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Analysis of DNA polymorphism in wild populations of herb-Paris (*Paris quadrifolia L.*, Trilliaceae) from Lithuania and Norway

V. Jogaitė[1], J. R. Lazutka[1],

A. Stapulionytė[1],

J. R. Naujalis[1], A. Odland[2],

D. K. Bjerketvedt[2]

[1] Department of Botany and Genetics, Vilnius University, M. K. Čiurlionio 21, 03101 Vilnius, Lithuania, E-mail: juozas.lazutka@gf.vu.lt

[2] Telemark University College, Hallvard Eikas plass, N-3800 B¸, Norway

Analysis of random amplified polymorphic DNA (RAPD) was used to determine genetic diversity within the populations of herb-Paris plant from Lithuania and Norway. RAPD analyses have shown 21.9 ± 5.2% of polymorphic loci in the total sample. Polymorphism level in Norwegian sample was 20.3 ± 5.0%, and in the Lithuanian one was 21.9 ± 5.2%. The proportion of distinguishable RAPD phenotypes in Lithuania was 0.52 ± 0.03 and in Norway 0.24 ± 0.03. For the total sample, Shannon's Information Index was 0.59 and Nei's gene diversity 0.41. The estimated total proportion of diversity among populations (G_{ST}) and gene flow (Nm) were 0.67 and 0.245, respectively. In Norway G_{ST} was 0.64 and Nm was 0.28, whereas in Lithuania G_{ST} was 0.42 and Nm was 0.68. The UPGMA analyses have revealed that Lithuanian and Norwegian populations of herb-Paris are clearly separated into two clusters. Analysis of molecular variance (AMOVA) showed that the majority of molecular variation (41%) was due to variation between geographical regions (Lithuania and Norway), 37% of molecular variation was due to variation among individuals within the populations, and only 22% of variation was estimated to be due to variation between populations. In Lithuania, 68% of molecular variation was due to variation within populations, 32% due to variation between populations (Φ_{PT} = 0.32), whereas in Norway the proportions were 49% and 51% (Φ_{PT} = 0.51), respectively. Thus, the estimated parameters indicated a higher genetic diversity in Lithuanian populations of herb-Paris than in Norwegian ones.

Key words: *Paris quadrifolia*, RAPD markers, genetic diversity

INTRODUCTION

Extensive studies of the genetic diversity of plants have demonstrated that geographic distribution range and biology (taxonomic status, life form, breeding system and seed dispersion) of the species are closely associated with genetic variation among and within populations [1, 2]. Due to different evolutionary history of the species (habitat fragmentation, population isolation, genetic drift, gene flow, mutations, natural selection, ecological conditions) genetic diversity within populations of the species can be different [3].

Genetic studies revealed an association of genetic diversity with the life history traits of the plants [2]. Clonal plants produce genetically identical ramets which form a genet or clone [4]. Owing to this life history trait, a low genetic diversity within but a high diversity among populations is expected [5]. On the other hand, according to Eriksson [6], in about 40% of clonal plant species propagation by seedlings is observed. Studies of genetic variation in *Vaccinium stamineum*, *Viola riviniana* and in other clonal plant species reported high levels of genetic diversity within populations [7, 8]. The mode of plant propagation often depends on environmental conditions: vegetative propagation through rhizome becomes particularly beneficial in infertile habitats, because persistent connections among long-lived and widely spaced plants allow to take up nutrients from more distant places [9]. Differences in the level of genetic diversity among different clonal plants can be explained by taxonomic status (gymnosperms or angiosperms), breeding system (outcrossing / selfing or mixed mating plants), seed dispersal type, mode of clonal spread [1, 2].

The aim of the present study was to investigate the genetic structure and polymorphism level in herb-Paris populations growing in Lithuania and Norway. For this purpose we used the method of random amplified polymorphic DNA (RAPD) which allows to detect genetic polymorphism over all genome (within coding and noncoding regions) without prior knowledge of genome nucleotide sequence [10]. This method is widely used in plant population biology due to its simplicity and informativity.

MATERIALS AND METHODS

Herb-Paris (*Paris quadrifolia* L., Trilliaceae) is a perennial herbaceous plant native of damp woodland in lime rich soils. It likes moist, leafy soil in partial or full shade. A more detailed description of this species could be found in our previous papers [11–13].

Plants were collected from 13 populations in Lithuania (240 plants) and 17 populations in Norway (164 plants), in total 404 plants (Table 1). The plants were placed into plastic bags containing ice, transported to the laboratory and stored for a few days at 4 °C prior to DNA extraction. Most of the collected plants were separated from each other at least for about 1 m to avoid sampling of the plants arising from the same rhizome. The populations of herb-Paris in Lithuania were more numerous and covered a larger area than in Norway. Therefore, the mean plant set from Lithuanian populations was 18.5 (range, from 7 to 50 per population), whereas it was 9.6 (range from 4 to 20 plants per population) in Norwegian populations.

DNA extraction and RAPD analysis were done exactly as previously described [12]. For DNA amplification by the RAPD method, seven (270-2, 270-7, 380-1, 380-6, 380-8, 470-6, 470-9) 10 nt long primers of random sequence (Roth, Germany) were used. DNA amplification was performed in a thermocycler (Mastercycler personal 5332, Eppendorf, Germany) under the following conditions: initial denaturation for 4 min at 94 °C, 45 cycles of denaturation for 1 min at 94 °C, primers annealing for 1 min at 35 °C, extension for 2 min at 72 °C followed by a final extension for 5 min at 72 °C.

Table 1. **Geographical location, sample size and some main genetic characteristics of herb-Paris populations from Lithuania and Norway**

Population	Geographic coordinates	Number of plants sampled	Number of polymorphic bands	Polymorphism (%)	Number of distinguishable RAPD phenotypes	Proportion of distinguishable RAPD phenotypes
			LITHUANIA			
Varėna	N 54°15'44.8'' E 24°35'2.3''	30	8	12.5 ± 4.1	10	0.33 ± 0.08
Kriukai	N 56°17'13.2'' E 23°50'03.3''	20	7	10.9 ± 3.9	17	0.85 ± 0.08
Pagramantis	N 55°22'17.9'' E 22°14'13.4''	14	9	14.1 ± 4.3	9	0.64 ± 0.12
Dūkštos	N 54°50' E 24°58'	11	5	7.8 ± 3.3	9	0.82 ± 0.11
Kurtuvėnai	N 55°50'12.7'' E 23°05'43.5''	8	6	9.4 ± 3.6	5	0.62 ± 0.17
Vingis	N 54°41' E 25°17'	17	5	7.8 ± 3.3	7	0.41 ± 0.12
Grigiškės	N 54°40'14.2'' E 24°54'45.2''	25	8	12.5 ± 4.1	14	0.56 ± 0.10
Þemaitkiemis	N 55°17'16.8'' E 24°53'36.0''	7	2	3.1 ± 2.2	3	0.43 ± 0.19
Varnikai	N 54°38' E 24°57'	18	11	17.2 ± 4.7	10	0.55 ± 0.12
Puškoriai	N 54°41'06.4'' E 25°22'06.9''	17	13	20.3 ± 5.0	12	0.71 ± 0.11
Uþtrakis	N 54°39'18.1'' E 24°56'53.9''	11	5	7.8 ± 3.3	3	0.27 ± 0.13
Prienai	N 54°34' E 24°16'	12	8	12.5 ± 4.1	10	0.83 ± 0.11
Kairėnai	N 54°42' E 25°18'	50	11	17.2 ± 4.7	31	0.62 ± 0.07

Table 1 continued

Population	Geographic coordinates	Number of plants sampled	Number of polymorphic bands	Polymorphism (%)	Number of distinguishable RAPD phenotypes	Proportion of distinguishable RAPD phenotypes
			NORWAY			
H¸rte A	N 58°38'27.9'' E 9°0'0.4''	4	4	6.2 ± 3.0	3	0.75 ± 0.22
H¸rte A1	N 58°38'27.9'' E 09°0'0,4''	13	7	10.9 ± 3.9	8	0.61 ± 0.13
H¸rte A2	N 58°38'27.9'' E 09°0'0.4''	5	2	3.1 ± 2.2	2	0.4 ± 0.22
H¸rte MI	N 58°38'27.9'' E 9°0'0.4''	13	4	6.2 ± 3.0	4	0.31 ± 0.13
Skien Marker	N 58°38'27.2'' E 09°0'2.2''	4	6	9.4 ± 3.6	4	1.00
Bø	N 58°38'27.9'' E 09°0'0.25''	16	4	6.2 ± 3.0	4	0.25 ± 0.11
Håtveit	N 58°38'27.8'' E 09°0'0.5''	9	5	7.8 ± 3.3	6	0.67 ± 0.16
Vinje Vågslid	N 59°31'35.9'' E 07°13'56.6''	12	2	3.1 ± 2.2	3	0.25 ± 0.12
Skvisla	N 58°38'27.9'' E 09°0'0.4''	6	0	0	0	0
¨sterb¸- Aurlandsdalen	N 60°25'27.5'' E 07°11'2.6''	4	3	4.7 ± 2.6	2	0.5 ± 0.2
Roldal	N 59°29'27.1'' E 05°28'6.5''	17	5	7.8 ± 3.3	7	0.41 ± 0.18
Ryggen	N 58°38'27.2'' E 09°0'2.2''	6	4	6.2 ± 3.0	2	0.33 ± 0.19
Gr¸nsdal Odda	N 59°29'27.6'' E 05°28'7.4''	5	1	1.6 ± 1.6	2	0.40 ± 0.22
Hildal Odda	N 59°29'27.7'' E 05°28'6.5''	8	0	0	0	0
Hjelset Brygge	N 58°38'27.5'' E 09°0'0.9''	10	1	1.6 ± 1.6	2	0.2 ± 0.13
Fj¿rland	N 61°25'32.8'' E 06°44'55.5''	12	4	6.1 ± 3.0	5	0.42 ± 0.14
B¸yabreen	N 61°16'58.3'' E 05°16'8.4''	20	7	10.9 ± 3.9	11	0.55 ± 0.11

Genetic distances among *P. quadrifolia* individuals were estimated by the method of Nei and Li [14]. Relationships among individuals were evaluated using a dendrogram based on Nei and Li's genetic distances. It was generated by the UPGMA (unweighted pair group method) cluster analysis method. Calculation of genetic distances and UPGMA cluster analyses were performed with the TREECON program for Windows V 1.3b [15]. The level of population genetic differentiation was estimated by AMOVA and calculated using GenAlEx software [16]. AMOVA was applied to estimate the ratio of variance among populations to total variance, Φ_{PT}, which is analogous to Fst and can be used to estimate the level of population genetic differentiation. Calculation of the observed number of alleles, Nei's [17] gene diversity (h), Shannon's Information Index ($I = -\Sigma p_i \ln p_i$), total gene diversity (Ht), gene diversity within populations (Hs), gene diversity among populations (Gst = (Ht–Hs) / Ht), gene flow (Nm = 0.5 (1–Gst) / Gst) and generation of a Nei's genetic distance based dendrogram were carried out with POPGENE V 1.31 software [18].

RESULTS AND DISCUSSION

The polymorphism level and proportion of distinguishable RAPD phenotypes in different populations of herb-Paris in Lithuania and Norway are shown in Table 1. For a total, 64 DNA fragments were ampli-

Table 2. **Calculated values of genetic diversity indices in Lithuanian and Norwegian populations of herb-Paris**

	Lithuania	Norway	Total sample
Shannon's Information Index	0.28	0.14	0.59
Nei's gene diversity	0.19	0.097	0.41
Observed mean number of alleles (na)	1.55	1.25	2.00
Gene diversity among populations (Gst)	0.42	0.64	0.67
Gene flow (Nm)	0.68	0.28	0.24

fied. The size of the amplified fragments ranged from 580 to 2400 bp, 14 of them were polymorphic (21.9 ± 5.2%). Polymorphism level in Norway was 20.3 ± 5.0%; in Lithuania it was 21.9 ± 5.2%. At the level of individual populations, polymorphism in Lithuanian populations varied from 3.1 ± 2.2% (Þemaitkiemis) to 20.3 ± 5.0% (Puškoriai), and in Norwegian populations it varied from 0% (Skvisla, Hildal Odda) to 10.9 ± 3.9% (Bøyabreen, Hørte A1). Thus, our study revealed a comparably low DNA polymorphism level in *P. quadrifolia*. It was substantially lower than in many outcrossing plant species [1, 19–22]. The observed DNA polymorphism level was more similar to that of wild autotetraploids [22–25]. These autotetraploids propagate by rhizome as well as by seeds, but many of them are sterile. The possible reason for the comparatively low DNA polymorphism level in our study could be the ability of *P. quadrifolia* to spread clonally.

The proportion of distinguishable RAPD phenotypes in Lithuania was 0.52 ± 0.03 and in Norway 0.24 ± 0.03. At the level of individual populations, the proportion of distinguishable RAPD phenotypes in Lithuanian populations varied from 0.27 ± 0.13 (Uþtrakis) to 0.85 ± 0.08 (Kriukai), and in Norwegian populations from 0 (Skvisla, Hildal Odda) to 1.0 (Skien Marker). Interestingly, part of distinguishable RAPD profiles in the whole Norwegian sample (0.24 ± 0.03) was very similar to those established for clonal plants with predominantly vegetative propagation [26]. However, several studies of clonal plant species have reported quite a wide variation in part of distinguishable RAPD profiles [8, 27]. Such differences could arise due to a different mode of clonal spread and seed distribution. It could be expected that the distance among the genets would be bigger if birds or mammalia disperse the seeds [28]. According to Ridley [29], ants and birds disperse seeds of *P. quadrifolia*, however, a colonization study did not show any evidence of seed dispersal by birds [30]. On the contrary, another study confirmed dispersal of seeds by ingestion [31]. In our opinion, birds as well as rodents or snails could ingest the seeds. In addition, a hypothesis of the dispersal of seeds through rain or snow melting water could not be rejected. However, further studies are needed to elucidate this issue.

To estimate genetic variation between populations, the values of Shannon's Information Index (I), Nei's gene diversity (h) and the observed number of alleles per locus (na) were calculated (Table 2). For the total sample, Shannon's Information Index was 0.59 and Nei's gene diversity 0.41. The observed number of alleles per locus ranged from 1.07 in Þemaitkiemis population to 1.93 in Puškoriai population in Lithuania (1.55 on average) and from 1.00 in Skvisla, Hildal Odda populations to 1.50 in Bøyabreen and H¸rte A1 populations in Norway (1.25 on average). The estimated total proportion of diversity among

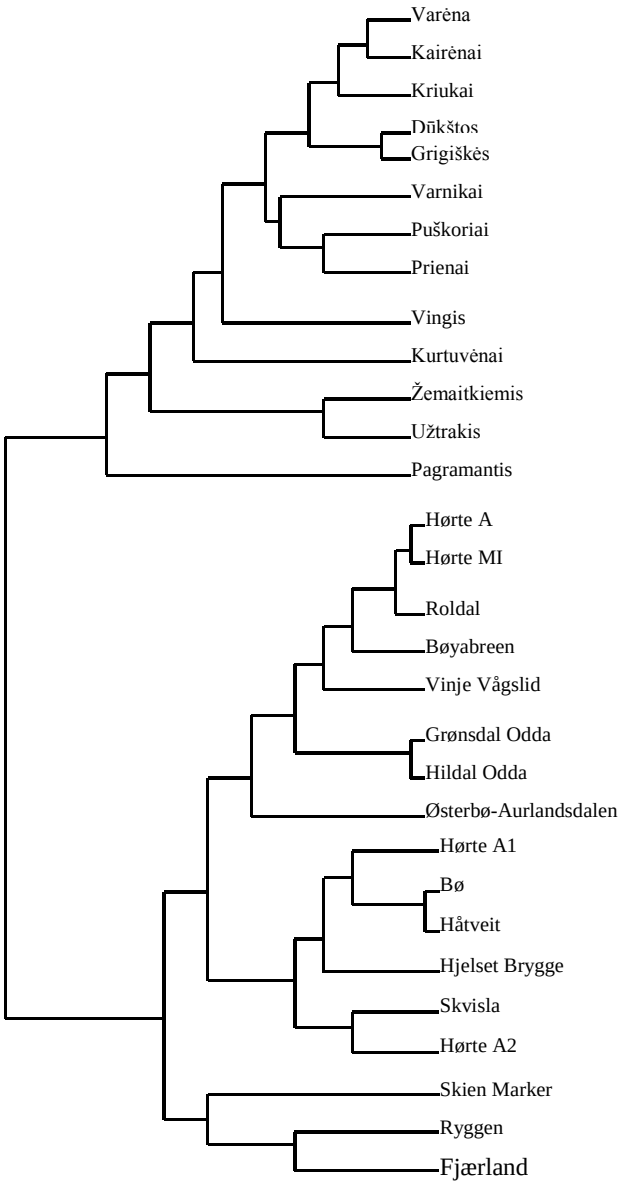

Fig. 1. UPGMA dendrogram based on Nei's genetic distances between different populations of herb-Paris from Lithuania and Norway

populations (G_{ST}) and gene flow (Nm) were 0.67 and 0.24, respectively. In Norway G_{ST} was 0.64 and Nm was 0.28, whereas in Lithuania G_{ST} was 0.42 and Nm was 0.68.

To estimate the relationship between herb-Paris populations, Nei's genetic distance between pairs of populations was calculated. Cluster analysis (UPGMA) was used to generate a dendrogram based on Nei's genetic distances among populations. The UPGMA analyses have revealed that Lithuanian and Norwegian populations of herb-Paris are clearly separated into two clusters (Fig. 1). Correlation analyses revealed a significant correlation between Nei's genetic distance and geographic distance in the total sample ($R = 0.775$, $P < 0.0001$). However, it was not significant when calculated separately for Lithuanian and Norwegian populations ($R = 0.283$, $P = 0.071$ and $R = 0.179$, $P = 0.079$, respectively).

Analysis of molecular variance (AMOVA) showed that the majority of molecular variation (41%) was due to variation between geographical regions (Lithuania and Norway), 37% of molecular variation was due to variation among individuals within the populations, and only 22% of variation was estimated to be due to variation between populations. Such a high proportion of molecular variation attributable to the variation between geographical regions could be explained by early separation, isolation and absence of gene flow between herb-Paris populations of Lithuania and Norway. The calculated values of $\Phi_{PT} = 0.625$ ($p = 0.001$) for the total sample was similar to the proportion of diversity ($G_{ST} = 0.67$) between the populations. In Lithuania, 68% of molecular variation was due to variation within populations, 32% due to variation between populations ($\Phi_{PT} = 0.32$), whereas in Norway the proportions were 49% and 51% ($\Phi_{PT} = 0.51$), respectively. The amount of molecular variation attributable to differences between the populations of *P. quadrifolia* was similar to that estimated for other outcrossing plant species [19, 32–34] such as *Digitalis minor* (26.5%), *Primula farinosa* (20.6%), *Aster tripolium* (17.5%) and *Digitalis obscura* (15.2%). Thus, the estimated higher molecular variation within populations than among them revealed a low genetic differentiation of herb-Paris populations, quite a significant gene flow and the importance of sexual propagation to the spread of herb-Paris.

Finally, it could be easily noted that the estimated genetic diversity of herb-Paris populations in Lithuania is higher than in Norway. The possible reason could be the different landscape of two regions: in Norway populations of herb-Paris are more isolated from each other by mountains, therefore the gene flow among them is lower than among populations in Lithuania. However, the hypothesis of a more frequent propagation of herb-Paris by seeds in Lithuania than in Norway could not be rejected, either.

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V. Jogaitė, J. R. Lazutka, A. Stapulionytė, J. R. Naujalis, A. Odland, D. K. Bjerketvedt

DNR POLIMORFIZMO TYRIMAI LIETUVOS IR NORVEGIJOS KETURLAPĖS VILKAUOGĖS (*PARIS QUADRIFOLIA* L., TRILLIACEAE) POPULIACIJOSE

S a n t r a u k a

Keturlapės vilkauogės Lietuvos ir Norvegijos populiacijų polimorfizmo tyrimai atlikti atsitiktinai amplifikuotos polimorfinės DNR (RAPD) metodu. Nustatyta, kad 21,9 ± 5,2% bendros imties lokusų buvo polimorfiniai. Norvegijos imtyje polimorfizmas siekė 20,3 ± 5,0%, o Lietuvos – 21,9 ± 5,2%. Unikalių RAPD fenotipų dalis Lietuvoje buvo 0,52 ± 0,03, Norvegijoje – 0,24 ± 0,03. Visai imčiai apskaičiuotas Šenono informacijos indeksas buvo lygus 0,59, o Nėjaus genų įvairovė – 0,41. Tarppopuliacinės įvairovės santykis (G_{ST}) ir genų srautas (Nm) buvo lygūs 0.67 ir 0.245. Norvegijos populiacijose G_{ST} buvo lygus 0,64, o Nm – 0,28. Lietuvoje šie parametrai buvo atitinkamai 0,42, ir 0,68. UPGMA analizė rodo, kad Lietuvos ir Norvegijos populiacijos formuoja dvi skirtingas sankaupas. Didžiausią molekulinės variacijos dalį (41%) sudaro variacija tarp geografinių regionų, 37% sudaro variacija tarp individų populiacijų viduje, o likusią dalį (22%) – variacija tarp populiacijų. Lietuvos populiacijose 68% molekulinės variacijos sudarė variacija tarp individų populiacijų viduje, o 32% – variacija tarp populiacijų (Φ_{PT} = 0,32). Norvegijos populiacijose šie dydžiai buvo atitinkamai 49% ir 51% (Φ_{PT} = 0,51). Taigi apskaičiuotieji parametrai rodo didesnę Lietuvos keturlapės vilkauogės populiacijų genetinę įvairovę.