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MORPHOLOGICAL AND GENETIC DIFFERENTIATION OF *SAXIFRAGA HIRCULUS* L. (*SAXIFRAGACEAE*) POPULATIONS IN LITHUANIA

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Summarized data of investigations of nine *Saxifraga hirculus* L. populations in Lithuania are presented. The abundance of populations is low, normally from one to fifteen floral shoots in a 0.25 m^2 plot. Ecologic density and morphologic features of *S. hirculus* highly varies throughout different habitats in Lithuania. *S. hirculus* populations in Lithuania are characterized by medium plasticity of phenotypic features of generative individuals.

The diversity of RAPD markers was studied in 76 plants of *Saxifraga hirculus* from five populations in Lithuania. Analysis of molecular variance (AMOVA) showed high level of differentiation among studied populations of *S. hirculus*. The interpopulation variance component accounted for 27%. The genetic distance between populations ranged from 0.168 to 0.258. We found no correlation between the genetic and geographic distances of the study *S. hirculus* populations. The level of average heterozygosity was highest in Galvydiske population (0.338) and the lowest in Juodle population (0.295).

Key words: RAPD, genetic differentiation, heterozigosity, morfological feature, density, *Saxifraga hirculus*

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INTRODUCTION

Fragmentation of distribution ranges of plant species due to anthropogenic impact or changed environmental conditions can reduce them to small isolated populations. Existence of the latter very much depends upon accidental environmental, demographic and genetic processes. Fragmented distribution of the species adds to the risk of local extinction (Lienert et al. 2002). Unfavourable demographic situation in isolated populations contribute to the probability of inbreeding depression. The inbreeding reduces heterozygosis and fitness. On the other hand, loss of genetic diversity could be caused by genetic drift (Wright 1951). Inbred individuals often show more morphological variation than outbred individuals but the loss of genetic diversity will restrict species capacity to adapt to environmental change (Dahlgaard & Warncke 1995). At the moment „there is an increasing need to better integrate genetic and ecological studies with the study of the processes that condition the viability of populations“ (Heywood & Iriondo 2003).

Due to fragmentation, changes can affect not the whole but only part of the species range. This situation is typical for *Saxifraga hirculus* L. It is a circumpolar species of wide distribution recorded in Europe, Asia and North America (Hulten & Fries 1986). In many European countries, including Lithuania, *S. hirculus* is a protected postglacial relict. Currently, due to climate changes and anthropogenic activities, only small isolated populations of this species have survived in many countries. So far quite comprehensive investigations on structure and long-term dynamics of *S. hirculus* populations were performed only in Galvydiškė, Kelmė distr. (Meškauskaitė & Naujalis, 2006). Some aspects of genetic diversity of *S. hirculus* populations in Lithuania were analyzed earlier (Naugžemys et al. 2007).

The aims of this work were the analyses of the variability of *S. hirculus* morphological characteristics in Lithuania, determination of genetic similarity and differentiation level as well as evaluation of the processes occurring inside larger populations

MATERIALS AND METHODS

Object of the research

S. hirculus is a perennial herbal rhizomatous plant. It can propagate by seeds and vegetatively developing plagiotrophic runners. The flowering period of *S. hirculus* starts at the end of July and lasts till beginning of October, flowers are yellow, about 2.5 cm in diameter. The populations are formed of compound individuals; the peculiarities of their formation are described in earlier article of Meškauskaitė & Naujalis (2006). Not separate individuals but floral shoots and runners are treated as elements constituting the structure of *S. hirculus* populations.

Research sites

Studies on *S. hirculus* were performed in 9 localities in Lithuania (Fig. 1): Dzūkija National Park (Kapiniškės, Merkinė), Gražutė Regional Park (Degučiai), Kurtuvėnai Regional Park (Galvydiškė, Juodlė, Jautmalkė), Labanoras Regional Park (Laukagalis, Girutiškis) and Sartai Regional Park (Jasai). The base of map was taken from Gudžinskas (1999). Short characteristics of the research sites are presented in Table 1.

Field and laboratory research

The investigations of *S. hirculus* populations were carried out in 2005-2006. Exact number of *S. hirculus* individuals was not determined in the studied populations. During the research, ecological density of *S. hirculus* shoots (floral shoots and runners) was determined, i.e. the number of shoots in the areas occupied by the species; from one to eight randomly chosen 0.25 m^2 plots in each population. In order to reveal variability of morphologic features, from 11 to 28 generative *S. hirculus* plants were studied in each population (Table 1). The main criteria for evaluation of *S. hirculus* populations were the numbers of floral shoots and runners, height or length of the shoots, number of flowers, number of leaves on floral shoots, length and width of the largest leaf. All data on the above-mentioned features was processed employing statistical

Table 1. The study populations of *S. hirculus* in Lithuania

Locality name	Population size m^2	Number of morphological samples	Number of genetic samples
Kapiniškės	300	20	20
Merkinė	900	20	20
Degučiai	300	17	-
Galvydiškė	500	28	20
Jautmalkė	600	11	20
Juodlė	200	13	10
Laukagalis	200	11	10
Girutiškis	200	17	-
Jasai	900	20	-

methods, using „STATISTICA v.7.0" program. *S. hirculus* populations were compared employing the non-parametric Kruskal-Wallis criterion. The correlation of features was evaluated applying the Pearson's correlation coefficient.

From ten to twenty *S. hirculus* runners from five populations were used for genetic investigations (Table 1). In each population the distances between the sampled individuals were at least 1 m. DNA isolation from plants, RAPD-PCR, agarose gel electrophoresis, result documentation and formation of binary matrix were performed following the earlier described methods (Naugžemys et al. 2007). Dependence of genetic and geographic distances in populations was calculated using the „STATISTICA v.7.0" software. The genetic diversity parameters were estimated with the PopGen v.1.31 (Yeh et al. 1999). Analysis of Molecular Variance (AMOVA) and level of *S. hirculus* heterozygosity in populations were determined using the GenAlEx v.6 (*Genetic Analysis in Excel*) program (Peakall & Smouse 2006). Genetic distances among individuals were evaluated employing TREECON v. 1.3b (Van de Peer & De Wachter 1994).

RESULTS AND DISCUSSION

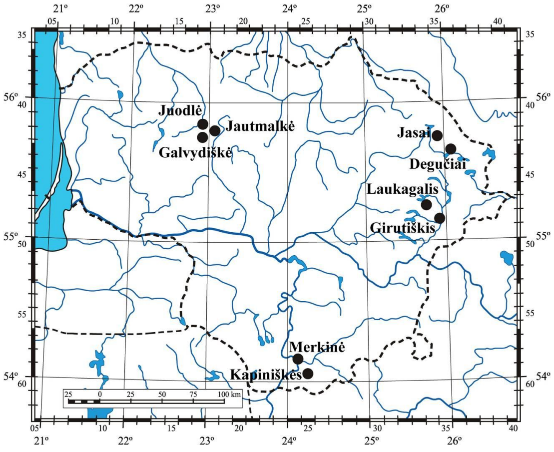

Fig. 1. The location of *S. hirculus* research sites in Lithuan

Ecologic density of *S. hirculus* populations

Ecologic density of *S. hirculus* highly varies throughout different habitats in Lithuania. The highest total number of shoots was recorded in Kapiniškės population; during the research up to 1200 shoots were recorded in one plot. High number of shoots was also recorded in Juodlė; in a 0.25 m^2 area about 800 shoots were registered. In Merkinė up to 170 *S. hirculus* shoots were recorded per plot. In the study plots of Degučiai, Jasai and Jautmalkė populations about 75–95 shoots were recorded. In a 0.25 m^2 plot in Galvydiškė population only 35 shoots were found. The lowest parameters of *S. hirculus* ecologic density were registered in Laukagalis and Girutiškis; 19 and 23 shoots per plot, respectively. Therefore, the maximum (Kapiniškės) ecological density of *S. hirculus* shoots was more than 63 times higher than the minimum (Laukagalis).

The ratio of the number of runners and floral shoots differed in the studied populations. In Jautmalkė runners comprised almost 99 % of all *S. hirculus* shoots; here in a 0.25 m^2 plot only 1–2 floral shoots were recorded. Low (2–3 per plot on average) ecologic density of floral shoots was also determined in Laukagalis population where they comprised 13 % of all shoots. In Galvydiškė, Juodlė, Kapiniškės, Merkinė and Degučiai runners made up more than 90 % of the whole population. The highest ecologic density of floral shoots was determined in Kapiniškės; here 60 flowering shoots were recorded per 0.25 m^2 plot. Meanwhile, at the moment of investigations, in Girutiškis floral shoots comprised almost 40 % of the whole population, but their ecologic density was only eight shoots per 0.25 m^2 plot.

In Lithuania, the abundance of *S. hirculus* populations is low comparing with some other countries. In Sweden (Ohlson 1986) in plots of the same size 15–40 floral shoots were recorded on an average; close to springs this number reached even 52 floral shoots. In Lithuania as much as 85 % of all study plots comprised less than 15 floral shoots.

Table 2. Statistical parameters of morphologic features of *S. hirculus* generative individuals in Lithuania

Features	Mean	Median	Standard error	Minimum	Maximum	St.dev.	Coef. of variation, %
Height of floral shoots, cm	20.4	26.5	0.5	10.0	40.0	5.6	27.5
Number of leaves	20.3	20.0	0.4	8.0	33.0	4.8	23.8
Length of longest leaf, mm	28.9	28.0	0.8	10.0	62.0	10.4	36.1
Width of longest leaf, mm	3.7	4.0	0.1	2.0	8.0	0.9	23.4
Number of flowers	2.3	2.0	0.1	1.0	6.0	1.2	53.1
Number of runners	2.2	2.0	0.1	0.0	7.0	1.41	63.0
Length of runners, cm	26.0	21.0	1.6	0.1	130.0	24.6	94.7

Variation in morphologic features of *S. hirculus* generative individuals

Comparative description of morphologic features of *S. hirculus* generative individuals in the studied populations is presented in Fig. 2. Maximum values of all features were not determined for any population. The largest average heights of floral shoots and leaf lengths were determined in Juodlė population, number of leaves – in Jasai, and the width of leaves – in Laukagalis populations. The highest average number of flowers was characteristic of Galvydiškė population. The number and length of runners, offshoots developed at basal parts of floral shoots, were determined only in six populations. The highest average number of runners was formed by *S. hirculus* in Jautmalkė population, but their largest length was in Degučiai. Very different variation of the same feature was determined in the studied populations. The highest variation of *S. hirculus* features was characteristic of Laukagalis, the lowest – of Juodlė populations. Variation in the leaf width was most evident (up to seven times): in Laukagalis the coefficient of variation of this feature was 31.7, while in Juodlė – only 4.7.Variation of the height of floral shoots in Laukagalis was three times higher than in Juodlė. Laukagalis population was characterized by both the highest (40 cm), and the lowest (10 cm) *S. hirculus* generative individuals.

Differences in the features of *S. hirculus* generative individuals in the studied populations were evaluated employing the non-parametric Kruskal-Wallis criterion. Differences of all estimated features are statistically significant (p<0,005).

Generalization of the data on variability of morphologic features of generative *S. hirculus* individuals in the studied populations allows the evaluation of the plasticity of the species in

Table 3. Spearmen‘s correlation between morphologic features of *S. hirculus* generative individuals

Features	Number of leaves	Length of longest leaf, mm	Width of longest leaf, mm	Number of flowers	Number of runners	Length of runners, cm
Height of floral shoots, cm	0.159 p=0.165	**0.382 p=0.001**	**0.510 p=0.000**	0.168 p=0.142	0.191 p=0.095	**0.291 p=0.010**
Number of leaves		**0.395 p=0.000**	**0.315 p=0.005**	**0.400 p=0.000**	**0.351 p=0.002**	0.208 p=0.068
Length of longest leaf, mm			**0.595 p=0.000**	**0.266 p=0.018**	**0.312 p=0.005**	**0.475 p=0.000**
Width of longest leaf, mm				**0.458 p=0.000**	**0.328 p=0.003**	**0.314 p=0.005**
Number of flowers					**0.351 p=0.002**	0.010 p=0.944
Number of runners						**0.267 p=0.018**

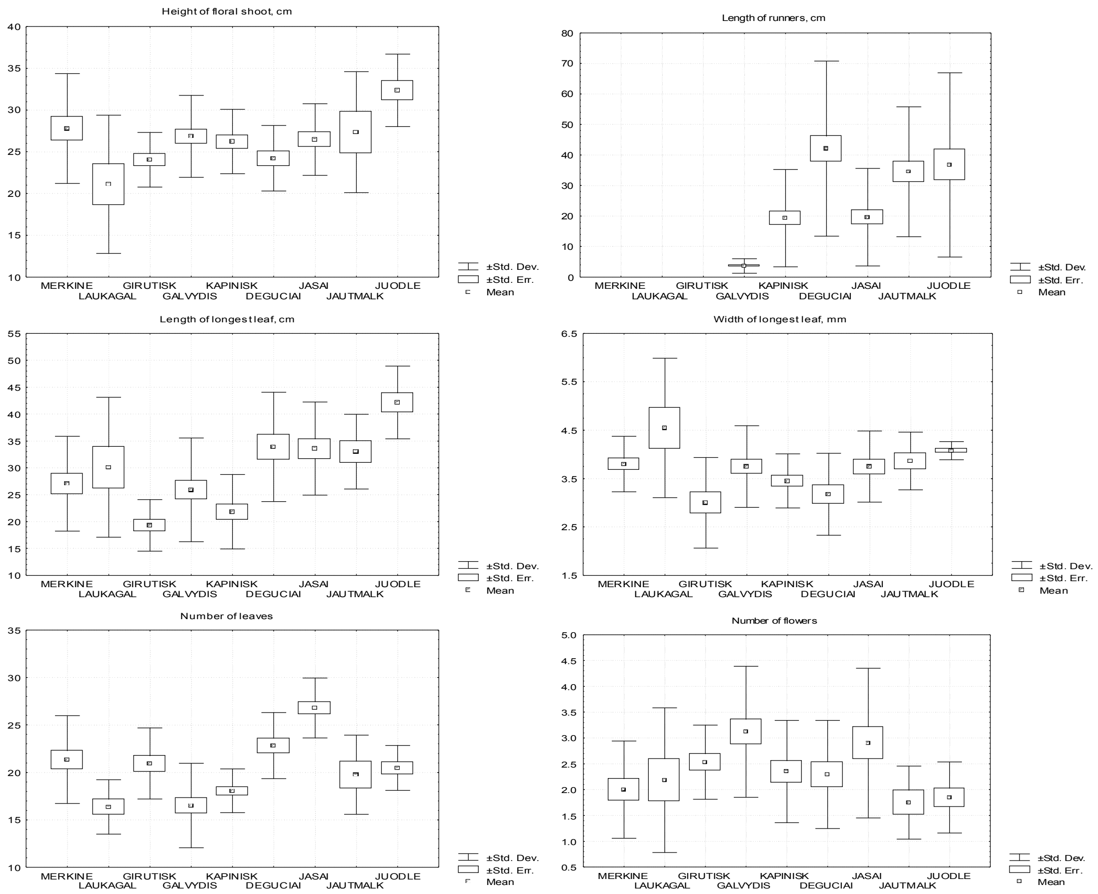

Fig. 2. Variation in morphologic features of *S. hirculus* generative individuals in Lithuania

Lithuania. Statistical parameters of all features are presented in Table 2. Average height of generative *S. hirculus* shoots was 23.3±0.5 cm; 20.3±0.4 leaves and 2.3±0.1 flowers formed on these shoots. The length of the longest leaf was 28.9±0.8 mm and width – 3.7±0.1 mm. The most varying of all features is the length of runners (variation coefficient 95 %). The numbers of runners (63 %) and flowers (53 %) also strongly vary. Variation coefficient of the length of the longest leaf of floral shoots is 36 %. General variation of other features is about 25 %. Therefore, it could be stated that *S. hirculus* populations in Lithuania are characterized by medium plasticity of phenotypic features of generative individuals.

During the research the correlations between different *S. hirculus* features were evaluated (Table 3). All the determined correlations were positive. The strongest correlations (r>0,5, p=0.000) were determined between heights of

Table 4. Genetic similarity (above *** diagonal) ir Nei‘s genetic distance (below *** diagonal) of *S. hirculus* populations

Population	Merkinė	Juodlė	Laukagalis	Galvydiškė	Jautmalkė
Merkinė	***	0.7727	0.7858	0.8025	0.8257
Juodlė	0.2579	***	**0.8455**	0.8053	0.8050
Laukagalis	0.2410	0.1679	***	0.8216	0.7802
Galvydiškė	0.2200	0.2162	0.1965	***	0.7729
Jautmalkė	0.1915	0.2169	0.2482	0.2576	***

floral shoots and leaf widths and between leaf lengths and widths. Mostly correlating features were leaf length and width; they statistically significantly correlate with all other evaluated features.

The height of *S. hirculus* plants in Lithuania is larger than of individuals studied in other countries. In Sweden (Ohlson 1986) and Denmark (Olesen & Warncke 1990) the average height of floral shoots was 19–24 cm, while during our research almost 70 % of *S. hirculus* shoots were higher. In Lithuania, the number of flowers of floral shoots was also higher. On one shoot from one to six flowers form; the average number of flowers in different populations varies from 1.9 to 3.5. In Denmark (Olesen & Warncke 1989, Dahlgaard & Warncke 1995) and Sweden (Ohlson 1986) *S. hirculus* plants usually form one, sometimes two flowers. Meanwhile in Lithuania only about 20 % of individuals had one flower, and even 43 % of the analyzed plants had three or even more flowers. The number of runners developed from basal parts of floral shoots also differs significantly. In Sweden (Ohlson 1986) about 80 % of such shoots had 2 – 3 runners, other plants – one, rarely four runners. Meanwhile in Lithuania about 10 % of the analyzed floral shoots had no runners, almost 17 % had one, about 55 % – 2 – 3 runners, and 10 % had four runners. In Lithuania more than 5 % of *S. hirculus* floral shoots had from five to seven runners. In different populations this feature is expressed very unevenly (Fig. 3), for example, in Jautmalkė floral shoot forms 3.3 runners on average, while in Galvydiškė – only 1.1.

Exhaustive investigations on *S. hirculus* populations, undertaken in the mire complex in Sweden (Ohlson 1986), showed that plant abundance and morphological features of individuals mostly depend on ecologic conditions. In the studied sites the most abundant and viable *S. hirculus* populations were located in spring area, with ground water at a depth of 10 cm, and fertile neutral to slightly alkaline soils (pH 6.7–7.5). In future all-round variation of this species under different ecologic conditions in Lithuania should be studied.

Genetic differentiation of *S. hirculus*

In order to determine whether the dependence between spatial position of plants in the population and genetic distance between plants exists, two situations were studied: 1) population formed of two groups of individuals separated by *Phragmites australis* overgrowth; 2) homogenic population, all individuals concentrated in one place but at different distances from one another. The first situation was observed in Merkinė population. This population covered the largest area and was the most abundant. The plants were sampled in two places. There were 12 individuals (N1, N2, N3, N7, N8, N9, N11, N12, N 14, N15, N19, N20) in the first group, and 8 individuals (N22, N23, N25, N26, N27, N28, N29, N30) – in the second group. The groups were situated at 100 m distance from each

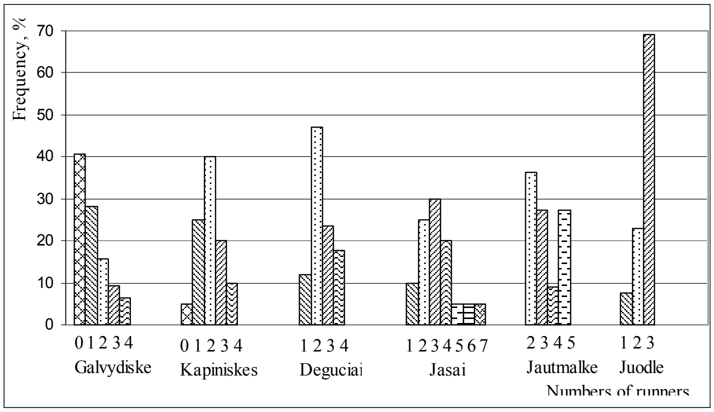

Fig. 3. Frequency distribution of runners per *S. hirculus* floral shoot in the studied populations

other and separated by *Phragmites australis* overgrowth. According to 93 RAPD loci (Naugžemys et al. 2007), genetic distances among all 20 individuals were calculated and genetic relationship dendrogram was compiled (Fig. 4). The research results show that plants from different fragments of Merkinė population form separate clusters, i.e. individuals of the same fragment of the population were closely related. For example, all eight plants of the second fragment of the population fall into one cluster. Individuals of the first fragment of the population are genetically more diverse, but majority of them (7 out of 12) form a very homogenic cluster.

Other four studied *S. hirculus* populations (Galvydiškė, Labanoras, Juodlė and Jautmalkė) are spatially undivided, territorially homogeneous. In the largest one – Galvydiškė population – the dependence of spatial arrangement of the individuals within the boundaries of the population on their genetic relatedness was investigated. Comparison of geographic and genetic distances among individuals of the Galvydiškė population revealed no correlation between these values (Fig. 5).

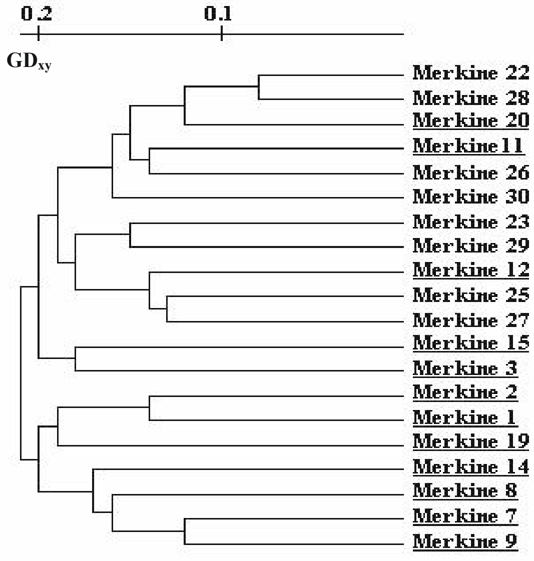

Fig. 4. UPGMA dendrogram of genetic relationships among 20 *S. hirculus* individuals from Merkinė population

In order to estimate genetic relatedness on the level of population, genetic distances and similarities among them were determined following Nei (1978) (Table 4). All studied populations were rather similar considering the analyzed parameters. Most genetically similar are Labanoras and Juodlė populations; their habitats are at a distance of about 188 km from each other. Genetic similarity of these populations reaches 84.55 %. The most different Juodlė and Merkinė populations (77.27 %) are at a distance of about 196 km from each other. Although Galvydiškė, Juodlė and Jautmalkė populations are rather close to each other, but genetically they are not most similar. For instance, geographical distance between Galvydiškė and Juodlė populations is only 3.4 km, but their genetic similarity is 80.53 %. These mismatches of genetic and geographic distances could be explained by genetic drift and other, not yet known, accidental processes occurring in small populations (Frankham & Ballou 2004). Much the same discrepancy in *S. hirculus* populations' relatedness was determined by other researchers (Oliver et al. 2006) who revealed significant differences in haplotypes in geographically close populations.

Analysis of Molecular Variance (AMOVA) revealed that the largest part of genetic (RAPD) diversity is attributed to individual plant differences in populations (73 %). Interpopulational component of molecular genetic diversity makes 27 % ($\ddot{O}_{PT}$ = 0.27). The obtained results show rather large differentiation among the studied populations. Using the software PopGen, Ht and Hs values were calculated for each studied locus as well as values of the coefficient of genetic differentiation (G_{ST}). The latter strongly varies among the studied loci. It shows that not all loci are equally affected by selection. It is possible that loci with high G_{ST} values are stronger affected by selection, therefore they are more differentiated in separate populations. Genetic differentiation coefficient of populations G_{ST}, calculated basing on average Ht and Hs values, is very similar to $\ddot{O}_{PT}$ ($G_{ST} = 0.26$). Therefore, both $\ddot{O}_{PT}$ and G_{ST} show rather high genetic differentiation of *S. hirculus* populations. The three geographically close Juodlė, Jautmalkė and Galvydiškė populations are characterized by even larger genetic differences ($\ddot{O}_{PT} = 0.30$). Similar level of genetic differentiation ($G_{ST} = 0.35$) was revealed for populations of other relict species *S. aizoides* (Lutz et al. 2000). The phenomenon when closely lying populations are characterized by high level of genetic differentiation was mentioned in Oliver et al. (2006) describing comprehensive phylogeographical investigations of *S. hirculus* using the cpDNA RFLP.

According to the data of some researchers (Hamrick & Godt 1989), average G_{ST} value, typical for endemic and rare plants, is usually 0.242–0.248. Nybon & Bartish (2000), while analyzing differentiation of populations according to RAPD markers, did not obtain statistically significant differences among G_{ST} values of plant species of various distribution. Based on their data, level of genetic differentiation in populations of endemic and rare species was 19–22 % ($G_{ST} = 0.19–0.22$).

Some researchers studying populations of vanishing plant species state that priority conservation should be undertaken for populations characterized by the largest diversity of molecular markers (Moritz et al. 1995) or those characterized by the highest heterozygosity (Vrijenhoek 1994). These criteria are proposed as principal ones while selecting populations for restoration of vanishing species (McKay et al. 2001). Among the studied populations Galvydiškė population was characterized by the highest average heterozygosity (Fig. 6), while Juodlė population – by the lowest. Moreover, in Juodlė and Merkinė populations some population-specific minor loci were identified (Naugžemys et al. 2007). So, higher heterozygosity of plant populations, presumably, does not reflect the whole spectrum of their genetic diversity.

The determined rather high genetic differentiation of *S. hirculus* populations shows that the RAPD markers can facilitate the disclosing of the peculiarity of populations of this relict species, and therefore, together with morphologic and protein (isoenzyme) markers they can be used for investigations of the processes occurring in *S. hirculus* populations.

CONCLUSIONS

S. hirculus runners dominated in all studied Lithuania's populations. Ecologic density of floral shoots is low, normally from one to fifteen floral

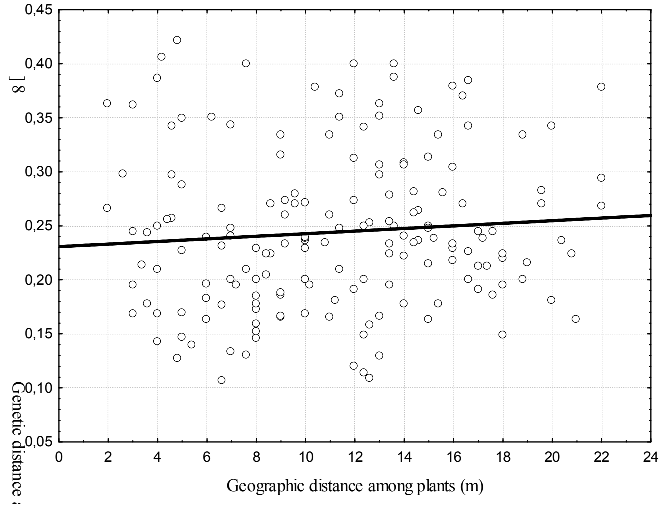

Fig. 5. Correlation between the geographic and genetic distances of *S. hirculus* plants in Galvydiðkë population ($R=0.083$, $p<0.283$)

shoots in a 0.25 m^2 plot. In optimal conditions about 5000 runners and 240 floral shoots were recorded in 1 m^2 plot.

S. hirculus populations in Lithuania are characterized by medium plasticity of phenotypic features of generative individuals. The most varying are features of vegetative propagation (number and length of runners).

The obtained results show rather large (G_{ST} = 0.26) differentiation among the studied *S. hirculus* populations. The largest part of genetic (RAPD) diversity is attributed to individual plant differences in populations (73 %). The level of average heterozygosity was highest in Galvydiske population (0.338) and the lowest in Juodle population (0.295).

ACKNOWLEDGMENTS

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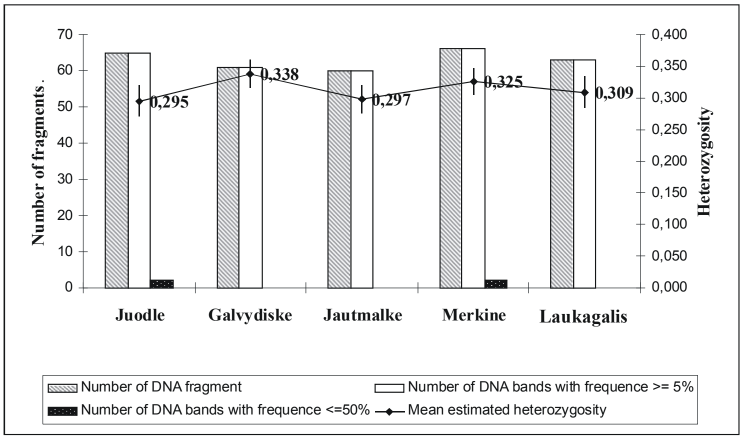

Fig. 6. RAPD band patterns and mean heterozygosity across *S. hirculus* populations in Lithuania

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