pollen pool produced by background populations. Therefore, any pattern of SGS for such seedlings should be attributed only to kinship resulting from restricted seed dispersal. Given the high levels of such SGS parameters, seed dispersal mechanisms (by gravity) seem to be the major driver for all studied species. We should note that all SGS parameters for seedlings with one local parent were higher for oak species than for beech (Table 1), while seedlings with two local parents exhibited even stronger results. Nevertheless, the proportional increase of SGS intensity (as measured by *Sp* and $b_{\log}$ statistics) between seedlings with one or two local parents was considerable, but similar in beech (45%) and oaks (34–50%). This indicates that pollen dispersal within the study populations was not random but spatially restricted, and it should not be neglected as an important determinant of SGS.

It seems that the SGS pattern might be facilitated by the clustered spatial distribution of individuals (Chung et al. 2007; Lara-Romero et al. 2016). However, profound insights into the effect of spatial aggregation on SGS could not be completed due to the inherent limitations of the study design. The clustered distribution of beech adults (Appendix Fig. 6) and seedlings/saplings (Appendix Fig. 8) was clearly related to their relatively strong SGS (Table 1). The tendency of spatial clustering in beech may indicate its competitive pressure (Oddou-Muratorio et al. 2010; Petritan et al. 2014). In fact, *F. sylvatica* under appropriate environmental conditions can successfully compete with *Q. robur* and *Q. petraea* (Packham et al. 2012; Petritan et al. 2014) due to its shade tolerance. Within the forest stand studied herein, the oaks seem to be a long-standing, but declining species, as evidenced by the distribution of DBH mostly exceeding 60 cm and a young cohort represented only by seedlings of 1–3 years of age. On the other hand, the beech population exhibited attributes typical of a rapidly growing population. Most beech adults have DBH below 60 cm, and the species is well represented across different age classes (Sandurska et al. 2017, 2019).

If spatial clustering results from limited seed dispersal, as might be expected in all three species, then the increased kinship among offspring established from restricted seed dispersal is a reasonable outcome (Doligez et al. 1998). Nevertheless, adult oak populations (sessile oak, in particular) exhibited significant SGS despite a lack of spatial aggregation of individuals (Table 1, Appendix Fig. 6). Population density decreases naturally with increasing age (mainly due to spatially dependent mortality), and the spatial structure of adult trees becomes generally more regular. Therefore, theoretically, the SGS observed in adults should reflect the SGS pattern formed at the seedling stage unless distances among near neighbors in adults extended beyond the distances of significant kinship among individuals at the seedlings stage. However, SGS is not expected to remain constant over time due to demographic changes as trees get older, and the populations become more scarce (Chung et al. 2003; Asuka et al. 2004; Hardesty et al. 2005). Demographic changes, interspecies competition, density-depended mortality, or even disastrous events (e.g., windbreaks) all might disrupt the stabilization of the local spatial structure, leading to a variable pattern of SGS in adult populations (Oddou-Muratorio et al. 2010).

5 Conclusion

We confirmed that naturally established populations develop SGS, and in species that predominantly disperse seed by gravity, such as beech and oaks, the formation of significant SGS may appear after even one generation. Therefore, the detection of significant SGS in plants may be good evidence for the natural establishment of populations (Rajendra et al. 2014). Our SGS results obtained in adult oak and beech populations (old-growth forest stand, close to natural) can be considered as a reference for comparisons with populations artificially created and managed by humans. Adult trees growing at distances of up to 60 m are likely to be genetically related. These results point to a minimum distance separating trees from which seeds should be harvested in forest management practices in order to avoid excessive relatedness among offspring, which seems to be a more conservative recommendation than recently published guidelines (Geburek et al. 2016). The similarities of SGS observed in this and other studies suggests that it develops in similar ways in both pure species-specific and mixed forest stands. However, the intensity of competition among species may change with the progress of global climate changes (Bontemps et al. 2012), possibly affecting the variance of reproductive success at individual and population levels. To address the impact of climate change and forest management on the SGS of forest trees, further studies should be conducted in natural populations not influenced by human activities.

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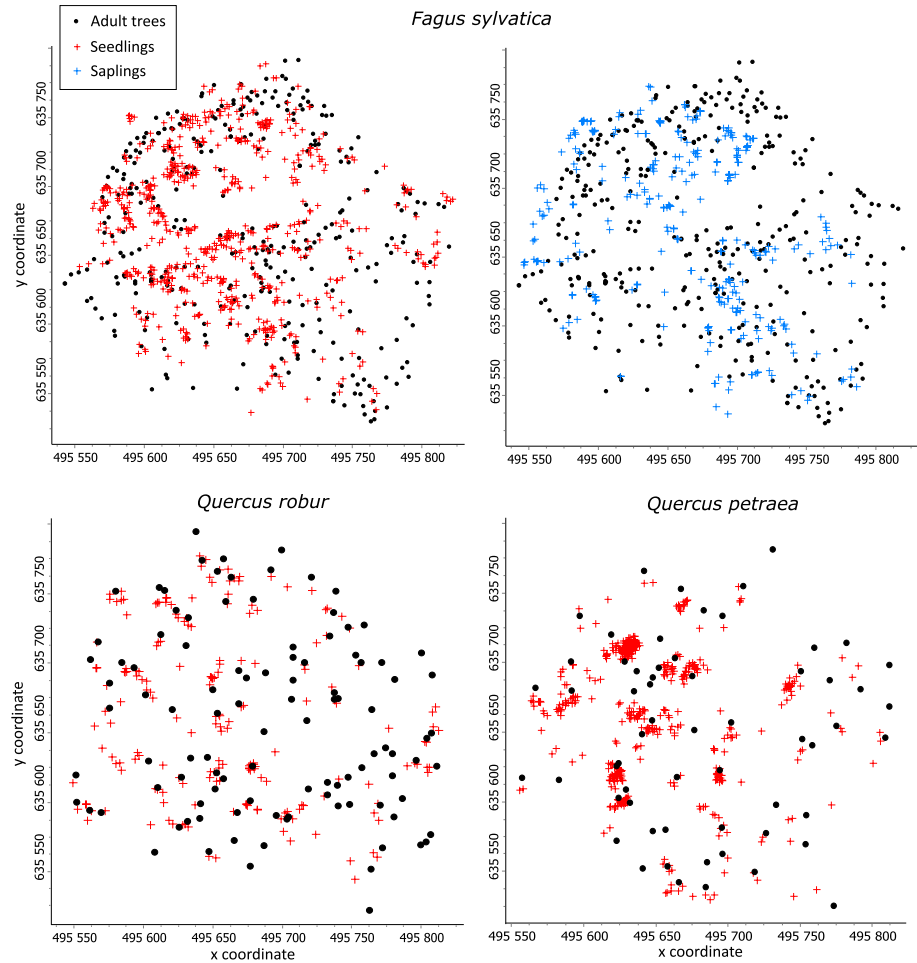
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Appendix

Fig. 4 Distribution of sampled adult trees (black circles) of *Fagus sylvatica, Quercus robur* and *Q. petraea* and their seedlings (red crosses) and saplings (blue crosses—*F. sylvatica* only). *Q. robur* and *Q. petraea* diagrams originate from our earlier work (Sandurska et al. 2019) (published with permission)
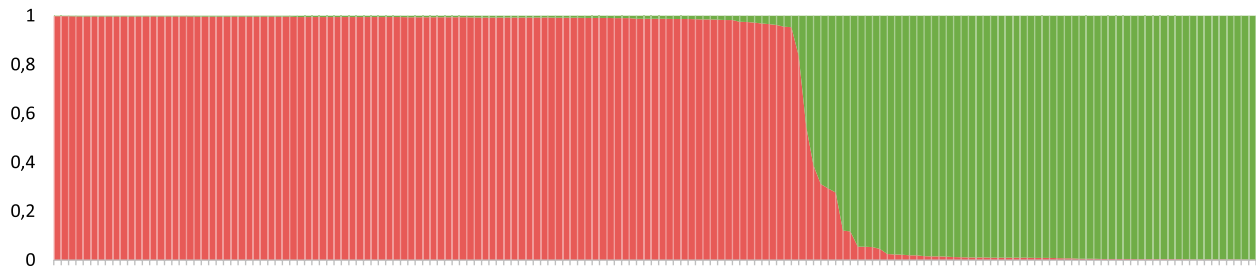

Fig. 5 Probability of assignment of individual adult oak trees to the two groups identified with STRUCTURE software (cluster 1: *Quercus robur*—red; cluster 2: *Q. petraea*—green)
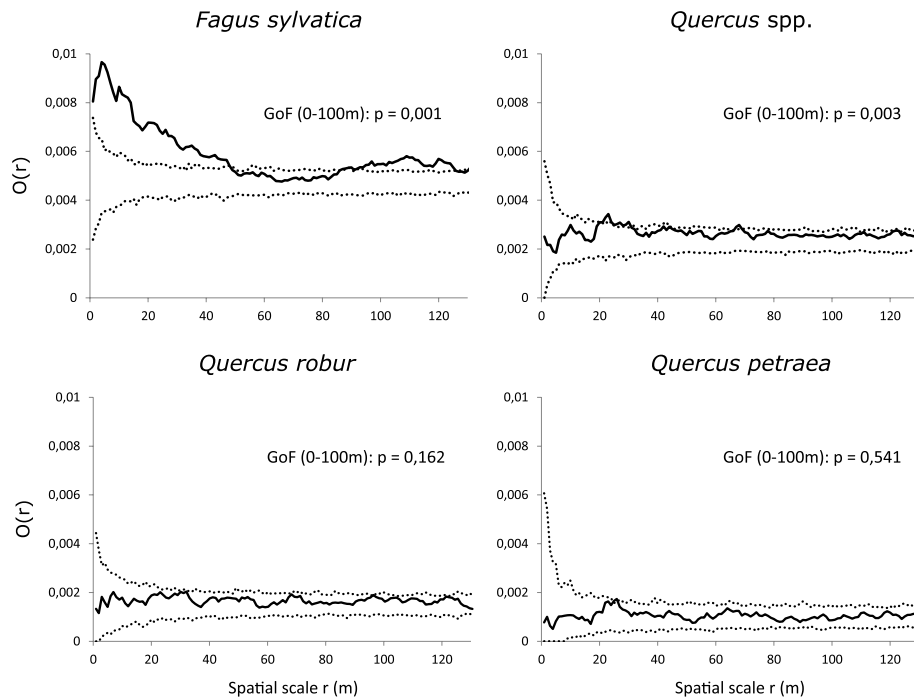
Fig. 6 Spatial demographic structure of all adult trees as measured using the O-ring statistic [O(r)]. Dotted lines indicate 95% confidence envelopes about the null hypothesis of random spatial structure. GoF(0–100 m) denotes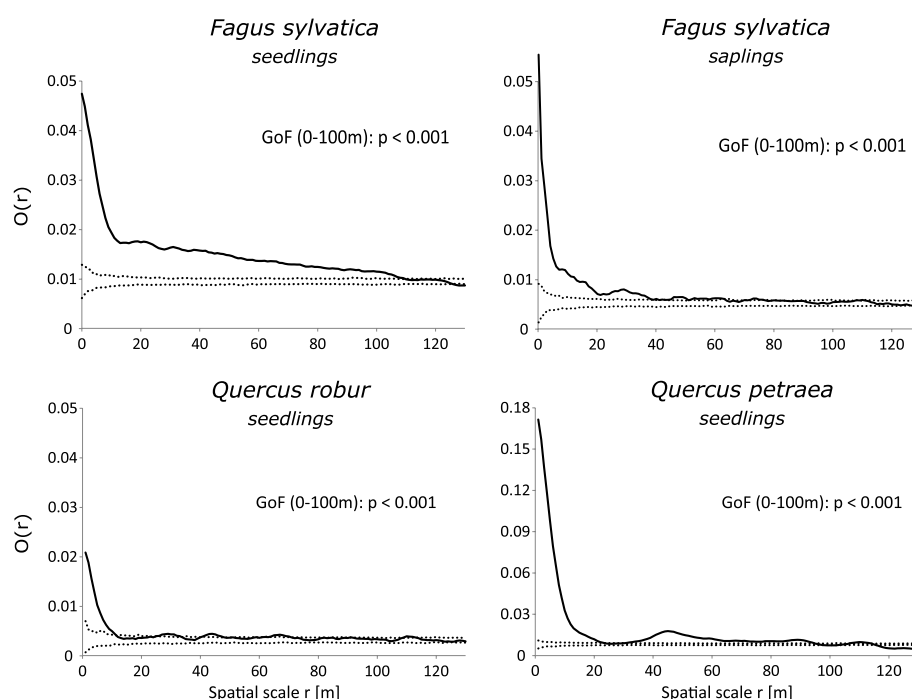 Goodness-of-Fit test results on the given distance. *Q. robur* and *Q. petraea* diagrams originate from our earlier work (Sandurska et al. 2019) (published with permission)
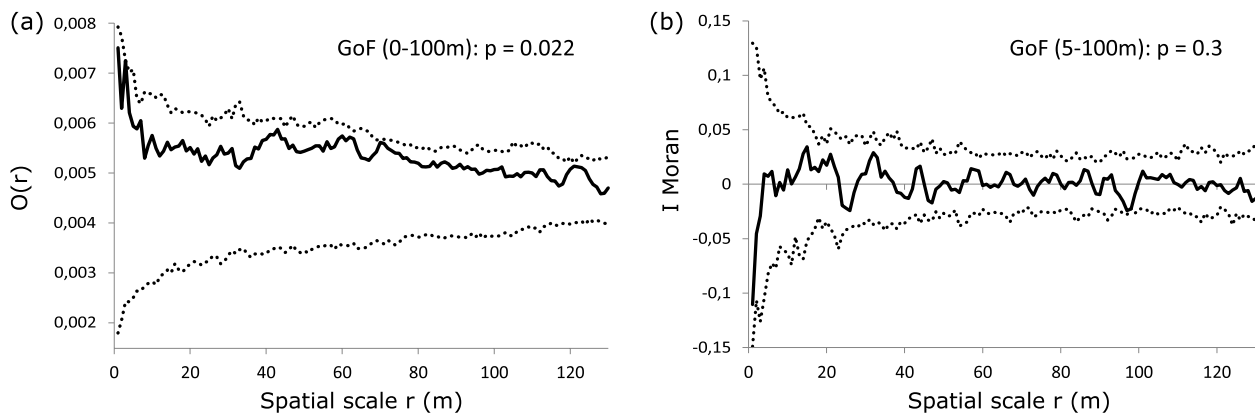
Fig. 7 The bivariate pair correlation functions between the distributions of beech and oaks (both oaks analyzed jointly): (a) bivariate O-ring statistics and (b) Moran's I autocorrelogram with DBH as a mark. Dotted lines indicate 95% confidence envelopes about the null hypothesis of random spatial structure. GoF(0–100 m) denotes Goodness-of-Fit test results on the given distance

Fig. 8 Spatial demographic structure of seedlings and saplings (*F. sylvatica* only) as measured using the O-ring statistic [O(r)]. Dotted lines indicate 95% confidence envelopes about the null hypothesis of random spatial structure. GoF(0–100 m) denotes Goodness-of-Fit test results on the given distance. Note that *y* axes show different scales of clarity for *Q. petraea* seedlings. *Q. robur* and *Q. petraea* diagrams originate from our earlier work (Sandurska et al. 2019) (published with permission)

Authors' contributions
Conceptualization: Elżbieta Sandurska and Jarosław Burczyk. Methodology: Elżbieta Sandurska and Jarosław Burczyk. Formal analysis: Elżbieta Sandurska, Bartosz Ulaszewski, and Jarosław Burczyk. Investigation: Elżbieta Sandurska, Bartosz Ulaszewski, Katarzyna Meyza, and Ewa Sztupecka. Resources: Katarzyna Meyza and Ewa Sztupecka. Writing—original draft preparation: Elżbieta Sandurska. Writing—review and editing: All authors. Funding acquisition: Jarosław Burczyk. Resources: Jarosław Burczyk. Supervision: Jarosław Burczyk. The authors read and approved the final manuscript.

Availability of data and materials
The datasets generated and analyzed during the current study are available in the RepOD repository, https://doi.org/10.18150/0CNXA1.

Declarations

Ethics approval and consent to participate
The authors declare that they follow the rules of good scientific practice. All the experiments conducted on plants were carried out in accordance with guidelines of National Science Center, Poland.

The authors declare that the plant material used in this study was sampled based on the permission WPN.6205.42.2013.KLD issued by the Regional Directorate for Environmental Protection in Bydgoszcz, Poland.
The authors declare that the diagrams of *Q. robur* and *Q. petraea* presented in Figs. 4, 6 and 8 (Appendix) originate from our earlier work (Sandurska et al. 2019), which are published with relevant permission.

Consent for publication
All authors gave their informed consent to this publication and its content.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Genetics, Faculty of Biological Sciences, Kazimierz Wielki University, Chodkiewicza 30, 85-064 Bydgoszcz, Poland.

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