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SPATIAL GENETIC STRUCTURE WITHIN POPULATIONS OF *SORBUS TORMINALIS* (L.) CRANTZ: COMPARATIVE ANALYSIS OF THE SELF-INCOMPATIBILITY LOCUS AND NUCLEAR MICROSATELLITES

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Distribution of genetic diversity among and within plant populations may depend on the mating system and the mechanisms underlying the efficiency of pollen and seed dispersal. In self-incompatible species, negative frequency-dependent selection acting on the self-incompatibility locus is expected to decrease intensity of spatial genetic structure (SGS) and to reduce population differentiation. We investigated two populations (peripheral and more central) of wild service tree (*Sorbus torminalis* (L.) Crantz), a self-incompatible, scattered tree species to test the differences in population differentiation and spatial genetic structure assessed at the self-incompatibility locus and neutral nuclear microsatellites. Although, both populations exhibited similar levels of genetic diversity regardless of the marker type, significant differentiation was noticed. Differences between F_{ST} and R_{ST} suggested that in the case of microsatellites both mutations and drift were responsible for the observed differentiation level, but in the case of the *S-RNase* locus drift played a major role. Microsatellites indicated a similar and significant level of spatial genetic structure in both populations; however, at the *S-RNase* locus significant spatial genetic structure was found only in the fragmented population located at the north-eastern species range limits. Differences in SGS between the populations detected at the self-incompatibility locus were attributed mainly to the differences in fragmentation and population history.

Keywords: *S-RNase* locus, microsatellites, wild service tree, spatial genetic structure, peripheral populations

INTRODUCTION

Spatial genetic structure (SGS) is defined as the non-random spatial distribution of genotypes and alleles within populations, meaning that genetic similarity is higher among neighbors than among more distant individuals (Gapare and Aitken, 2005). SGS has been described in various plant groups, usually in the form of within population fine-scale aggregation (Vekemans and Hardy, 2004). Many evolutionary and ecological factors can affect the development of genetic structure within plant populations, including pollen and seed dispersal patterns (Fenster et al., 2003; Latta et al., 1998), micro-environmental selection (Epperson and Allard, 1989), adult population density, temporal and spatial patterns of seedling establishment (Knowles et al., 1992), extent of clonality (Dering et al., 2015) and stand age and history of a population (Schnabel et al., 1998). However, the expected patterns of SGS at target loci will strongly depend on the mating system (Vekemans and Hardy, 2004) and the type of selection involved (Leducq et al., 2011).

Nearly half of plant species reproduce sexually using a self-incompatibility system (SI) (Barrett, 2002), which is an important genetically controlled mechanism to prevent inbreeding. SI allows the pistil of a flower to distinguish between genetically related (self) and unrelated (non-self) pollen (Kao and Tsukamoto, 2004). RNase-based gametophytic self-incompatibility (GSI) is the most phylogenetically widespread system (60% of self-incompatible species) and is controlled by the multi-allelic S-locus (Stoeckel et al., 2012). Usually, this complex locus

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contains two linked genes, an *S-RNase* that controls pistil specificity, and *SFB* gene, that is determinant of pollen (Ikeda et al., 2005; Ushijima et al., 2003).

Genomic regions involved in self-incompatibility are under negative frequency dependent selection (NFDS) (Lawrence, 2000). The immediate consequence of NFDS is that any new S-allele in a population has a selective advantage in mating, which promotes a high number of alleles within populations (Stoeckel et al., 2012; Wright, 1939). Another consequence at the population level is that allelic frequencies should be equal at an equilibrium state (Boucher, 1993; Stoeckel et al., 2008). Strong NFDS acting on the S-locus within subpopulations counteracts allele frequency divergence due to random genetic drift and causes a higher effective migration rate for S-alleles than for neutral loci (Schierup et al., 2000). Kamau et al. (2007) reported a weaker population structure in the genomic region surrounding the S-locus in *Arabidopsis lyrata*, when compared with more distant or unlinked regions. This problem has been tested in *Prunus lannesiana* across the whole range of the species distribution (Shuri et al., 2012). In accordance with the theoretical predictions Shuri et al. (2012) found smaller genetic differentiation at the S-locus than at the unlinked SSR loci. Similarly, Stoeckel et al. (2008) in *Prunus avium* and Holderegger et al. (2008) in *Pyrus pyraster* found less genetic differentiation between populations at the S-locus than at SSR loci.

Population structure and size have different implications for the evolution of balanced genetic variation relative to neutral variation. Richman (2000) suggested that genetic variation subjected to balancing selection may provide additional information for the inference of history of populations. Unequal S allele frequencies may be maintained in disturbed habitats and in evolutionarily young, recently established and sub-structured populations. Especially in perennial woody species that have long generation times, unequal distribution of S alleles among populations would likely remain detectable for a long time (Kato et al., 2007). Schierup et al. (2006) proposed that non-equilibrium allele frequency contributed to strengthening of the spatial genetic structure at the S-locus and limited dispersal through variation in individual reproduction. Additionally, Suarez-Gonzalez and Good (2013) showed significant spatial genetic structure in a fragmented population of *Prunus virginiana* as compared with a continuous population, which suggested limited pollen dispersal despite high diversity at the self-incompatibility locus.

Vekemans and Hardy (2004) showed that patterns of SGS in plant species are very similar in predominantly outcrossing species with (Sp=0.0134±0.0077) or without SI systems (Sp=0.0126±0.0101). However, spatial genetic structure at the S-locus is expected to be lower than at neutral markers, as a consequence of the higher effective dispersal of alleles at the S-locus (Schierup et al., 2000; Leducq et al., 2011). This paradigm has not been truly resolved based on empirical studies. Several authors found evidence for spatial genetic structure at both the *S-RNase* locus and microsatellite markers, but they could not ascertain clear differences in the extent of spatial genetic structure between these types of loci (Jolivet et al., 2010; Schueler et al., 2006). The difference between spatial genetic structure at the S-locus and at unlinked neutral markers is expected to strongly depend on the number of S-alleles and on the distance of pollen and seed dispersal (Leducq et al., 2011), but other factors, such as population history and the degree of population fragmentation might also be important.

In this study, we investigated genetic diversity and spatial genetic structure in *Sorbus torminalis* (L.) Crantz based on the *S-RNase* locus and 12 nuclear microsatellite loci. We analyzed two populations located at the northern edge of the species geographic distribution, which exhibited differences in size, area covered, population history and the degree of fragmentation. We compared our empirical results with theoretical predictions for loci under negative frequency dependent selection and neutral loci, discussing how ecological and genetic factors can influence spatial genetic structure in self-incompatible species.

MATERIALS AND METHODS

STUDY SPECIES

Wild service tree (*Sorbus torminalis* (L.) Crantz) is a rare, scattered species that occurs in small, isolated populations (Demesure-Musch and Oddou-Muratorio, 2004; Hoebee et al., 2006; Rasmussen and Kollmann, 2004). It is described as diploid 2n=34 (Liljefors, 1955) and the main method of reproduction is generative propagation. The species is characterized by the gametophytic self-incompatibility system, which occasionally may be broken (Hoebee et al., 2007). Wild service tree has the ability for vegetative reproduction by root suckers, which often occurs in natural conditions (Hoebee et al., 2006; Rasmussen and Kollmann, 2004; Rasmussen and Kollmann, 2007; Tarnawski, 2001). Clonal propagation via root suckers can be an effective strategy for species survival and compensation for limited sexual reproduction (Bednorz, 2009; Bednorz, 2010; Vallejo-Marin et al., 2010). In Poland *S. torminalis* reaches north-eastern distribution limits and because it is considered endangered it is protected by law (Bednorz, 2007; 2010).

STUDY SITES AND PLANT MATERIAL

The study included two populations of *S. torminalis* growing in Poland. One population was located in the Forest District Jamy (N53°34'33", E18°55'11") on the north-eastern range limit of the species and the other one was located in the Forest District Jarocin (N51°55'59", E17°21'43"), about 210 km south-west from the population Jamy (Fig. 1a). Individuals in Jamy are grouped into 5 spatially distinct subpopulations (Fig. 1c), whereas trees in Jarocin are clustered in 2 subpopulations (Fig. 1b). These populations were described in details by Bednorz (2004). We attempted to include in the study all adult individuals (DBH > 10 cm). In 2012 fresh leaves were sampled from 172 trees of the population Jamy and in 2014 from 188 trees of the population Jarocin. Because *S. torminalis* is known to form clonal groups (Jankowska-Wroblewska et al., 2016), genotypes of the sampled individuals were verified based on microsatellite loci (see below) if they belonged to the same clone, and only individuals with distinct microsatellite genotypes were included in genetic analyses. All the sampled individuals were georeferenced using a GPS mapping system Pathfinder® ProXT™ (Trimble, Sunnyvale, USA). Clonal coordinates were determined as means of coordinates of all the individuals belonging to a particular clone.

LABORATORY METHODS

Total genomic DNA was extracted from 20 mg of 1–2 dried leaves. The plant material was frozen and ground in a Mixer Mill MM301 (Retsch, Haan, Germany). DNA was extracted according to two methods: GeneMATRIX Plant & Fungi DNA Purification Kit (EURx Sp. z o.o.) and CTAB (Doyle and Doyle, 1990). The amount and quality of DNA were evaluated using a DNA calculator (BiophotometerR, Eppendorf, Hamburg, Germany). The set of 13 nuclear microsatellite markers: *Sa01*, *Sa07*, *Sa14* (González-González et al., 2010); *Mss1*, *Mss4*, *Mss5*, *Mss6*, *Mss9*, *Mss13*, *Mss16* (Oddou-Muratorio et al. 2001); *CH01h01* (Gianfranceschi et al., 1998); *CH02c09*, *Ms14H03* (Liebhard et al., 2002) was used for genotyping. Also a pair of markers flanking the *S-RNase* first intron *PaConsI-F*, *PaCconsI-R2* developed for *Prunus avium* (Sonneveld et al., 2003, 2006) was used to detect polymorphism of the *S-RNase* locus. In order to amplify the selected microsatellite loci we applied two multiplex-PCR protocols developed previously (Jankowska-Wroblewska et al., 2016). In the case of *S-RNase*, a single PCR reaction was used according to Sonneveld et al. (2006). PCR products were separated using an ABI PRISM 3130XL sequencer (Applied Biosystems, Foster City, USA) with LIZ600 as an internal size standard. The identification of alleles based on their size was performed using GENESCAN 3.7 and GENOTYPER 3.7 software provided by Applied Biosystems.

STATISTICAL ANALYSES

Genetic analyses were done based on individuals with unique microsatellite genotypes. Finally, the sample included 77 individuals from Jamy and 143 individuals from Jarocin. CERVUS 3.0.3 software (Kalinowski et al., 2007) was used to estimate standard genetic diversity parameters at population and locus level: number of alleles (A), effective number of alleles (A_e), observed (H_o) and expected (H_e) heterozygosities, Hardy-Weinberg equilibrium (*H-W*). All the loci were also tested for the presence of *null* alleles (Chybicki and Burczyk, 2009) with their frequencies estimated jointly with F_{IS} (Wright's fixation index). Allelic richness (AR) was estimated using FSTAT 2.9.3.2 (Goudet, 1995). Genetic differentiation between the populations was assessed by estimating F_{ST} and R_{ST} parameters using SPAGeDi v. 1.4 software (Hardy and Vekemans, 2002). GENEPOP v. 4.5.1 (Rousset, 2008) was used to estimate the mean frequency of private alleles (A_p) and linkage disequilibrium between the *S-RNase* locus and microsatellite loci.

Spatial autocorrelation analysis of kinship within populations was conducted using SPAGeDi v. 1.4 (Hardy and Vekemans, 2002). The average multilocus pairwise kinship coefficients (F_{ij}) (Loiselle et al., 1995) were computed for each distance class (0–10, 30, 60, 120, 250, 500, 1000, 2500, >2500), relative to local (within population) allele frequencies, as suggested by Wang (2011). The average F_{ij} values were regressed on the logarithm of the spatial distance to estimate the regression slope (b_{log}). The significance of b_{log} was tested based on 10000 permutations of spatial positions of individuals within the populations. The extent of SGS was investigated through the *Sp* statistic developed by Vekemans and Hardy (2004) and computed according to the formula $Sp = -b_{log}/(1–F(10,m))$, where b_{log} is the regression slope and $F(10,m)$ is the mean pairwise kinship coefficient between individuals within the first distance class (in this case 0–10 m).

The matrices of pairwise spatial physical distances and genetic distances based on kinship coefficients within each population were obtained for SSR and the *S-RNase* locus using SPAGeDi. Within each population the relationship among matrices was studied using the Mantel test implemented in the PASSaGE 2 software (Rosenberg and Anderson, 2011). The significance of the Mantel test was evaluated based on 1000 permutations.

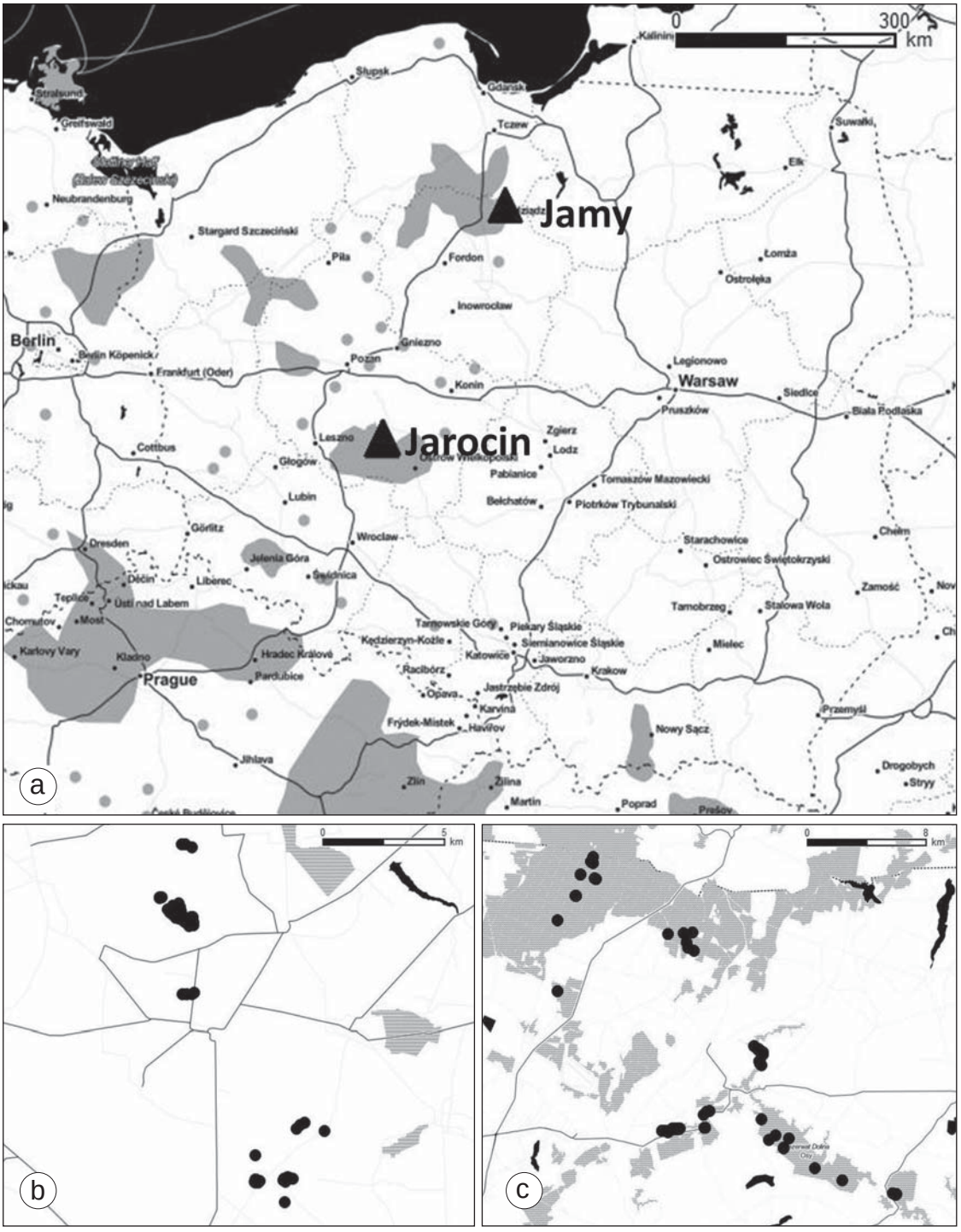

Fig. 1. Location of the studied populations of *S. torminalis* at the north-eastern range limit (**a**), dark-shadowed areas indicate the species distribution, dots indicate isolated small populations based on *S. torminalis* distribution maps available at *www.euforgen.org/distribution-maps*. Spatial distribution of individuals in populations Jarocin (**b**) and Jamy (**c**).

RESULTS

GENETIC DIVERSITY

All 13 microsatellite loci appeared to be polymorphic in each population but *Mss1* locus exhibited significant frequency of *null* alleles and was removed from further analyses. The detailed information on genetic diversity measures are presented in Table 1. In general, both populations were similar for parameters obtained based on microsatellites. Here, *null* alleles were at a low and acceptable level. Notably, the fixation index (F_{IS}) indicated a small excess of heterozygotes, which might be expected for species with self-incompatibility systems. Nevertheless, considering SSR loci, the two populations were in H-W equilibrium. On the other hand, the *S-RNase* locus exhibited low polymorphism (see discussion for possible reasons). While the population Jamy showed slightly higher diversity at the *S-RNase* locus, both populations appeared to be quite comparable. However, the fixation indices (F_{IS}) calculated based on the *S-RNase* locus indicated a higher excess of heterozygosity than the indices obtained based on SSR loci. Consequently, significant departures from Hardy-Weinberg equilibrium were found in both populations based on the *S-RNase* locus. The numbers of alleles within populations were not related to the sample size. Analyses of linkage disequilibrium (LD) between the *S-RNase* locus and microsatellites indicated that this locus exhibited significant LD with four SSR loci in Jamy, however with only one locus in Jarocin.

Despite comparable levels of genetic diversity within populations, the differentiation between the populations was significant for SSR loci with the average F_{ST} = 0.091 (SE 0.013) and R_{ST} = 0.084 (SE 0.020). In contrast, for the *S-RNase* locus, while F_{ST} value (0.092) was similar to that observed for microsatellites, the estimate of R_{ST} showed no differences.

SPATIAL GENETIC STRUCTURE

We found evidence of significant spatial genetic structure within both populations when microsatellites were used as genetic markers (Tab. 3, Fig. 2). However, while kinship coefficients in the first distance class (parameter *F*(10,m)) were similar in the two populations suggesting similar mechanisms underlying the levels of relatedness among near neighbors, other parameters (*Sp*, b_{log}) were higher

TABLE 1. Genetic diversity of *S. torminalis* in Forest District Jamy and Jarocin (for SSR – mean values across loci): *N* – mean sample size, *A* – mean number of alleles, A_e – effective number of alleles, *AR* – allelic richness, A_p – mean frequency of private alleles, H_o – observed heterozygosity, H_e – expected heterozygosity, F_{is} – inbreeding coefficient, *Null* – frequency of null alleles, *H-W* – significance of departure from Hardy-Weinberg equilibrium.

Population	*N*	*A*	A_e	*AR*	A_p	H_o	H_e	F_{is}	*Null*	*H-W*
nSSR										
Jamy	76.917	10.333	5.022	10.312	0.056	0.778	0.758	-0.018	0.005	
Jarocin	142.167	10.333	4.989	10.272	0.040	0.751	0.745	-0.007	0.009	
S-RNase										
Jamy	77	5	3.226	5.000	0.188	0.792	0.690	-0.098	0.001	***
Jarocin	143	4	2.950	4.000	0.009	0.748	0.661	-0.076	0	***

*** Significant at the $p < 0.001$ level.

TABLE 2. Genetic differentiation between populations (standard error across loci in parentheses)

Loci	F_{ST}	R_{ST}
SSR	0.091 (0.013)	0.084 (0.020)
S-RNase	0.092	-0.001
SSR* and *S-RNase	0.097 (0.014)	0.085 (0.026)

TABLE 3. The parameters of spatial genetic structure of the populations

Population	Marker	*Sp*	b_{log}	*F*(10,m)	*Mantel test* (*r*)
Jamy	*SSR*	0.0173	-0.0162 ***	0.0656 ***	-0.174 ***
	S-RNase	0.0463	-0.0417 ***	0.1001 ns	-0.268 ***
Jarocin	*SSR*	0.0067	-0.0063 ***	0.0508 **	-0.133 ***
	S-RNase	0.0002	-0.0002 ns	0.0047 ns	-0.024 *

Significance: * p <0.05; ** p <0.01; *** p <0.001; ns – not significant at p <0.05.

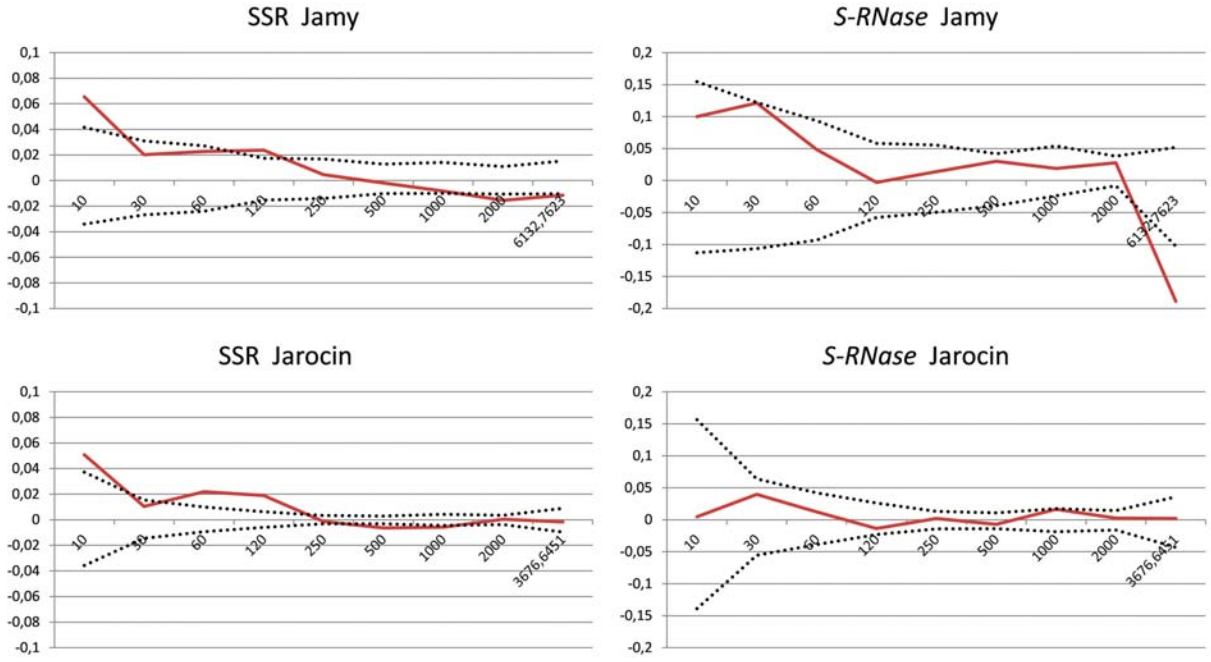

Fig. 2. Autocorrelograms of kinship coefficients (F_{ij}) against logarithm of distance estimated for the populations Jamy and Jarocin based on microsatellites (SSR) and the *S-RNase* locus. The dotted lines represent the upper and lower 95% confidence envelopes around the null distribution of kinship coefficients (note the difference in scale of kinship coefficients – Y axis, between nSSRs and *S-RNase* data).

in Jamy than Jarocin, suggesting more efficient gene dispersal at larger distances in the population Jarocin.

On the other hand, correlograms of kinship coefficients obtained based on the *S-RNase* locus were not significantly different from the null distribution in any of the populations, but it is worth noting that the range of null distribution in the case of the *S-RNase* locus is much broader than for microsatellites, partly because it was estimated based on a single locus. However, taking into account SGS parameters (*Sp*, b_{log} and *F*(10,m)), it was evident that spatial structure based on the *S-RNase* locus was present only in the population Jamy (Tab. 3).

The results of SGS analyses were generally supported by the Mantel test (Table 3). In the population Jamy, the Mantel test revealed significant correlations between spatial and genetic distances for SSR markers (r = -0.174; p <0.001) and even stronger for the *S-RNase* locus (r = -0.268; p < 0.001). These correlations were lower, although still significant in Jarocin (r = -0.133; p <0.001 for SSRs; r = -0.024; p <0.05 for *S-RNase*). Note, that while correlations for SSR markers were quite

similar in the two populations, the correlation for the *S-RNase* locus was stronger in the population Jamy. Additionally, the Mantel test revealed that the relationships between genetic distances calculated based on SSRs and *S-RNase* data were weak but significant in both populations; however, this relationship appeared to be stronger in the population Jamy (r = 0.058; p <0.001) than Jarocin (r = 0.022; p <0.05).

DISCUSSION

Genetic diversity among and within populations ensures their survival and species persistence in changing environmental conditions, and the knowledge of the distribution of genetic diversity may be useful in designing gene conservation programs, in particular for threatened species. In this study, we investigated two populations of *Sorbus torminalis* focusing on genetic diversity and spatial genetic structure revealed at neutral microsatellite loci and the *S-RNase* locus.

METHODOLOGICAL CONCERNS OF POLYMORPHISM AT THE *S-RNASE* LOCUS

The number of alleles identified in our populations of *S. torminalis* does not fall within the range observed in previously studied species having a gametophytic self-incompatibility system. Lawrence (2000) showed that typically 12–45 *S*-alleles are maintained within natural populations and that multiple populations of the same species can have up to 45 different *S*-alleles. Raspé and Kohn (2002) detected 17 *S*-alleles among 13 individuals of *Crataegus monogyna* and the same authors found 30 *S*-alleles in 2 populations of *Sorbus aucuparia* each with 20 individuals sampled (Raspé and Kohn, 2007). It is likely that low number of *S*-alleles in the studied populations of *S. torminalis* is a consequence of their small population size. Theoretical predictions have shown that in large populations many *S*-alleles should be maintained by negative frequency dependent selection, but in moderately sized or small populations a number of *S*-alleles can be lost owing to genetic drift (Busch and Schoen, 2008). Additionally, small peripheral populations in fragmented landscapes do not have all *S*-alleles included in the gene pool of central populations of the species. Richman et al. (1996) suggested that variation in allele numbers among populations might be related to variation in ecological conditions and/or extent of clonality, where lower allele numbers are more likely in disturbed habitats. However, one important reason for a low number of S-alleles detected in this study is that the consensus primers that we used for amplification of *S-RNase* were originally designed for *Prunus* and not for *Sorbus* species (Sonneveld et al., 2003). It is important to mention, that alleles that could not be distinguished unambiguously according to their electrophoretic profiles were binned together, which is a common but conservative procedure affecting the ability to detect a large number of alleles. Binning of alleles could also have an influence on the ability to distinguish between homo- and heterozygotes at the *S-RNase* locus. While for this locus H_o is expected to be close to unity (eg. Ganopoulos et al., 2012), the estimate of the observed heterozygosity in this study was about 0.75-0.79. Therefore, the comparisons of genetic diversity of the *S-RNase* locus between this and other studies may be problematic; however, the comparisons of genetic diversity and spatial genetic structure between populations genotyped in a consistent way within this study seem justified.

GENETIC DIVERSITY WITHIN AND BETWEEN POPULATIONS

The observed pattern of genetic diversity at microsatellite loci (high expected hetorozygosity and slightly negative fixation indices) shares the general pattern of variability typical of outcrossing tree species with the self-incompatibility system (Oddou-Muratorio et al., 2001; Kamm et al., 2009; George et al., 2015). The self-incompatibility system promotes both the avoidance of inbreeding, and the maintenance of intra-population genetic variability, therefore it is considered the main reason for the excess of heterozygosity in *Sorbus torminalis* (Hoebee et al., 2006).

The level of population genetic structure observed among the *S. torminalis* populations at microsatellite loci (F_{ST} = 0.091) was moderate and slightly higher than expected for typical outcrossing temperate tree species, probably due to a fragmented nature of species' distribution (Hamrick and Godt, 1990). In earlier studies of *S. torminalis* higher F_{ST} values were found. For example, Bednorz et al. (2006) and Demesure et al. (2000), who used allozymes as genetic markers, reported F_{ST} values equal to 0.167 and 0.15 based on country-wide studies with 20 (Poland) and 73 (France) populations, respectively. Distinctly higher values were reported based on microsatellites for a group of populations at the northern range limits (F_{ST} = 0.341; Rasmussen and Kollmann, 2007) or for populations sampled at a larger geographic scale in Eastern Europe (F_{ST} = 0.228; Kučerová et al., 2010).

Our data allowed for comparisons of F_{ST} and R_{ST} estimates between *S-RNase* and microsatellite loci. The *S-RNase* locus showed a similar F_{ST} estimate as microsatellite loci. Thus, this result is

not consistent with predictions from theoretical models of strong balancing selection in subdivided populations (Schierup et al., 2000; Muirhead, 2001) or empirical observations in other species (Ganopoulos et al., 2012). However, the consistency in genetic differentiation between the S-locus and neutral loci may depend on the migration levels (Stoeckel et al., 2008). Effective migration at the S-locus prevents the development of isolation by distance at microsatellite loci. Other studies reported a low population genetic structure at the S-locus (F_{ST} = 0.014 in *Prunus lannesiana*, Kato et al., 2007) or a high degree of the overlap of alleles at the S-locus among populations (*Sorbus acuparia*; Raspé and Kohn, 2007). However, these studies did not report differentiation levels at neutral genetic markers.

Comparing F_{ST} and R_{ST} values estimated for the same sets of individuals can provide valuable insights into the main causes of population differentiation (drift or mutation; Hardy et al., 2003). The microsatellite loci showed similar R_{ST} (0.084) and F_{ST} (0.091) estimates, which suggests that in this case both drift and mutation are responsible for the observed level of population differentiation (Balloux and Lugon-Moulin, 2002; Hardy et al., 2003). Conversely, the *S-RNase* locus indicated higher values for F_{ST} (0.092) than for R_{ST} (-0.001). The R_{ST} estimate indicated that most of the variance in allele sizes of the *S-RNase* locus was retained within the population, with no apparent differentiation among populations. Comparing F_{ST} and R_{ST} estimates suggests that the size variation of the studied intron of S-RNase may not follow the stepwise mutation model assumed for R_{ST} estimator, thus this statistics may not be appropriate for assessing population differentiation based on this locus. Nevertheless, although the mutation rate of a particular *S-RNase* locus is usually unknown, it is assumed that most S loci have low mutation rates of approximately 10^{-6} (Uyenoyama and Zhang, 2001). This suggests that the differentiation between the two populations at the *S-RNase* locus is caused mainly by genetic drift and not mutations.

EFFECT OF BALANCING SELECTION ON SPATIAL GENETIC STRUCTURE

We observed significant extent of SGS at microsatellite loci in both populations. It was more strongly emphasized in the population Jamy. The level of spatial genetic structure (*Sp* parameter) detected in this study at SSR loci falls within the range expected for species characterized with some extent of clonality, insect pollination and animal-mediated seed dispersal, as recently summarized by Dering et al. (2015). Our results turned out to be comparable to a French population (*Sp* = 0.0187; Oddou-Muratorio and Klein, 2008). However, the existence of SGS pattern is not a universal feature of this species (Hoebee et al., 2006). Considering the *S-RNase* locus, some evidence of SGS was found only in the population Jamy, while no signatures of SGS were found in Jarocin. Nevertheless, in the latter population the Mantel test detected barely significant relationship between the physical distance and genetic similarity based on the *S-RNase* locus. Given that *Sp* statistics is expected to be inversely proportional to the effective neighborhood size (Vekemans and Hardy, 2004), low *Sp* value for the *S-RNase* locus in Jarocin suggests relatively large neighborhoods. However, nonrandom distribution of *S*-alleles in Jamy (relatively high *Sp*) may be a limiting factor decreasing effective sizes of neighborhoods in Jamy.

There are only few studies on trees (mostly *Prunus avium*), where spatial genetic structure was assessed based on the *S-RNase* locus and neutral loci, such as microsatellites (Schueler et al., 2006; Jolivet et al., 2010). However, so far comparisons of SGS between the *S-RNase* locus and microsatellites have provided inconsistent results. Schueler et al. (2006) found similar *Sp* parameters for SSR and *S-RNase* equal to 0.0094 and 0.0078, respectively. Jolivet et al. (2011) found similar *Sp* values among three out of four populations studied, but one population characterized with high density and extensive clonal propagation showed distinctly higher *Sp* for *S-RNase* than for SSRs (0.0941 vs. 0.0596). However, reasons for these differences were not ascertained.

There are some possible reasons for a difference in SGS pattern at the *S-RNase* locus between populations Jamy and Jarocin. The population Jamy is located further to the north-eastern distribution limits, and assuming species range expansion towards this direction, it should be considered as established more recently. It is more fragmented and its census populations size of genets is smaller than in the population Jarocin. In recently established fragmented populations linkage disequilibrium (LD) among loci is usually emphasized as a result of low effective population sizes (Waples and Do, 2010). However, in larger, well established continuous populations approaching linkage equilibrium among unlinked loci, negative frequency-dependent selection acting upon the *S-RNase* locus may cause a divergent pattern of SGS between *S-RNase* and selectively neutral loci such as microsatellites, which could be the reason for difference in SGS pattern of the *S-RNase* locus between the Jamy and Jarocin populations. Indeed, in our study LD between the *S-RNase* locus and microsatellites was more evident in Jamy than in Jarocin.

CONCLUSION

Spatial distribution of genetic diversity within and among populations of self-incompatible plant species might be affected by the self-incompatibility system, which promotes more random gene distribution. We indicated, that spatial genetic structure at the self-incompatibility locus in peripheral fragmented populations may be stronger than in other more central and larger populations, as compared to neutral microsatellite loci which may show insignificant differences. However, this finding needs to be confirmed based on a larger number of populations where different population characteristics and ecological conditions could be tested for their effect on the efficiency of negative frequency dependent selection.

AUTHORS' CONTRIBUTIONS

SJ-W, JW and JB designed the research, contributed to statistical analyses and wrote the article. SJ-W and JW performed laboratory analyses on microsatellites and *S-RNase* locus, respectively. JB obtained funding.

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