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GENETIC INSIGHTS INTO ECOLOGICAL SUCCESSION FROM OAK- (*QUERCUS ROBUR* L.) TO BEECH- (*FAGUS SYLVATICA* L.) DOMINATED FOREST STANDS

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Genetic diversity is often considered a major determinant of long term population persistence and its potential to adapt to variable environmental conditions. The ability of populations to maintain their genetic diversity across generations seems to be a major prerequisite for their sustainability, which is particularly important for keystone forest tree species. However, little is known about genetic consequences of demographic alterations occurring during natural processes of ecological succession involving changes in the species composition. Using microsatellites, we investigated genetic diversity of adult and offspring generations in beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) populations coexisting in a naturally established old-growth forest stand, showing some symptoms of ongoing ecological succession from oak- to beech- dominated forest. In general, adult generations of both species exhibited high levels of genetic diversity (0.657 for beech; 0.821 for oak), which, however, depended on the sets of selected genetic markers. Nevertheless, several symptoms such as differences in genetic diversity indices between generations, significant levels of inbreeding (up to 0.029) and low estimates of effective population size (48–80) confirmed the declining status of the oak population. On the other hand, the uniform distribution of genetic diversity indices across generations, low levels of inbreeding (0.004), low genetic differentiation among adults and offspring and, most importantly, large estimates of effective population size (119–716), all supported beech as a successive and successful tree species in the studied forest stand.

Keywords: nature reserve, ecological succession, genetic diversity, effective population size, *Fagus sylvatica*, *Quercus robur*

INTRODUCTION

Establishment of protected areas such as nature reserves is one of the major efforts of conservation of terrestrial ecosystems, attempting to protect specific species, habitats or communities (Prendergast et al., 1999). Because nature reserves usually embrace diverse species communities and conserve ecological and evolutionary processes underlying the persistence of such communities, they are considered attractive research objects for studying biological processes existing in fairly undisturbed ecosystems (Soulé, 1985; Balmford et al., 2002).

Long term sustainability of nature reserves is one of the main goals of their conservation (Grumbine, 1994; Bengtsson et al., 2003). However, despite increasing knowledge of ecology (in a broad sense) of terrestrial ecosystems, little is known about the role of genetic diversity of foundation species in the persistence of natural populations (Lande and Shannon, 1996; Hughes et al., 2008). Genetic diversity of keystone tree species is believed to be one of the drivers determining species and genetic diversity of the coexisting communities in forest ecosystems (Whitham et al., 2003, 2006; Gugerli et al., 2013). In temperate zones, forest ecosystems dominated by two or more key-stone tree species are of particular interest, because they may promote greater diversity of coexisting organisms. In this respect, forest stands composed of beech and oak are of particular interest.

Pedunculate oak (*Quercus robur* L.) and common beech (*Fagus sylvatica* L.) are among the most important tree species in Central Europe, both from ecological and economical points of view (Packham et al., 2012). Beech and oak form pure and mixed stands, but productivity of mixed beech-oak forests might be superior to pure stands

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depending on site conditions (Pretzsch et al., 2013), and the importance of mixed stands might increase with the consequences of climate changes (Pretzsch et al., 2013). However, the future status of beech and oak forests affected by climate changes remains unclear and it is currently under intensive debate (Geßler et al., 2006; Kramer et al., 2010; Czucz et al., 2011; Scharnweber et al., 2011; Mette et al., 2013; Zimmermann et al., 2015). Succession from oak- to beech-dominated forest stands is often observed (Rohner et al., 2012; Petritan et al., 2014), but this process is usually long and complex, involving a variable pattern of competition for light, moisture and nutrient resources at the stage of regeneration, but also a variable pattern of mortality of senescent individuals (Vera et al., 2006; Bontemps et al., 2012; Ligot et al., 2013). However, the role of genetic diversity in the succession process is largely unknown.

Loss of genetic diversity may be an important factor of extinction (Spielman et al., 2004), particularly in isolated populations (Frankham, 2005). However, the loss of genetic diversity in forest tree populations is unlikely, especially in common tree species growing across large areas spanning over continents. Nevertheless, forest management and global climate changes are expected to influence the levels and distribution of genetic diversity of forest trees (Koskela et al., 2007, 2014; Ratnam et al., 2014). The predicted changes in temperature and precipitation regimes might affect particularly broadleaf tree species such as oak and beech (Bussotti et al., 2015).

The extent to which populations can adapt to variable climate conditions and succeed in a competitive environment depends on the within-population genetic diversity and how this diversity is distributed within and among populations (Aitken et al., 2008; Bolte and Degen, 2010). However, one of the major concerns of intensive management of forest tree population is mixing of gene pools from different populations due to long distance commercial seed transfer (Koskela et al., 2014). Such uncontrolled seed movement may lead to establishment of populations maladapted to new environments. Historical seed transfer, extensive particularly in the 19th century, altered the distribution of genetic diversity of several forest trees in Europe (Koskela et al., 2014; Jansen and Geburek, 2016; Myking et al., 2016). It is expected that the exchange of forest reproductive material had a strong impact on the levels of genetic diversity of forest trees and the spatial distribution of genetic diversity, even stronger than natural processes (Geburek and Turok, 2005). Today, natural and introduced populations might be largely intermixed (Konig et al., 2002; Lewandowski et al., 2012, 2014). On the other hand, populations composed of old trees (> 200 years old) usually represent native gene pools which may have gene variants and their combinations important for a future success in adaptation to variable (and unpredictable) environmental conditions. Such intact old-growth forest stands, often conserved as nature reserves, are therefore of special interest. Despite the long lifespan of majority of forest trees and their complex age structure, the question remains whether the genetic diversity existing in adult populations is maintained in young, naturally regenerating generations. The maintenance of genetic diversity across generations is considered a major prerequisite for sustainability and persistence of populations of forest trees (Aitken et al., 2008) and is particularly important in nature reserves. This issue becomes fundamental in the context of ecological succession, when one key-stone tree species might be replaced by another. Here, the old-growth forest stands evolving from oak- to beech-dominated forests (Rohner et al., 2012; Petritan et al., 2014) may serve as an excellent study system to investigate genetic aspects of ecological succession.

Here we present a case study focused on genetic diversity of two key-stone tree species (*Fagus sylvatica* L. and *Quercus robur* L.) coexisting in a single forest stand showing symptoms of ongoing ecological succession form oak- to beech- dominated forest. Using nuclear microsatellites as genetic markers we investigated the levels of genetic diversity and inbreeding in adult generations, and explored to what extent genetic diversity is transferred to the cohorts of subsequent generations represented by naturally established seedlings. We also performed comparative analyses of effective population sizes. Finally, we provide recommendations for conservation of genetic resources of the studied populations.

METHODS

The study was conducted in a forest stand being a part of the Jamy Nature Reserve, established in 1968 in the Jamy Forest District in North-Central Poland. In terms of phytosociology, the area is classified as a subcontinental hornbeam forest (*Tilio-Carpinetum*) with participation of beech, for which it is the northeastern boundary of the species range. The oldest individuals, according to forest management plans, are at least 218 years old, with a maximum height of 35 meters. For this study the centrally located plot was designed (φ18°56'6.07"E, λ53°35'9.67"N) with a round-shaped area of about 5.5 ha. The studied forest stand shows clear symptoms of ecological succession form oak- to beech-dominated forest. The oak population is represented only by an even-aged adult cohort (ca. 220 years old)

and young seedlings, with no individuals at intermediate age classes. On the other hand, the beech population consists of a small fraction (11 ind.) of old trees (DBH > 80 cm), followed by a large number of younger trees (DBH: 30–60 cm) (Fig. 1). Beech is also strongly represented by juvenile individuals and a large number of seedlings. All cohorts of oak and beech seem to be established naturally, which is exemplified by a strong spatial genetic structure observed in this stand (Sandurska et al., *in preparation*).

Plant material was sampled from three cohorts: 1) *adults* – trees with DBH > 25 cm (all individuals sampled); 2) *juveniles* – trees with DBH < 20 cm (a sample of 300 individuals, only *F. sylvatica*; no juveniles for *Q. robur*); and 3) *seedlings* – < 30 cm tall (a sample of about 640 individuals within each species). Leaves from individuals were collected in August of 2013 and 2014. The numbers of individuals sampled in each cohort and species are presented in Tables 1 and 2.

DNA from dried leaves was isolated with GeneMATRIXPlant & Fungi DNA Purification Kit (EURx, Poland). For *Fagus sylvatica* the set of 20 nuclear microsatellite markers: *Fc3* (*FcC00468*), *Fc5* (*FcC00730*), *Fc6* (*FcC00927*), *Fc9* (*FcC03095*) (Ueno et al., 2009); *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* (Lefevre et al., 2012); *sfc_0036*, *fc_1143* (Asuka et al., 2004); *FS1_15* (Pastorelli et al., 2003) was used for genotyping of the samples. The *Quercus robur* genotyping was done by the set of 19 microsatellite markers: *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* (Guichoux et al., 2011b); *ssrQrZAG 30*, *ssrQrZAG 65*, *ssrQrZAG 87*, *ssrQrZAG 101*, *ssrQrZAG 112* (Kampfer et al., 1998); *ssrQpZAG 9* – modified (Steinkellner et al., 1997). PCR products were separated using the ABI PRISM 3130XL sequencer (Applied Biosystems, Foster City, USA). Allele identification was performed with GENESCAN 3.7 and GENOTYPER 3.7 software provided by Applied Biosystems.

Parameters of genetic structure were calculated using CERVUS v3.0 (Kalinowski et al., 2007). Allelic richness was estimated using FSTAT 2.9.3.2 (Goudet, 1995). Differentiation measures (F_{st}, R_{st}) between different cohorts were calculated using SPAGEDI v.1.5 (Hardy and Vekemans, 2002). The frequencies of *null* alleles and inbreeding coefficients accounting for the presence of *null* alleles and genotyping errors were calculated within each cohort using INEST v.2.0 software (Chybicki and Burczyk, 2009). Effective population size (*Ne*) was estimated for individual cohorts based on the linkage disequilibrium method (Waples and Do, 2008). The temporal method (Waples, 1989) for estimating *Ne* was also used employing pairs of different cohorts, based on Pollak's method for computing the standardized variance in allele frequency (Pollak, 1983). The estimates were calculated using NEESTIMATOR v2 (Do et al., 2014); however, we screened out alleles with frequencies lower then 0.02 (P_{Crit} = 0.02) as suggested for microsatellite data (Do et al., 2014).

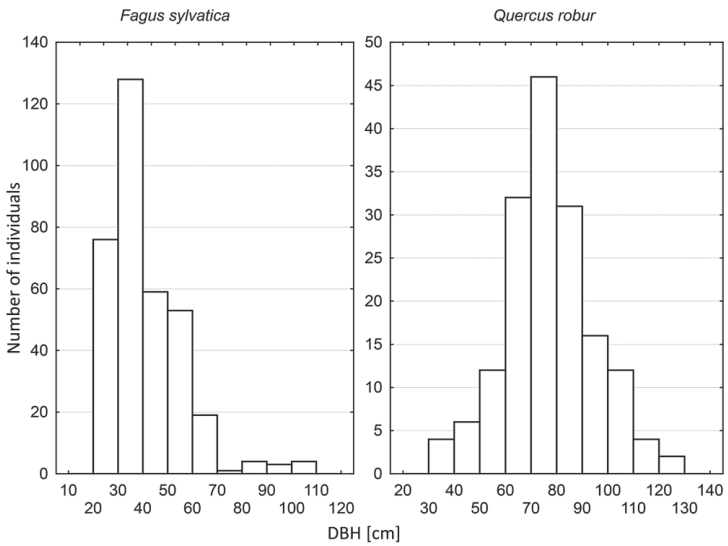

Fig. 1. The distribution of diameter at breast height (DBH, in cm) in adult populations of *Fagus sylvatica* and *Quercus robur*.

TABLE 1. Genetic diversity of *Fagus sylvatica* in the reserve Jamy. Details for each locus are presented only for adult population, mean values are given for adults (N=333), juveniles (N=300) and seedlings (N=644). (*A*): mean number of alleles; (*Ae*): effective number of alleles; (*AR*): allelic richness; (*Ho*): observed heterozygosity; (*He*): expected heterozygosity; (*F*is): inbreeding coefficient; (*Null*): frequency of null alleles; (*H-W*): significance of departure from Hardy-Weinberg equilibrium. Standard errors in parentheses.

Locus	*A*	*Ae*	*AR*	*Ho*	*He*	*Fis*	*Null*	*HW*
Cf29	3	1.389	3.000	0.288	0.280	-0.028	0	ns
Cf31	12	5.780	11.895	0.925	0.827	-0.119	0	***
Cf5	7	2.778	7.000	0.631	0.640	0.015	0.0047	ns
Cf6	11	5.682	10.789	0.859	0.824	-0.042	0	ns
Concat	4	1.437	4.000	0.276	0.304	0.09	0.0217	ns
Fc5	4	2.092	4.000	0.550	0.522	-0.052	0	ns
Fc9	11	2.801	10.790	0.637	0.643	0.011	0.0086	ns
Fs115	13	4.274	12.684	0.739	0.766	0.036	0	ns
Sfc0036	7	2.695	7.000	0.628	0.629	0.002	0.0005	ns
Sfc1143	14	5.650	13.846	0.801	0.823	0.026	0.0100	ns
Cf19	12	6.410	11.796	0.837	0.844	0.008	0	ns
De576	7	2.618	6.988	0.601	0.618	0.029	0.0011	ns
Dukct	7	2.506	7.000	0.565	0.601	0.061	0.0132	ns
Dz447	5	2.123	4.895	0.541	0.529	-0.021	0	ns
Ejv8t	6	2.841	6.000	0.432	0.648	0.332	0.1360*	***
Emily	7	4.545	6.999	0.820	0.780	-0.051	0	ns
Erhbi	4	1.869	4.000	0.447	0.465	0.038	0.0222	ns
Fc3	11	4.587	10.884	0.784	0.782	-0.002	0	ns
Fc6	12	5.076	11.903	0.815	0.803	-0.015	0.0015	ns
Geu75	11	5.405	10.989	0.823	0.815	-0.010	0	ns
Mean	8.4	3.628	8.323	0.650	0.657	0.015	0.0110	
adults	(0.782)	(0.366)	(0.767)	(0.042)	(0.038)	(0.019)	(0.0068)	
Mean	8.5	3.629	8.498	0.656	0.659	0.007	0.0084	
saplings	(0.724)	(0.366)	(0.723)	(0.042)	(0.037)	(0.019)	(0.0062)	
Mean	8.9	3.670	8.291	0.659	0.667	0.013	0.0110	
seedlings	(0.757)	(0.353)	(0.684)	(0.038)	(0.036)	(0.016)	(0.0058)	

Significance: * $p < 0.05$; *** $p < 0.001$; ns – not significant at $p < 0.05$

RESULTS

GENETIC DIVERSITY

All loci used in the analyses of beech were polymorphic with numbers of alleles per locus ranging from 3 to 14 (mean 8.4), however, the mean effective number of alleles was found to be 3.628. The observed and expected heterozygosities appeared to be quite similar (Table 1). The levels of *null* allele frequencies was low, in general. For adult and juvenile cohorts, only locus *Ejv8t* exhibited considerable and significant frequency of *null* alleles (Table 1), but in seedlings significant level of *null* alleles was observed additionally at *Fs115* locus. Inbreeding was found to be low. It averaged across loci at 0.015, 0.007 and 0.013 for adults, juveniles and seedlings, respectively. Nevertheless, when accounting for the presence of *null* alleles, a low but significant level of inbreeding was observed only for seedlings (0.0044; 95% CI: 0.0008-0.0083). The estimates of population genetic parameters were found to be similar across different cohorts, with a slight increase observed for seedlings.

All loci used for oak analyses were highly polymorphic, with 7 to 33 alleles per locus (17.89 on average) (Table 2). The effective number of alleles varied widely across loci from 2.92 to 21.23.

TABLE 2. Genetic diversity of *Quercus robur* in the reserve Jamy. Details for each locus are presented only for adult population, mean values are given for adults (N=163) and seedlings (N=647). (*A*): mean number of alleles; (*Ae*): effective number of alleles; (*AR*): allelic richness; (*Ho*): observed heterozygosity; (*He*): expected heterozygosity; (*Fis*): inbreeding coefficient; (*Null*): frequency of null alleles; (*H-W*): significance of departure from Hardy-Weinberg equilibrium. Standard errors in parentheses.

Locus	A	Ae	AR	Ho	He	Fis	Null	HW
Pie102	12	2.865	11.969	0.632	0.651	0.029	0.0216	ns
Pie20	11	4.115	10.968	0.693	0.757	0.084	0.0346	ns
Pie215	9	3.906	8.908	0.761	0.744	-0.023	0	ns
Pie223	8	4.098	7.969	0.730	0.756	0.035	0.0019	ns
Pie242	13	6.024	12.938	0.804	0.834	0.036	0.0152	ns
Pie243	7	2.924	7.000	0.620	0.658	0.058	0.0049	ns
Pie267	13	5.291	12.997	0.834	0.811	-0.028	0	ns
Zag65	29	21.277	29.000	0.899	0.953	0.057	0.0226	ns
Zag11	29	9.524	28.962	0.800	0.895	0.106	0.0402*	ns
Zag112	17	5.556	16.907	0.791	0.820	0.035	0	ns
Zag20	20	7.634	19.813	0.810	0.869	0.068	0.0223	ns
Zag30	33	14.925	32.876	0.883	0.933	0.053	0.0098	**
Zag9	13	6.849	12.908	0.853	0.854	0.002	0	ns
Zag96	21	4.082	20.907	0.675	0.755	0.106	0.0177	ns
Zag101	23	10.417	22.888	0.876	0.904	0.031	0.0155	ns
Zag110	26	4.000	25.812	0.755	0.750	-0.006	0	ns
Zag15	14	4.219	13.997	0.755	0.763	0.010	0.0044	ns
Zag7	20	16.129	19.939	0.859	0.938	0.084	0.0409*	ns
Zag87	22	11.364	21.876	0.859	0.912	0.058	0.0179	ns
Mean	17.894	7.642	17.823	0.784	0.821	0.042	0.0142	
adults	(1.767)	(1.173)	(1.761)	(0.019)	(0.021)	(0.009)	(0.0032)	
Mean	20.158	6.599	17.128	0.759	0.803	0.056	0.0187	
seedlings	(2.082)	(0.911)	(1.654)	(0.022)	(0.021)	(0.010)	(0.0031)	

Significance: * $p < 0.05$; ** $p < 0.01$; ns – not significant at $p < 0.05$

Apparently, some loci with large numbers of alleles had relatively low effective numbers of alleles (e.g., *Zag110*, *Zag96*). Expected heterozygosity was higher than observed one, resulting in an increased level of fixation index. Interestingly, while the mean number of alleles was distinctly higher for seedlings, the effective number of alleles was higher for adults. Given that both types of heterozygosity were lower in seedlings, the increase of mean number of alleles in this cohort could result from new alleles observed in seedlings at low frequencies, likely as a result of background pollination. In general, the frequency of null alleles in oak was low, but on average it was slightly lower in adults (0.0142) than in seedlings (0.0187). In adults, significant levels of nulls were observed for two loci (*Zag11*, *Zag7*; Table 2); however, in seedlings, low but significant levels of *null* alleles were observed for most loci, except *Pie102*, *Pie215*, *Pie242*, *Pie267*, *Zag30*, *Zag9*, *Zag101*. The mean levels of inbreeding averaged across loci for adults and seedlings were equal to 0.042 and 0.056, respectively (Table 2). However, the levels of inbreeding estimated when accounting for the presence of *null* alleles were found to be significant for adults (0.0235; 95% CI: 0.0010–0.0401), as well as for seedlings (0.0288; 95% CI: 0.0219–0.0361).

GENETIC DIFFERENTIATION AMONG COHORTS

Genetic differences between different cohorts within species were generally low (Table 3); however, the level of differentiation between seedlings and adults was distinctly greater for oak than for beech across both estimated parameters (F_{st} and R_{st}). Within

TABLE 3. Levels of genetic differences between pairs of populations representing different cohorts within each of the studied species.

Population pair	F_{st}	R_{st}
Fagus sylvatica		
adults-seedlings	0.00072*	0.00071 ns
adults-juveniles	0.00052 ns	0.00004 ns
juveniles-seedlins	0.00206*	0.00193*
Quercus robur		
adults-seedlings	0.01282*	0.01918*

Significance: * $p < 0.05$; ns - not significant at $p < 0.05$

TABLE 4. The estimates of effective populations size obtained based on single sample (linkage disequilibrium) and temporal methods. (95% CI in parentheses).

	Fagus sylvatica	*Quercus robur*
Single sample		
Adults	119.0 (100.7–141.8)	80.3 (72.2–89.7)
Juveniles	91.3 (78.7–106.4)	–
Seedlings	149.2 (118.8–188.3)	49.7 (46.0–53.3)
Temporal		
Adults-seedlings	716.3 (413.4–1562.2)	47.8 (37.5–60.3)
Adults-juveniles	801.3 (382.0–3894.5)	–
Juveniles-seedlings	224.6 (144.2–359.9)	–

beech, the smallest differentiation was observed between adults and juveniles, as well as between adults and seedlings (Table 3). The consistency between F_{st} and R_{st} suggests that genetic drift is the main reason for differentiation between the cohorts.

EFFECTIVE POPULATION SIZE

The estimates of effective population size (EPS) appeared to be significantly greater for beech than for oak (Table 4). However, while in beech the effective populations size of adults equaled only 35.74% of the sampled census size ($Ne/N = 0.3574$), in oak adults the effective population size was 49.26% of the number of sampled individuals. Considering seedlings, which were sampled at comparable quantities in both species, beech appeared to have the estimates of effective population size three times greater than oak (Table 4). Interestingly, in oak the temporal method applied to the generations of adults and seedlings provided the EPS estimates quite similar to those obtained based on single sample approach in seedlings, which cross-validates generally low level of EPS in oak seedlings. Such similarity was not observed in beech. In beech, all possible pairs of generations used for the temporal method provided relatively high estimates of EPS. The lowest estimate, however still considerable (224.6), was observed for the pair of juveniles and seedlings, but it should be mentioned that the generation of juveniles is rather unlikely to be the parental generation of the studied seedlings. The high estimates of EPS obtained based on the temporal method in beech suggest a high similarity of gene pools among different beech cohorts.

DISCUSSION

Monitoring genetic diversity of populations with the aid of genetic markers may provide important insights into the current status of populations (Schwartz et al., 2007; Fussi et al., 2016). Forest management may affect genetic diversity (Rajendra et al., 2014) and several studies were focused on the effect of forest management on genetic diversity (Buiteveld et al., 2007; Piotti et al., 2013; Ratnam et al., 2014; Westergren et al., 2015). However, in order to monitor the impacts of forest management and climate changes on genetic diversity of forest trees, intact populations not affected by human activities are needed to serve as a reference autochthonous populations. Ultimately, the results of genetic diversity obtained in this study in adult populations (old-growth natural stand) may serve as a reference for future comparisons with managed populations.

Validated multiplexes of microsatellite loci developed for beech (Lefevre et al., 2012; Pluess and Maattanen, 2013) and oaks (Guichoux et al., 2011b) recently became available, and they might be considered standard sets of marker loci for population genetic studies of these species. Nevertheless, there were only a few studies employing these loci which could demonstrate their utility and robustness in the context of genotyping problems (e.g., the presence of *null* alleles) (Guichoux et al., 2011a) Here we found that both sets of microsatellite loci showed low and acceptable levels of *null* allele frequencies, and all of them (with the exception of *Ejv8t* locus in beech) might be efficiently used in population genetic surveys. However, when estimating inbreeding levels, which are sensitive to the presence of *null* alleles, we advise using appropriate tools for accounting for *null* alleles (Chybicki and Burczyk, 2009).

The loci optimized for beech have rarely been used so far (de Lafontaine et al., 2013; Gomory et al., 2015). At European scale, these loci were represented on average by 12.1 alleles per locus (de Lafontaine et al., 2013), while in this study we found on average only 8.125 alleles per locus. Similarly,

the expected and observed heterozygosities were found to be 0.702 and 0.694 at European scale, but in our case they were smaller and equaled 0.650 and 0.638, respectively. These differences might obviously result from the fact that we sampled only a single population, located in the north-eastern distribution limit, presumably in the front edge of an expanding population (Magri et al., 2006). However, similar levels of genetic diversity, as in our study, were found in other beech populations (Westergren et al., 2015; Kempf and Konnert, 2016).

The presence of *null* alleles is a common problem in beech (Paffetti et al., 2012; Gomory et al., 2015), particularly when estimating the levels of inbreeding (Chybicki and Burczyk, 2009). Elevated levels of inbreeding in beech were found in several studies (Vornam et al., 2004; Buiteveld et al., 2007; Paffetti et al., 2012), but in most cases it was a likely effect of the presence of *null* alleles. Other authors (Rajendra et al., 2014; Westergren et al., 2015; Kempf and Konnert, 2016) observed relatively low levels of inbreeding. Apparently, when F_{is} is estimated along with accounting for the presence of *null* alleles, the levels of inbreeding in beech are usually found to be insignificant (Piotti et al., 2013; Pluess and Maattanen, 2013).

In the case of oaks, the recently proposed validated multiplexes of microsatellites were used mostly to study mating patterns (Lagache et al., 2014), interspecific hybridization processes (Lander et al., 2013) or species identification (Neophytou, 2013; Rellstab et al., 2016) rather than genetic diversity (Curtu et al., 2015; Moracho et al., 2016). Nevertheless, the levels of population genetic parameters found in our study were similar to those observed by other authors (Streiff et al., 1998; Cottrell et al., 2003; Curtu et al., 2015).

Investigating temporal changes of genetic diversity between adult and offspring populations is interesting in the context of population sustainability, and is of great importance for nature reserves. Recently, such studies have been conducted in beech (Westergren et al., 2015) and oak (Dering and Chybicki, 2012; Vranckx et al., 2014) forest stands. Westergren et al. (2015) reported for beech, that actual and effective numbers of alleles, as well as the levels of expected heterozygosity were not different between adult and saplings cohorts; however, they noted slight excess of inbreeding in the offspring, but the estimates were not adjusted for the presence of *null* alleles. Those results closely resemble the findings obtained in this study. Here, the uniformly high levels of genetic parameters across different life-stages suggest that the population probably attains the maximum available levels of genetic diversity (given the used marker set) and the gene pool of parental generation is transferred to offspring generation to a great extent. This is supported by the relatively high estimates of effective population size and low levels of genetic differentiation between adults and offspring.

Similarly, in pedunculate oak, Vranckx et al. (2014) found no difference between adults and seedlings based on various parameters of population genetic structure. Dering and Chybicki (2012) noticed increase or decrease of genetic diversity of naturally regenerated seedlings as compared to adult generations, depending on the population studied. They also found differences in *Ne* estimates (from 49 to 237), which corresponded well to the differentiation levels revealed by F_{st} indices. Nevertheless, some of the mentioned studies (Vranckx et al., 2014; Westergren et al., 2015) were done based on relatively small numbers of adults and seedlings/saplings per population, making the detection of possible differences difficult. Also, the above mentioned studies focused only on single-species stands.

In this study we investigated genetic changes between parental and offspring generations in beech and oak populations coexisting in a mixed stand composed of the two species. The studied forest stand seems to be at the stage of transition from oak- to beech- dominated forest. Here, beech is well represented across various age classes and demonstrates successful regeneration. On the other hand, oak is represented only by an adult cohort and 1–3 years old seedlings, suggesting the decline of this population. Indeed, beech has the tendency to dominate the forest stands where it occurs, competing commonly with pedunculate oak (Packham et al., 2012).

The competitive advantage of beech may result from its shade tolerance (Ligot et al., 2013) and specific root development (Leuschner et al., 2001), but here we demonstrate that genetic diversity and the degree to which it is transferred from adults to offspring may also favor beech, as compared to oak. At first sight, oak shows greater genetic diversity than beech (Tables 1 and 2). However, genetic diversity measures such as expected heterozygosity or allelic richness depend on the selected genetic markers, therefore are not appropriate for comparisons between species. However, the estimates of inbreeding or effective population size are independent of the specific marker set and they are comparable across different species.

CONCLUSIONS

From a genetic point of view, beech population seems to perform very well in the studied stand: indices of genetic diversity were uniformly high across the cohorts, suggesting that the population reaches its maximum variability, given the set of

used genetic markers. Additionally, the adult generation had no signs of inbreeding, there was no differentiation between adults and offspring, and effective population sizes were large. On the other hand, the oak population demonstrated some symptoms of decline. Adults and offspring showed significant levels of inbreeding. Notably, while the mean number of alleles increased from adults to the generation of seedlings, the effective number of alleles was decreasing. Genetic differentiation between adults and seedlings, as measured based on F_{st} and R_{st}, was much greater than in beech. Finally, effective population sizes appeared to be low, which was particularly evident for seedlings. All these results suggest a restricted number of local oaks participating in reproduction.

Genetic diversity cannot be considered the major determinant of beech success or oak decline in the Jamy Nature Reserve. However, low inbreeding levels and high genetic diversity revealed by large effective population sizes may support beech as a successive species in the studied forest stand. From this point of view, no action needs to be taken to conserve genetic diversity of the beech population. However, the future of oak in the forest stand of the Jamy Reserve seems foregone. Given the reserve status of the studied stand, possible actions to be undertaken to protect genetic diversity of the oak population are limited to *ex-situ* conservation measures. In order to conserve the potentially valuable gene pool of the oak population, it is advised that seeds from as many as possible oak trees should be collected and used for establishing *ex-situ* plantations to preserve yet available gene diversity of oaks.

AUTHORS' CONTRIBUTIONS

ES, BU and JB designed the research and wrote the article. ES and BU collected samples, genotyped plant material and contributed to statistical analyses. JB obtained funding.

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