

# GENETIC DIVERSITY AND SPATIAL GENETIC STRUCTURE OF *STELLARIA HOLOSTEA* POPULATIONS FROM URBAN FOREST ISLANDS

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*Stellaria holostea* is a clonal herb highly confined to well-established deciduous forests. This study examined whether its genetic diversity and spatial genetic organization in urban forest islands is similar to the values from well-established populations outside urban landscape. We studied four populations in Warsaw and two populations from well preserved forests outside the city. Genetic diversity was greater in populations from well-preserved forests than from forests heavily exploited in the past. High clonal diversity indices indicate that the studied populations did not lose the ability to reproduce sexually. The small populations in urban forests differed from the remaining sites in spatial organization. High overall  $F_{ST}$  (0.24) and the lack of correlation between genetic and geographic distance between the studied populations indicate limited gene flow. Urban forests may be of great value for conservation of *S. holostea* and other ancient forest species as they may still harbor substantial genetic variability despite their isolation.

**Key words:** Genetic variability, ancient forest plant species, sexual reproduction, vegetative propagation, conservation value, urban forests.

## INTRODUCTION

One of the main obstacles to restoration of European forests is the limited ability of recent forests to develop an herb layer composed of ancient forest plant species, mostly attributable to the poor colonization ability of species associated with ancient forests, such as *Stellaria holostea* L., due to limited seed availability (Dzwonko and Loster, 1992) and low seed recruitment (Baeten et al., 2009). The recolonization process takes a long time and is sometimes not possible without proper conservation efforts (Honnay et al., 2002). Situations in which the target community borders an ancient forest, enabling forest species to migrate to the recent forest patch, favor successful restoration (Peterken and Game, 1984; Dzwonko and Loster, 1992; Bossuyt et al., 1999; Honnay et al., 2002). Even when recent forest borders with old forests the difference between them may still be visible after a considerable time (Baeten et al., 2010). The most effective way to conserve such communities is to sustain already established local populations of ancient forest species which may serve as sources for reforestation. The value of these source populations

depends on their having sufficient genetic diversity, and that is influenced by edaphic conditions as well as natural and anthropogenic disturbances.

The forest environment imposes limitations on plant development, the most important being limited light availability at the forest floor, which negatively affects the growth and sexual reproduction of many plant species. Because of these restrictions it is thought that most forest herb layer species propagate mainly vegetatively, being unable to reproduce sexually. Low seed productivity (Falińska, 1971; Bierzychudek, 1982) together with low seed germination (Baeten et al., 2009) leads to low reproductive success. With time, such a scenario should lead to reduction of the genetic diversity of local plant populations (Kudoh et al., 1999). Ancient forest species may disappear from fragmented forest patches due to changes in edaphic conditions or to decreased genetic diversity caused by limited recruitment and limited mate availability, especially in the case of outcrossing plants (van Rossum, 2008).

This paper focuses on urban forests in which species are constrained not only by the forest environment but also by urban development. Typically,

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urban and suburban forests are surrounded by drastically altered environments intensively exploited by man. They can be regarded as environmental islands on a hostile landscape, constantly subjected to strong anthropogenic disturbance (Honnay et al., 1999a; Honnay et al., 1999b; McKinney, 2006). Recreational uses, the introduction of alien species, eutrophication, and pollution of the air, water and soil are examples of such disturbances. A dense system of roads and paths favors the spread of alien species and trampling, leading to soil degradation and destruction of the forest herb layer (Honnay et al., 1999a). Human-caused forest fragmentation leads to spatial isolation and reduction of the populations of many forest species, degrading their genetic variability (DiBattista, 2007). On the other hand, urban forests have substantial educational value and present an opportunity for the public to become acquainted with conservation issues.

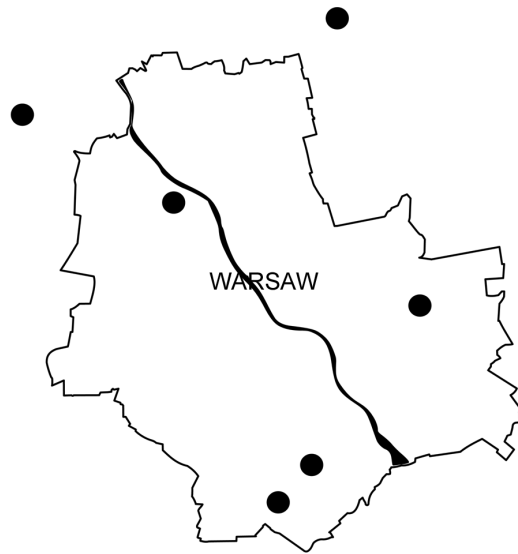
In a review of the effects of fragmentation on the genetic and demographic structure of forest plant populations, Honnay et al. (2005) stated that forest fragmentation does not automatically lead to extinction of species on a large scale. They concluded that prolonged clonal growth and long generation times help forest species escape or postpone extinction and reflect the historical landscape configuration rather than the present one. This time-delayed extinction constitutes the extinction debt at the community level (Jackson and Sax, 2010).

We wanted to know whether *S. holostea* in urban forests forms remnant populations with limited genetic variability prone to stochastic extinction or rather populations as variable genetically as other populations in large forest complexes, that is, whether diversity loss associated with forest fragmentation would be evident in the level of its genetic variability. The working hypotheses were that urban populations of *S. holostea* would be less genetically diverse than populations occupying large forest complexes, and that the populations from isolated urban forests would be dominated by a few distinct genotypes, indicating that they propagate mainly vegetatively. We assessed the intra- and inter-population genetic diversity of *S. holostea* in urban forests, characterized the local spatial genetic pattern of *S. holostea* at patch level, and measured the individual area of a genet.

## MATERIALS AND METHODS

### SPECIES

*Stellaria holostea* is a polycarpic, perennial diploid ( $2n = 26$ ) (Löve and Löve, 1961) with a West European–Mid-Siberian distribution (Hultén and Fries, 1986). It occurs mainly in deciduous forests



**Fig. 1.** Study area. Location of studied plots (filled circles) in relation to Warsaw city limits.

and is a characteristic species of the *Carpinion betuli* alliance (Matuszkiewicz, 2005). Many authors consider it an ancient forest species (Peterken and Game, 1984; Wulf, 1997; Hermy et al., 1999; Dzwonko and Loster, 2001). It is most abundant on moist, mildly acidic, moderately infertile soils. It shows low tolerance of full shade (Werner et al., 1982). In Poland *S. holostea* is not rare and is associated with old forests (Dzwonko and Loster, 2001), a vegetation type of high conservation value.

According to Grime et al. (1986), *S. holostea* is a low-growing, sprawling, evergreen herb with a creeping stock. Shoots are often supported by the surrounding plants; when the support dies back the shoot drops to the soil surface and may form adventitious roots in the vegetation gaps. In this way *S. holostea* may form large clonal patches. Fryxell (1957) regarded *S. holostea* as partially self- and partially cross-fertilized. Studies of its self-incompatibility system revealed that it is self-incompatible but that the barrier may be broken for a small fraction of matings for some combinations of S-alleles (Lundqvist, 1994). The flowers are entomogamous and protandrous, the latter promoting cross-pollination. Seed germination is stimulated by diurnal temperature fluctuations (Vandelook et al., 2008).

### STUDY AREA

We studied four *S. holostea* populations in oak-hornbeam urban forests within the city limits of Warsaw, central Poland (Fig. 1). Our selection of sites was based on the occurrence of *S. holostea* in forest patches within Warsaw (Sudnik-Wójcikowska,

TABLE 1. Size of populations at each studied location

Forest	Location	Patch area	Disturbance
Słupecka	21° 04' 53" E 52° 21' 53" N	c.a. 3000 m <sup>2</sup>	Before WWII a managed forest. Well-preserved herb layer
Kampinos	20° 49' 56" E 52° 20' 05" N	c.a. 1000 m <sup>2</sup>	Undisturbed forest
Bielany	20° 57' 57" E 52° 17' 27" N	c.a. 2000 m <sup>2</sup>	Decrease in water table, human penetration
Natolin	21° 04' 44" E 52° 08' 38" N	c.a. 2000 m <sup>2</sup>	Relatively undisturbed
Kabaty	21° 02' 39" E 52° 07' 00" N	c.a. 600 m <sup>2</sup>	Tree felling during XIX and XX centuries; clearcutting during and after WWI, arable cultivation
Sobieski	21° 10' 59" E 52° 14' 06" N	c.a. 600 m <sup>2</sup>	Heavily exploited for timber at the beginning of the XX century, not converted to pasture

1987). They were located in the Las Kabacki forest (called "Kabaty" here), Las Jana III Sobieskiego forest (Sobieski), Las Bielański forest (Bielany) and Las Natoliński forest (Natolin). Now all of these forests are used for recreational purposes, but they differ in history. The Kabaty and Sobieski forests were heavily exploited for timber before World War II, and the Bielany and Natolin forests were used mainly for recreation (Nowakowska and Żak, 2010). The Bielany forest is a remnant of the Las Mazowiecki forest, with continuous forest cover for at least 400 years (Luniak, 2005). The Natolin forest has been fenced and inaccessible to the public for a longer time, enabling natural regeneration processes (Zielony, 2005). The estimated population sizes and locations of the sampling plots are shown in Table 1.

As natural reference sites we chose two sites in large forest complexes outside Warsaw, in the Puszcza Słupecka forest (called "Słupecka" here) and Puszcza Kampinoska forest (Kampinos). Słupecka was exploited for timber before World War II but the understorey is very well preserved. The Kampinos forest is the largest forest complex near Warsaw (~27,000 ha forest area). The complex has been under intensive management since the 18th century and suffered much during the 19th and early 20th centuries (Zielony, 2004). Pine was propagated and broadleaf tree stands were destroyed. The study site is in the Sieraków Reserve, protected since 1937 (Zielony, 2004), with tree stands ~150 years old and a very well preserved herb layer.

#### SAMPLING PROCEDURE

In order to describe stable *S. holostea* populations, at each site we selected an area where the species was abundant, the herb layer was not disturbed, and a unified sampling scheme could be applied (Fig. 2). For each population we established two 20 m transects crossing in the middle, with sampling points spaced every meter. At the center of the

transects we established an additional 20 sampling points spaced 0.5 m apart on a grid. This sampling scheme permits two-dimensional spatial analysis (Arnaud-Haond et al., 2007). In each population we sampled leaf fragments from ~61 ramets. At two of the studied sites (Słupecka, Kampinos) we found no plants at some of the grid points. That is why the total of number of analyzed plant samples is 363. The samples were stored at -80°C until analyzed by allozyme electrophoresis.

#### ALLOZYME ELECTROPHORESIS

Samples were extracted using Tris-HCl pH 7.5 with 0.1% 2-mercaptoethanol extraction buffer (Wendel and Weeden, 1989). Allozyme electrophoresis was performed on 10% starch gels. Nine out of 14 systems (AAT, ACO, ADH, DIA, EST, GPI, IDH, MDH, ME, PGD, PGM, SKD, SOD, TPI) gave clear banding patterns. Six enzymatic systems (AAT, GPI, MDH, ME, PGD and SKD) were resolved with a morpholine-citrate buffer (pH 6.1) system, and three (EST, IDH and PGM) with a tris-citrate buffer (pH 8) system. Isozyme staining followed the method described by Wendel and Weeden (1989).

#### DATA ANALYSIS

Genets were identified based on the uniqueness of multilocus electrophoretic banding patterns of polymorphic loci. The probability that any two sampled ramets within a population had the same multilocus genotype by chance was  $PI < 0.0005$  for Bielany, Słupecka, Kampinos and Natolin, and  $PI < 0.002$  for the Kabaty and Sobieski populations. This probability was calculated in GenALEX (Peakall and Smouse, 2006) based on all samples collected per site, including ramets with the same multilocus banding pattern. We assumed that ramets with an identical banding pattern belonged to the same genet.

TABLE 2. Allele frequencies for polymorphic loci in six studied populations of *Stellaria holostea*

Locus	allele	Stupecka	Kampinos	Bielany	Natolin	Kabaty	Sobieski
EST	1	0	0	0.090	0.066	0.5	0
	2	0.390	0.417	0.779	0.148	0	0.5
	3	0.585	0.583	0.131	0.787	0.5	0.5
	4	0.025	0	0	0	0	0
GPI1	1	0.339	0.167	0.328	0.484	0	0
	2	0.661	0.833	0.5	0.516	1	1
	3	0	0	0.172	0	0	0
IDH	1	0.059	0.842	0.180	0.803	0	0
	2	0.636	0.050	0.262	0.082	0	1
	3	0.305	0.108	0.557	0.115	1	0
SKD	1	0.170	0.042	0.180	0.087	0.020	0.398
	2	0.110	0.177	0.250	0.135	0.451	0.082
	3	0.720	0.781	0.570	0.779	0.529	0.520
ME	1	0.195	0.142	0.156	0.041	0.000	0.008
	2	0.407	0.417	0.582	0.344	0.287	0.262
	3	0.398	0.442	0.246	0.516	0.566	0.730
	4	0	0	0.016	0.098	0.148	0
AAT1	1	0	0	0.049	0.008	0	0
	2	0.025	0.054	0.074	0.369	0	0.180
	3	0.093	0.491	0.189	0.189	0.205	0.664
	4	0.881	0.455	0.689	0.434	0.795	0.156
AAT2	1	0.110	0	0.016	0.369	0.475	0.016
	2	0.254	0.267	0.197	0.467	0.475	0.328
	3	0.636	0.733	0.787	0.164	0.049	0.656
PGM	1	0	0.025	0.008	0.016	0	0
	2	0.975	0.925	0.992	0.934	0.992	0.992
	3	0.025	0.050	0	0.049	0.008	0.008
MDH1	1	0.009	0	0	0	0	0
	2	0.259	0.353	0.276	0.410	0.241	0.480
	3	0.152	0.362	0.103	0.156	0.157	0.133
	4	0.580	0.284	0.621	0.434	0.602	0.388

Intrapopulation genetic variability is expressed as percentage of polymorphic loci, number of alleles per polymorphic locus, expected and observed heterozygosity, Shannon's index of allelic diversity (Legendre and Legendre, 1998), and the inbreeding coefficient,  $F_{IS}$  (Weir and Cockerham, 1984) using GenAlEx (Peakall and Smouse, 2006). To assess how clonal propagation affects local genetic variability (Halkett et al., 2005) we calculated expected and observed heterozygosity, Shannon's index of clonal diversity, expected and observed heterozygosity and  $F_{IS}$  after exclusion of repeated multilocus genotypes. To analyze deviations from Hardy-Weinberg equilibrium (HWE) for each population we used global tests for deficit and excess of heterozygotes available in

Genepop (Rousset, 2008), with p values calculated by the Markov chain method with 10,000 dememorizations, 20 batches and 5000 permutations.

To investigate intrapopulation genotypic diversity we calculated the clonal diversity index ( $G/N$ ), the ratio of distinct genet number to sampled ramet number (Arnaud-Haond et al., 2007). This index equals 1 when all samples represent different genets, and  $1/N$  when all sampled ramets belong to the same genet. We also calculated Simpson's (Pielou, 1969) and Shannon's index of clonal diversity (Arnaud-Haond et al., 2007), and Fager's evenness (Fager, 1972).

To assess interpopulation differentiation we calculated  $F_{ST}$  (Weir and Cockerham, 1984) using



TABLE 3. Intrapopulation genetic diversity of studied populations of *Stellaria holostea* calculated for all sampled ramets. N – number of sampled ramets; P – percentage of polymorphic loci (0.95 criterion); Ap – number of alleles per polymorphic locus; Ap5% – number of alleles per locus with 5% threshold for polymorphism;  $S_A$  – Shannon's index of allelic diversity;  $H_o$  – observed heterozygosity;  $H_e$  – expected heterozygosity; HWE departure from Hardy-Weinberg equilibrium; D – deficiency of heterozygotes, E – excess of heterozygotes, \* –  $p < 0.05$ , \*\* –  $p < 0.001$ , \*\*\* –  $p < 0.0001$ ;  $F_{IS}$  – inbreeding coefficient

Population	N	P	Ap	Ap5%	$S_A$	$H_o$	$H_e$	HWE	$F_{IS}$
Słupecka	59	67	2.89	2.44	0.72	0.42	0.43	D**	0.068
Kampinos	60	75	2.67	2.44	0.68	0.39	0.42	D**	0.089
Bielany	61	67	3.11	2.67	0.79	0.44	0.46	D*	0.060
Natolin	61	75	3.11	2.67	0.79	0.61	0.46	E***	-0.228
Kabaty	61	50	2.22	1.89	0.51	0.45	0.32	E***	-0.239
Sobieski	61	50	2.33	2.00	0.54	0.44	0.34	E***	-0.216

Genepop (Rousset, 2008) and unbiased Nei's genetic distance between populations (Nei, 1978) with GenAlEx (Peakall and Smouse, 2006). We constructed a UPGMA dendrogram based on unbiased Nei's genetic distance between populations and checked the stability of the tree by 10,000 bootstraps using TFPGA (Miller, 1997). We applied the Mantel test (Legendre and Legendre, 1998) in GenAlEx (Peakall and Smouse, 2006) to test whether genetic distance between populations is correlated with geographic distance.

To estimate the area of an individual genet in each population we calculated the maximum distance between ramets belonging to the same genet. We used Mantel test correlograms for spatial autocorrelation analysis within study sites. The distance classes corresponded to distance in meters between sampling points: the first distance class was from 0 to 1 m, the second from 1 to 2 m, etc. The tests were run with 999 permutations, and 95% confidence limits for the correlations were assessed with 1000 bootstraps. To examine differences in spatial genetic structure among the studied populations we used the test for homogeneity of spatial correlograms (Smouse et al., 2008) in GenAlEx (Peakall and Smouse, 2006). This test computes Omega statistics based on matrices of correlations between genetic and spatial distance for different lags and populations. The p value is assessed by bootstrapping. We based the assessment of p value in our tests on 1000 bootstraps.

## RESULTS

### INTRAPOPULATION GENETIC AND GENOTYPIC VARIATION

Three of 12 analyzed isozyme loci (MDH2, PGD1, PGD2) proved to be monomorphic. The nine remaining loci were polymorphic in most populations (Tab. 2). The highest intrapopulation genetic diversity, as indicated by the high percentage of

polymorphic loci, number of alleles per polymorphic locus and Shannon's index of allelic diversity, was in the populations from Kampinos, Słupecka, Bielany and Natolin (Tab. 3). The populations from Kabaty and Sobieski showed lower genetic variation. In the Bielany, Słupecka and Kampinos populations the  $F_{IS}$  values and the relation between  $H_o$  and  $H_e$  indicated inbreeding, while the remaining three populations showed an excess of heterozygotes versus HWE. The analysis after exclusion of repeated multilocus genotypes gave similar results (data not shown).

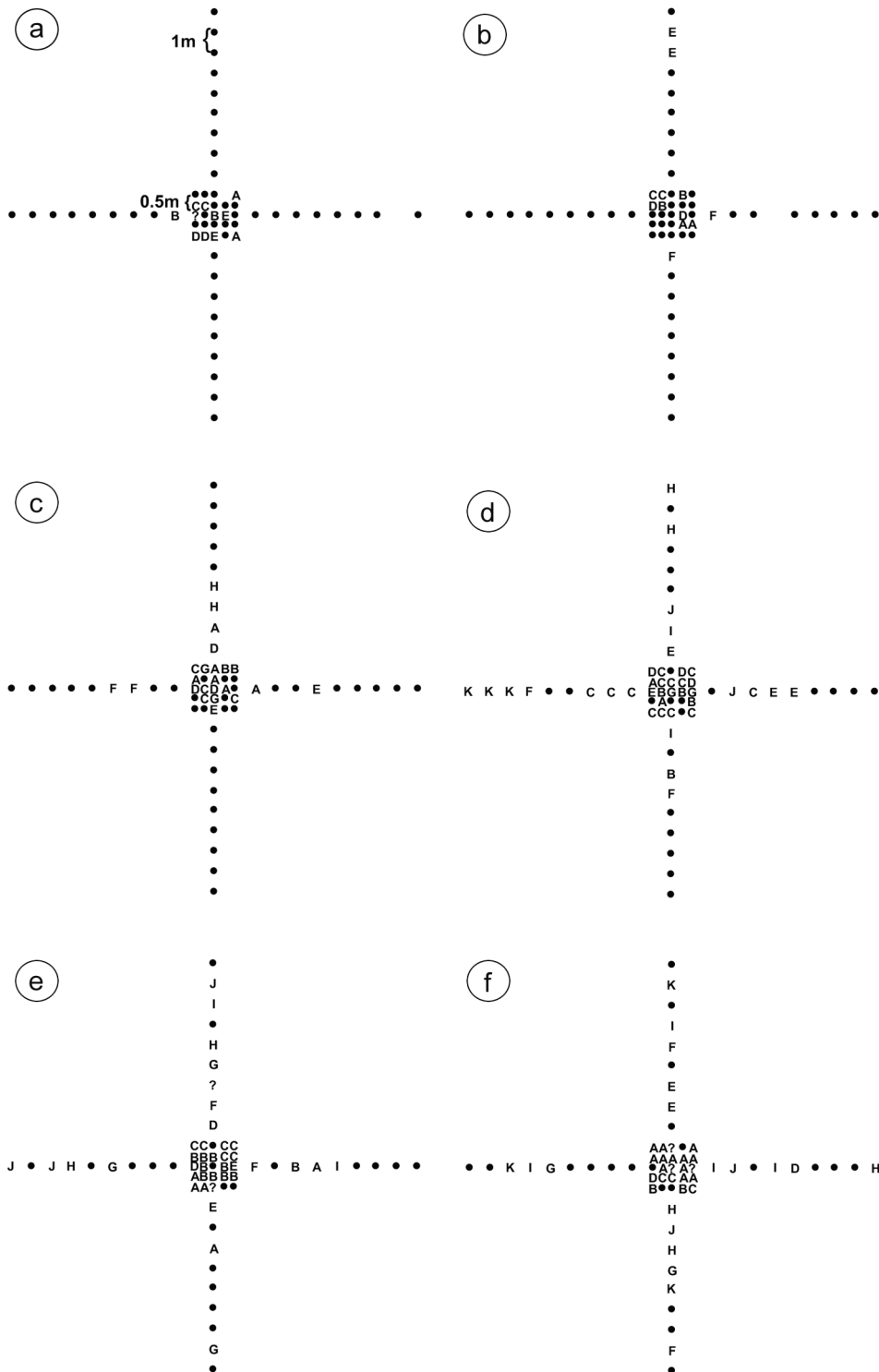
In the Słupecka and Kampinos populations the clones were represented by fewer ramets within the sampling area than in those from the other studied sites (Fig. 2). The fraction of distinctly genotyped individuals ( $G/N$ ) and Shannon's index of clonal diversity for the Sobieski, Kabaty and Natolin populations indicated lower clonal diversity than in those from Bielany, Kampinos and Słupecka (Tab. 4).

### INTRAPOPULATION SPATIAL PATTERN

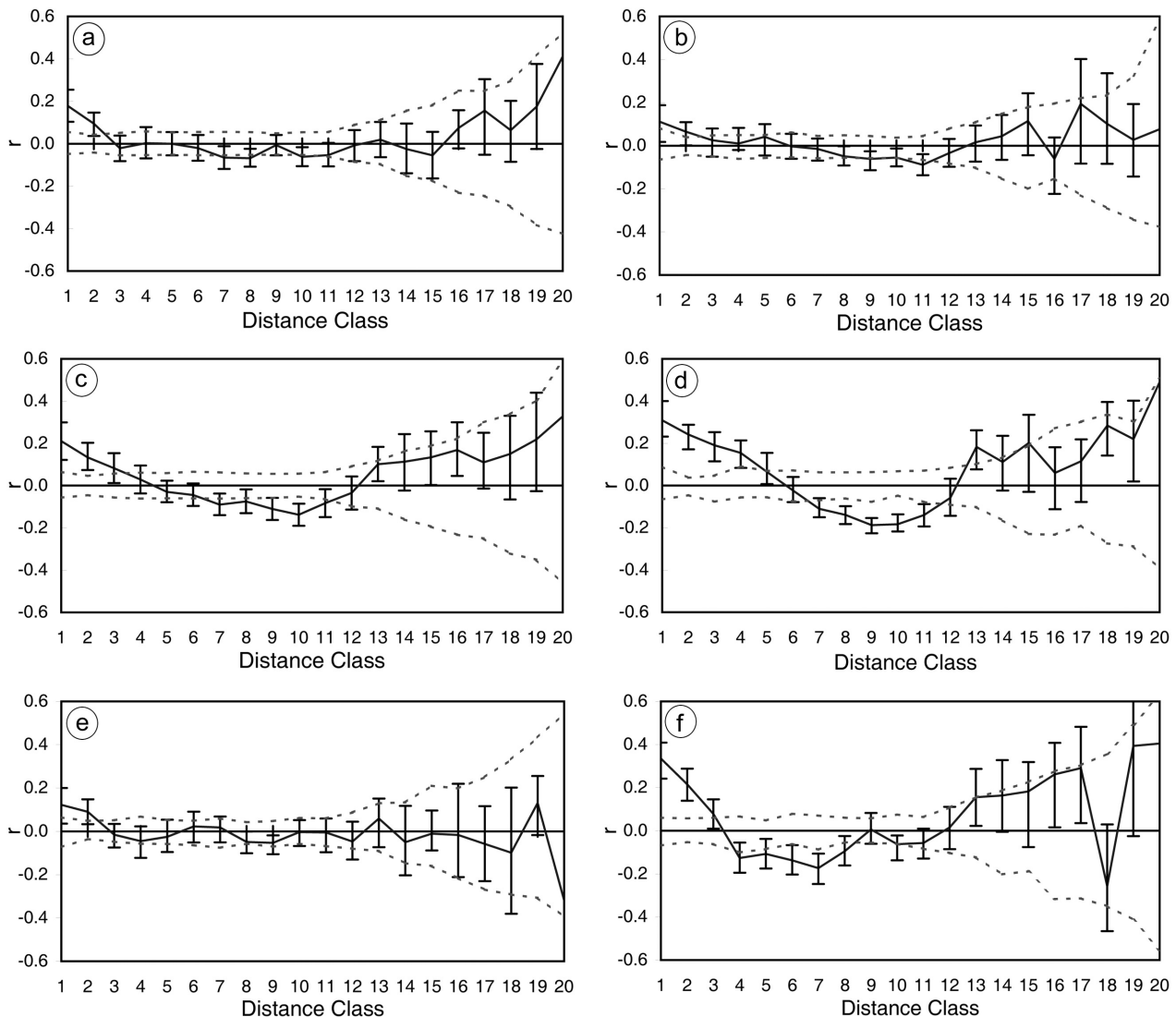
We did not find a significant spatial genetic autocorrelation for the large diversified populations from large forest complexes surrounding Warsaw

TABLE 4. Intrapopulation genotypic diversity of studied populations of *Stellaria holostea*.  $N_G$  – number of genets;  $G/N$  – clonal diversity index; D – Simpson's index; E – Fager's evenness;  $S_{MLG}$  – Shannon's index of clonal diversity.  $G/N$ , D, E,  $S_{MLG}$  were calculated based on ramets that could be positively assigned to specific clones

Population	$N_G$	$G/N$	D	E	$S_{MLG}$
Słupecka	53	0.91	1.00	0.81	1.71
Kampinos	54	0.90	1.00	0.85	1.73
Bielany	46	0.75	0.98	0.83	1.60
Natolin	33	0.54	0.94	0.80	1.36
Kabaty	32	0.54	0.95	0.84	1.36
Sobieski	31	0.53	0.95	0.83	1.36



**Fig. 2.** Distribution of clones of *Stellaria holostea* in each study area. (a) Słupecka, (b) Kampinos, (c) Bielany, (d) Natolin, (e) Kabaty, (f) Sobieski. Two 20 m transects crossing in the middle were sampled every 1 m, with a central grid sampled every 0.5 m. Dots represent clones with one ramet within the sampling area; letters represent clones with multiple ramets within the sampling area. Each letter represents a different clone within one population; question mark – ramets that could not be assigned to a specific clone.



**Fig. 3.** Spatial correlograms for each of the studied *Stellaria holostea* population. (a) Słupecka, (b) Kampinos, (c) Bielany, (d) Natolin, (e) Kabaty, (f) Sobieski. Solid line – Mantel  $r$ ; dotted line – 95% confidence interval for the null hypothesis of no spatial structure for the combined data set, as determined by permutation; error bars – 95% confidence interval for  $r$ , as determined by bootstrap re-sampling.

(Kampinos, Słupecka; Tab. 5). In the Bielany and Natolin populations, which occupy the best-preserved oak-hornbeam stands within Warsaw, we found a significant spatial autocorrelation of genetic data (Tab. 5). Spatial correlograms showed diversified genetic structure in all populations (Fig. 3).

In our study the area occupied by an individual from the highly variable populations from Kampinos and Słupecka (large number of unique multilocus genotypes and high index of clonal variability) was relatively small, not exceeding 3 m. In the less vari-

able populations from Kabaty and Sobieski the area of an individual was much larger, more than 10 m (Tab. 5).

#### INTERPOPULATION GENETIC VARIATION AND SPATIAL PATTERN

The genetic differentiation index ( $F_{ST}$ ) among all studied populations, based on all polymorphic loci, was 0.24. UPGMA cluster analysis (Fig. 4) performed on unbiased Nei's genetic distance (Tab. 6)

TABLE 5. Spatial characteristics of studied populations. r – results of Mantel test for spatial autocorrelation, genetic patch size – interpreted from x-intercept of correlograms; individual size – maximum distance between ramets with the same multilocus genotype

Population	r	Genetic patch size (m)	Individual size (m)
Słupecka	0.09 <sup>ns</sup>	2.8	2.0
Kampinos	0.12 <sup>ns</sup>	5.9	2.8
Bielany	0.24 <sup>**</sup>	4.5	5.1
Natolin	0.49 <sup>**</sup>	5.7	8.6
Kabaty	0.08 <sup>ns</sup>	2.9	13.5
Sobieski	0.20 <sup>*</sup>	3.4	15.0

ns – not significant, \* – p<0.01, \*\* – p<0.001

TABLE 6. Pairwise Nei's unbiased genetic distance between populations

	Słupecka	Kampinos	Bielany	Natolin	Kabaty
Kampinos	0.160				
Bielany	0.079	0.174			
Natolin	0.194	0.110	0.291		
Kabaty	0.231	0.342	0.281	0.279	
Sobieski	0.160	0.217	0.262	0.333	0.428

indicated that the populations from Słupecka and Bielany were the most similar to each other. The populations from Kampinos and Natolin form another distinct cluster, and the remaining populations from Sobieski and Kabaty differ most from the others. Nei's genetic distance between populations was not correlated with geographical distance (Mantel test  $r = -0.13$ ,  $p = 0.404$ ; 9999 permutations). Similar results were obtained for pairwise  $F_{ST}$  analysis (data not shown).

Analysis of correlograms showed differences in spatial pattern between the Natolin and Sobieski populations on the one hand and all the remaining populations on the other. The spatial population structure was generally similar between Bielany, Słupecka, Kampinos and Kabaty (Tab. 7).

## DISCUSSION

### INTRAPOPULATION GENETIC VARIATION

The high variability of the studied populations may indicate a significant impact of generative reproduction or recent or historic gene flow. Recent gene flow is less probable because the sites were highly isolated by being in very urbanized areas. Relatively high genetic variability indicating significant generative

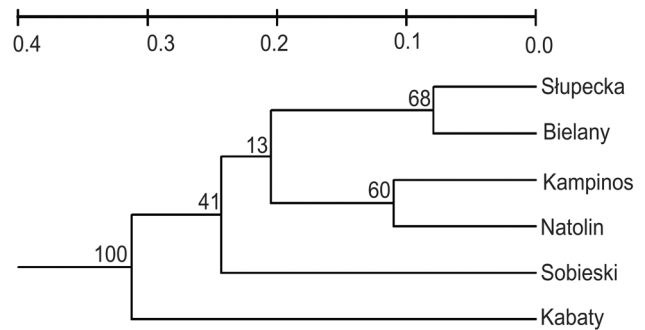


Fig. 4. UPGMA tree of *Stellaria holostea* studied populations based on Nei's unbiased genetic distance. Numbers at nodes represent percentage support for a node after bootstrapping.

reproduction and limited seed dispersal has been noted in other forest species, both clonal ones such as *Anemone nemorosa* (Stehlik and Holderegger, 2000) and *Galium odoratum* (Ziegenhagen et al., 2003) and nonclonal ones such as *Cryptotaenia canadensis*, *Osmorhiza claytonii* and *Sanicula odorata* (Williams, 1994). In well preserved populations, vegetative propagation allows a particular genet to hold its space, while generative reproduction counterbalances the loss of genetic diversity. In *S. holostea* such a scheme might be indicated by high clonal variability and small clone area. The less diverse populations from Kabaty and Sobieski (Tab. 3) still do not show domination of single genets. This indicates that generative reproduction (now or in the past) allowed the genetic variability of these populations to be maintained. The data for other long-lived forest species suggests that even minor reproductive success can maintain a population's genetic variability, counterbalancing domination of individual genotypes (Watkinson and Powell, 1993).

The lowest genetic variation found in the Sobieski and Kabaty populations may be due to the small size of these populations in spite of the large forest area. This might be associated with the history of those forests, as they were both exploited for timber in the past. Clonal plants associated with ancient forests are capable of forming remnant populations (Honnay et al., 2005). Such populations can be characterized by considerable genetic variation due to long generations (Van Rossum, 2008). Although small and endangered, they may be valuable for restoring genetic variability in neighboring populations (Wehling and Diekmann, 2008).

### INTRAPOPULATION GENOTYPIC VARIATION

The genotypic diversity of *S. holostea* populations reported in our study (G/N: 0.53 – 0.91; D: 0.944 – 0.997; E: 0.80 – 0.85; Tab. 3) was higher than the mean reported for other clonal species (G/N: 0.44,



TABLE 7. Comparison of spatial structure across populations of *Stellaria holostea*. Omega values above diagonal. Probability: P (Omega-rand  $\geq$  Omega-data) below diagonal

	Słupecka	Kampinos	Bielany	Natolin	Kabaty	Sobieski
Słupecka		32.777	48.307	113.585	44.036	96.078
Kampinos	0.795		53.910	117.532	40.812	122.979
Bielany	0.163	0.056		65.057	80.910	81.190
Natolin	0.001	0.001	0.006		158.807	111.091
Kabaty	0.299	0.440	0.001	0.001		129.663
Sobieski	0.001	0.001	0.001	0.001	0.001	

D: 0.85, E: 0.74; Honnay and Jacquemyn, 2008). Surprisingly, clonal plant species exhibit quite substantial levels of genetic variation (Ellstrand and Roose, 1987; Widén et al., 1994). In forest species this may be the result of high diversity of local microhabitats shaping population genetic structure (Jacquemyn et al., 2005) via various mechanisms operating on plant populations (for review see, e.g., Linhart and Grant, 1996). In self-incompatible forest species such as *S. holostea* the variability might also be driven by heterozygote-enhanced mutation (Amos and Harwood, 1998). Thus the species may exhibit significant population variability even on a microscale (Kudoh et al., 1999).

Our results show that *S. holostea* populations do not reproduce exclusively by vegetative propagation. However, the rate of clonal propagation indicated by  $F_{IS}$  and G/N (Halkett et al., 2005) seems to be higher in the Natolin, Sobieski and Kabaty populations than in those from Kampinos, Słupecka and Bielany. The high negative  $F_{IS}$  and low G/N (Tab. 3) in the Natolin, Sobieski and Kabaty populations indicate a higher ratio of vegetative propagation to sexual reproduction. This in turn may affect the genetic diversity of the population, as a smaller number of large genets may reduce mate availability and impair pollination between compatible individuals, leading to diminished fecundity (Charpentier, 2002).

In the Kabaty, Sobieski and Natolin populations the low G/N ratio together with the excess of heterozygotes might indicate limited mate availability. In these populations there might be few compatible mates, *S. holostea* being a self-incompatible species. This might lead to the production of heterozygous offspring, but with limited genotypic variability. As an outcome, generative reproduction might still be strongly limited in subsequent generations. Such a scenario, leading to major constraints on population regeneration, has been reported for urban populations of *Primula elatior* (Van Rossum, 2008). On the other hand, matings between unrelated individuals, although limited, might produce highly heterozygous off-

spring, avoiding inbreeding depression (Ellstrand and Roose, 1987; Amos and Harwood, 1998).

The area occupied by an individual in the less variable populations (Kabaty and Sobieski) was larger than in the highly variable populations (Kampinos, Słupecka). This could be a result of dispersal of fragmented clones (Widén et al., 1994), or may indicate higher clonal spread in populations with smaller numbers of genets. Since *Stellaria holostea* is self-incompatible (Lundqvist, 1994), the probability that two ramets exhibiting the same multilocus banding pattern belong to different genets is low, though they may originate from matings between the same parents producing genetically similar offspring.

#### INTERPOPULATION GENETIC VARIATION

Genetic similarity among the studied populations did not correlate with distance, indicating limited gene flow between geographically close populations. This is not surprising, as urban landscape hampers the exchange of pollen and propagules between island populations. Similar findings were reported for urban populations of *Primula elatior* (van Rossum, 2008) and for *Geum urbanum* in an agricultural landscape (Vandepitte et al., 2007). Cluster analysis showed that the Sobieski and Kabaty populations differ from the other ones (Fig. 4), possibly indicating the importance of local conditions such as habitat quality and sufficient spatial area that enable maintenance of a genetically variable population even under restricted gene flow between populations. Our expectations of high similarity between the Bielany and Kampinos populations (as these forests are linked by an ecological corridor) were not met, despite the possibility that seeds of *S. holostea* could be transported between these forests by small animals.

#### VARIATION OF SPATIAL PATTERN

Our results show that the Natolin and Sobieski populations differ in spatial pattern from the other four populations. Individual ramets growing closer

together are more similar than ramets growing farther apart in the Natolin and Sobieski populations (Fig. 3). This is not necessarily associated only with small population size, because in Kabaty, also a small population, the pattern was clearly different. Perhaps the intrapopulation spatial pattern is correlated with environmental heterogeneity, which generates a different response of the clones to environmental variables acting at a local scale (Jacquemyn et al., 2005; Hutchings and Wijesinghe, 2007). Environmental factors have been reported to shape population spatial genetic structure (e.g., Vandepitte et al., 2009). However, these differences may also be stochastic or caused by the presence of genetically different clones differently responding to particular environmental variables.

A cause of differences in spatial autocorrelation between the studied populations may also be the difference in population genetic variability. Populations with high levels of genetic variability (Kampinos, Ślupecka) may exhibit such levels of diversity that the spatial autocorrelation is not evident. Populations with moderate genetic variability (Bielany, Natolin) may exhibit a spatial autocorrelation due to increased clonal propagation. Populations with low variability (Kabaty) may be so uniform that the spatial autocorrelation is again not evident.

#### VALUE OF URBAN FORESTS FOR CONSERVATION OF *STELLARIA HOLOSTEA*

A major factor affecting successful conservation of ancient forest species in urban forests is the size of a forest patch (Dzwonko and Loster, 1992; Honnay et al., 1999a), along with its environmental diversity which enables species richness to be sustained. Even in small forest patches, however, one can find plant species associated with ancient forests (Honnay et al., 1999b). This reflects their stress tolerance, clonal propagation and long lifespan, enabling some species to persist in suboptimal conditions for a long time. In the latter case there is a threat of sexual extinction (Eckert, 2002; Honnay et al., 2005): individuals are still present in the community but unable to reproduce generatively due to low genetic variability and thus limited mate availability.

Though enclosed by the urban matrix, urban forests are still of high conservation value for species such as *S. holostea* if the original habitat has not been greatly altered. The population occupying a relatively small forest patch in Bielany was as diversified as the populations from Kampinos and Ślupecka, our reference areas. Similarly, the Natolin population, occupying an even smaller and more isolated forest patch, harbors substantial levels of genetic variability. The species is also present in the Kabaty and Sobieski forests, which have suffered major anthropogenic dis-

turbance in the past. This had an impact on habitat quality; consequently the genetic variability of the species is lower at those sites.

#### CONCLUSIONS

The life history traits of *S. holostea* and other ancient forest species – low dispersability and colonization ability, low survival under suboptimal conditions, prolonged clonal growth leading to the formation of remnant populations, and the risk of losing the capacity to reproduce sexually – pose a threat to these organisms in a rapidly changing anthropogenic landscape. Populations of such species may still form part of the community but because of the already lost genetic variability their sexual reproduction may be restricted. However, even in city forests surrounded by such a hostile environment as the urban matrix, small remnant populations of ancient forest species may still harbor substantial levels of genetic diversity. The management history seems to be the key factor influencing habitat quality and naturalness, which enable ancient forest plant species to thrive in these island populations. The *S. holostea* populations in the Bielany and Natolin forests, used for recreation, were more diverse than in the Sobieski and Kabaty forests, which were previously exploited for timber. With the expansion of urban landscape such island forests may be of considerable value for conservation of species such as *S. holostea*. They can be valuable sites for restoration of target species, based on local populations, as their large area is protected from further timber harvest and the associated disturbance. To prevent the genetic variability of these target species from being lost, more attention should be paid to effective protection of forest habitats in highly urbanized landscape.

#### AUTHORS' CONTRIBUTIONS

MW devised the concept of the study, analyzed the data, elaborated the results and wrote the paper; BG collected the material, acquired the data and elaborated the results. The authors declare that there are no conflicts of interests.

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