BIOLOGIJA. 2005. Nr. 2. P. 5–12
© Lietuvos mokslø akademija, 2005
© Lietuvos mokslø akademijos leidykla, 2005

Genetic diversity in fragmented population of herb-Paris (*Paris quadrifolia* L., Trilliaceae)

Vaida Jogaitë*,

Jonas R. Naujalis,

Juozas R. Lazutka

Department of Botany and Genetics, Vilnius University, 21 M. K. Èiurlionio, 03101 Vilnius, Lithuania

Analysis of random amplified polymorphic DNA (RAPD) was used to determine genetic diversity within fragmented population of plant herb-Paris (*Paris quadrifolia* L.). RAPD analyses have shown 21.9% of polymorphic loci. Part of distinguishable RAPD phenotypes in five groups was 0.68 (range from 0.37 to 1.00). The lowest part of distinguishable RAPD phenotypes (0.37) was found in the mown part of the population. Analysis of molecular variance (AMOVA) revealed 76% of the overall genetic variation within plant groups and 24% among plant groups (F_{PT} = 0.23, p = 0.001). An UPGMA dendrogram based on Nei's genetic distance showed quite a good correspondence between the genetic distances and the degree of isolation among the plant groups. The lowest genetic distance (0.083) was calculated among the groups that were closely located to each other and not separated by any natural barriers. The relatively high genetic variation within the groups of *Paris quadrifolia* L. population revealed the importance of sexual propagation to the spread of this plant.

Key words: *Paris quadrifolia*, RAPD markers, natural barriers, fragmented populations, genetic diversity

INTRODUCTION

Clonal propagation is widespread throughout plant kingdom. It occurs in two–thirds of all plant species in Central Europe [1]. Due to ability to produce genetically identical plants (clones), low genetic diversity within and high genetic diversity among clonal plant populations is expected [2]. However, plant species that propagate only clonally are not common in nature – most of them can spread both by vegetative and sexual propagation [3]. 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 [4]. Williams [5] suggested that dispersal of seeds with a genetically variable offspring is an adaptive trait, which helps to evade competition among clonal plants as a result of colonization of new enviroments. Probably sexual propogation is important for arising of new plant populations in unstabile natural conditions, while normally clonal propogation prevails. Some studies of clonal plants indicated a high level of intrapopulation genetic diversity which may occur as a result of repeated recruitment of new seedlings [6]. Differences in the level of genetic diversity among different clonal plants can be explained by taxonomic status (gymnosperms or angiosperms), life form (perennial or annual), geografic range (endemic or widespread), breeding system (outcrossing / selfing or mixed), seed dispersal type, or mode of clonal spread [7].

Different plant studies a reported positive relationship between population size and genetic diversity within a population. Reduction of gene flow among populations increases a random genetic drift and inbreeding as well as decreases genetic diversity within a population [8]. However, there is almost no information about the influence of small natural barriers (runlets, hills, roads, etc) on genetic variations within a population.

The aim of the present study was to investigate genetic structure and polymorphism level within herb-Paris (*Paris quadrifolia* L., Trilliaceae) population, the influence of small natural barriers on the spread and the importance of sexual and clonal propagation in the life history of this plant. For this purpose we used the method of random amplified polymorphic DNA (RAPD) which allows to detect genetic poly-

* Corresponding author. E-mail: Vaida.Jogaite@gf.vu.lt

morphism over all genome (within coding and noncoding regions) without prior knowledge of genome nucleotide sequence [9]. This method is widely used in plant population biology due to its simplicity and informativity.

MATERIALS AND METHODS

Species description

Herb-Paris (*Paris quadrifolia* L., Trilliaceae) is a herbaceous plant 20–40 cm high, with an erect single stalk and an underground rhizome. It has four (sometimes 3–6) oval leaves. Herb-Paris is clonal, tetraploid (4n = 20), gramineous, ancient-forest plant species, widespread in deciduous and mixed forests of Europe and Central Asia [10–13]. It is a perennial plant species wich takes 10–15 years to come into flower [14]. The flower is yellowish-green, single, four sepals and four petals, appearing at the top of the stalk, blooming from May to July. It is pollinated predominantly by wind [15] and by insects, because flowers have a fetid smell which, together with the dark purple stigmas and frequently also with stamens and petals, attracts carrion-loving flies [16]. The fruit is globose berry, 10–15 mm in diameter, bluish-black, surrounded by the calyx, bitter-tasting, reaching maturity from July to September. Seeds are dispersed by ants and birds [10, 17]. However, Karpisonova [11] suggested that *Paris quadrifolia* is spread predominantly by rhizome, because its seedlings are rarely found in nature.

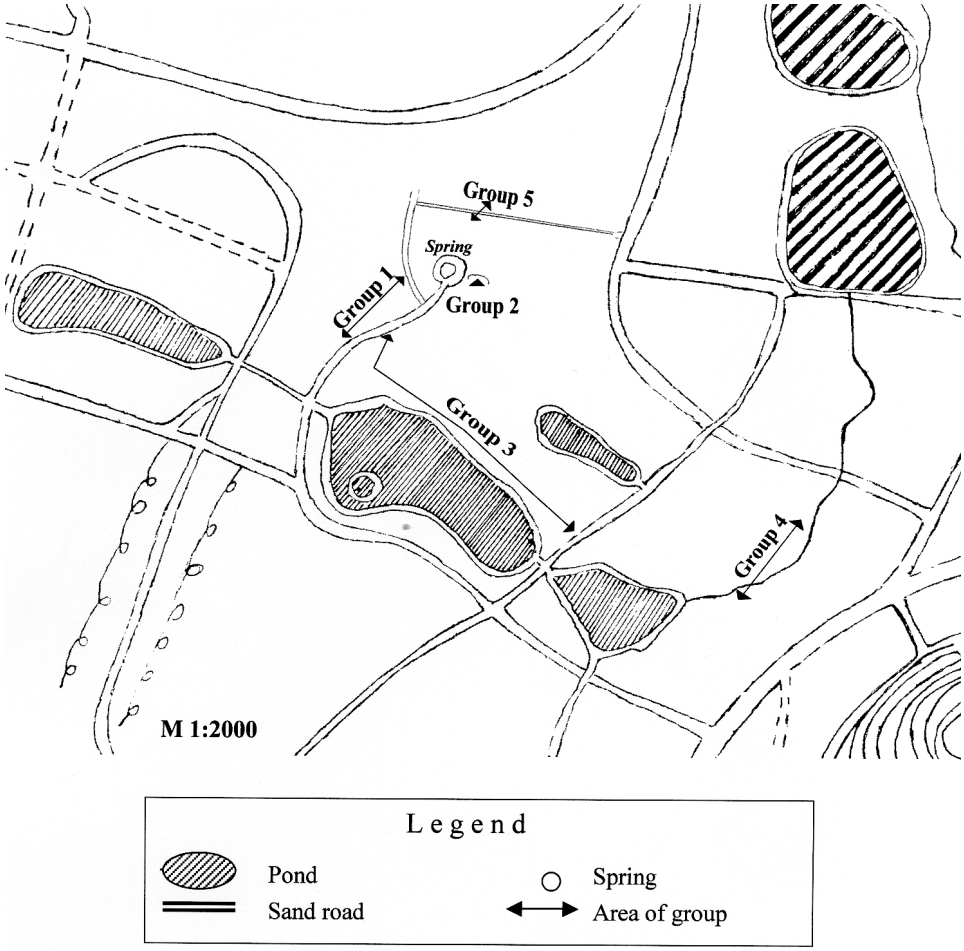

Fig. 1. Location of the five *Paris quadrifolia* groups studied in Kairënai Botanical Garden of Vilnius University population

Study site and sampling condition

The study site was at Kairënai Botanical Garden of Vilnius University, Vilnius, SE Lithuania (54°42'N, 25°18'E), *ca*. 80 m above sea level. The population was located in an Aegopodio-Fraxinetum forest. It covered about an area of 1 ha and was fragmented by small natural barriers: a hill, a spring, sand roads. Plants from five groups separated by any natural barriers were sampled (Fig. 1).

Plants of group 1 were located in mown grassland and separated from the other plant groups by a sand road and a spring. The other four groups were located in unmown areas. The groups varied in size: group 1, group 2 and group 3 contained more individuals than did groups 4 and 5 (Table 1). Abundance of gross, flowering *Paris quadrifolia* plants (some of them having 5 leaves) suggests favourable conditions for the growth as well as a longstanding origin of these *Paris quadrifolia* groups. Depending on

Table 1. **Some characteristics of studied herb–Paris (*Paris quadrifolia* L.) groups in population from Kairënai Botanical Garden of Vilnius University**

Group	Number of plantsin the group	Minimal distance among plants in the group (m)	Maximal distance among plants in the group (m)
1	16	0.3	20
2	16	0.3	10
3	7	1	60
4	5	0.2	20
5	6	0.3	3

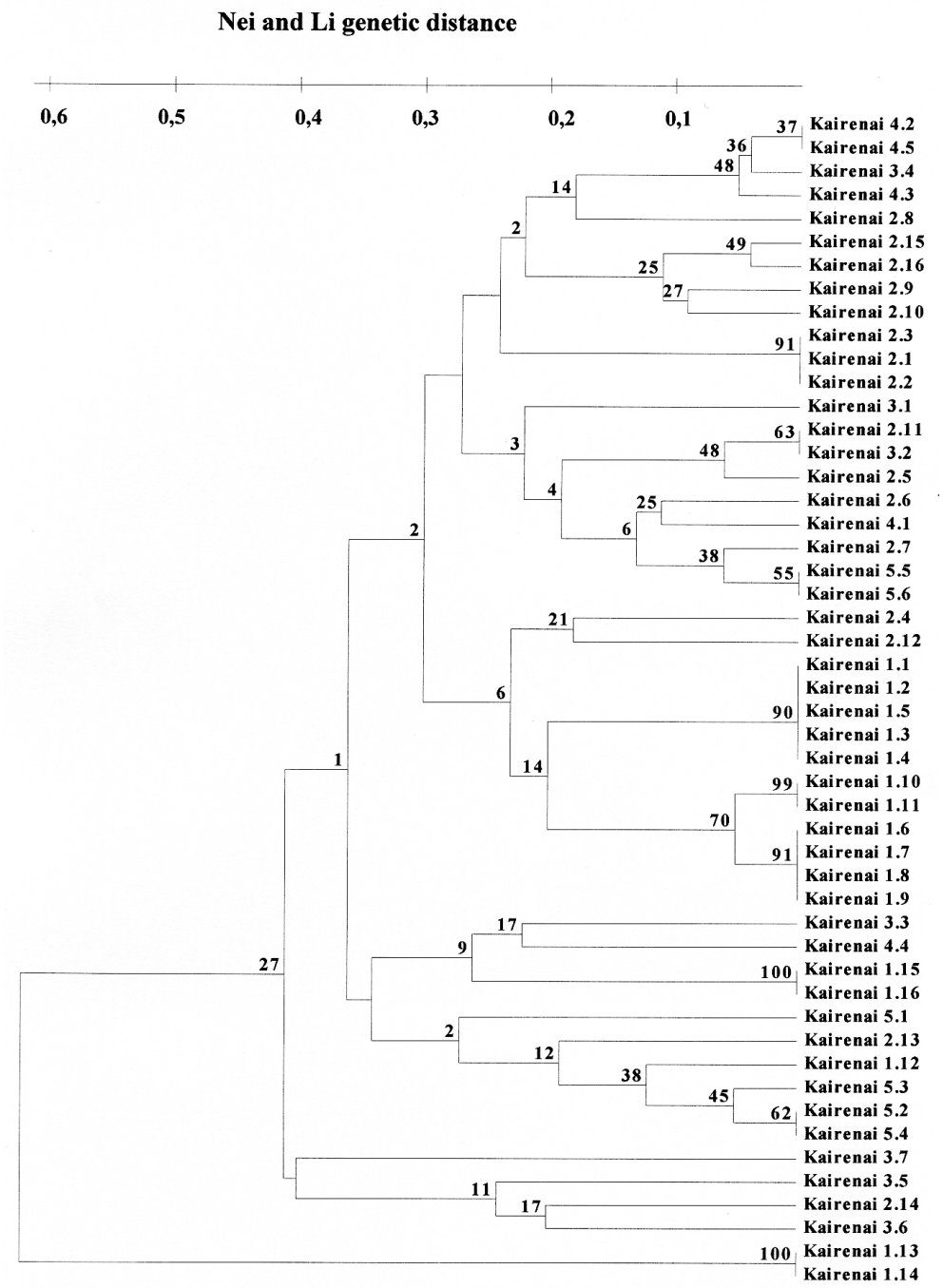

Fig. 2. UPGMA dendrogram based on Nei and Li [16] genetic distances among individuals from *Paris quadrifolia* L. population in Kairënai Botanical Garden of Vilnius University. The plants were labelled according to their origin (the number before the dot means the group from which this plant was picked up). Numbers at nodes indicate bootstrap support (1.000 iterations)

the group size, 5–16 plants (in total 50 individuals) were picked up per group. 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.

DNA isolation and amplification

DNA was extracted from fresh leaves of *Paris quadrifolia* using Genomic DNA Purification Kit (Fermentas, Lithuania) in accordance with manufacturer's recommendations. DNA concentration and purity was estimated spectrophotometrically (BioPhotometer, Eppendorf, Germany). Extracted DNA samples were stored in a freezer at –45 °C.

For DNA amplification by the RAPD method, 10 nt length primers of random sequence (Roth, Germany) were used. Three Random Primer Kits, in total 30 primers, were screened with a subset of *Paris quadrifolia* DNA samples to select primers that generated clear, reproducible, and polymorphic RAPD band patterns. DNA amplification was carried out in a 25 μl final volume of reaction mixture containing 1× *Taq* buffer with KCl, 3 mM $MgCl_2$, 0.2 mM dNTP mix, 0.75 U recombinant *Taq* DNA polymerase (Fermentas, Lithuania), 0.4 μM primer (Roth, Germany), 200 ng genomic DNA. The prepared samples were overlaid with 14 μl of mineral oil (SIGMA, USA) to prevent evaporation.

The 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 [18]. DNA amplification of each sample was repeated at least twice. Amplification products were analysed by horizontal electrophoresis in 1.5% aga-

Table 2. **Selected primers used in the survey of *Paris quadrifolia*, minimal and maximal sizes of amplified DNA bands, total number of DNA bands, number of polymorphic bands, number of monomorphic bands in RAPD patterns and the proportion of polymorphic bands**

Primer	Primer sequence) (5'→3'	Band size (bp)	Total	Monomorphic bands	Polymorphic bands	Polymorphism (%)
270-2	GGCCTACTCG	580–1600	9	7	2	22.2
270-7	GAGACCTCCG	1000–2100	9	7	2	22.2
380-1	ACGCGCCAGG	650–2100	9	7	2	22.2
380-3	GGCCCCATCG	400–2000	11	9	2	18.2
380-6	CCCGACTGCC	580–2400	10	8	2	20.0
380-8	CGCACCGCAC	600–2400	10	8	2	20.0
470-6	GCACGTGAGG	480–2300	15	12	3	20.0
470-9	CCGGGGTTAC	580–1100	9	6	3	33.3
Total			**82**	**64**	**18**	**21.9**

Table 3. **Polymorphism of DNA bands and part of distinguishable RAPD phenotypes in groups of *Paris quadrifolia* plants from Kairënai population**

Group	Polymorphic bands	Polymorphism (%)	Total number of phenotypes	Number of distinguishable phenotypes	Part of distinguishable phenotypes
1	15	18.3	16	6	0.37
2	14	17.1	16	14	0.87
3	14	17.1	7	7	1.00
4	9	11.0	5	4	0.80
5	8	9.7	6	4	0.68

Table 4. **Nei's genetic distances estimated among *Paris quadrifolia* L. groups in Kairënai population**

Groups	**1**			
2	0.205	**2**		
3	0.165	0.083	**3**	
4	0.246	0.147	0.179	**4**
5	0.281	0.125	0.105	0.116

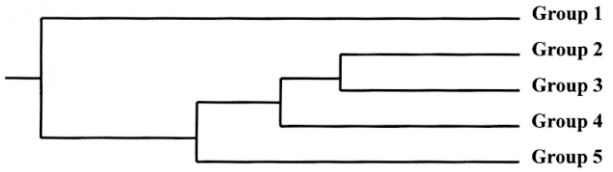

Fig. 3. UPGMA dendrogram based on Nei's genetic distances among five groups of *Paris quadrifolia* plants from Kairënai population. Genetic distances are shown in Table 4

rose gel with etidium bromide in 1 × TBE (Tris-borate-EDTA) buffer at 90 V. DNA was visualized in UV light. The size of DNA fragments was estimated using a DNA marker – GeneRuler DNA Ladder Mix (Fermentas, Lithuania).

Data analyses

Each DNA band was estimated as a distinct DNA locus with two alleles. Monomorphic loci had only one allele (present), while polymorphic loci had both (present and absent) alleles. Clear reproducible DNA bands were scored across all individuals as 1 if the band was present or 0 if the band was absent. For the estimation of the level of DNA polymorphism, all clear, reproducible bands were scored, whereas in other data analyses only bands with a frequency 5–95% were included.

The level of polymorphism (percentage of polymorphic bands) was calculated across all groups as well as in each separate group of plants. Part of the distinguishable RAPD phenotypes in each group of *Paris quadrifolia* was calculated dividing the number of unique RAPD profiles by the total number of RAPD profiles. Genetic distances between *Paris quadrifolia* individuals were estimated by the method of Nei and Li [19].

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 [20].

The level of population genetic differentiation was estimated by AMOVA and calculated using GenAlEx software [21]. For the calculations, all five plant groups were considered as five populations from one region. 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 [22] 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 [23].

RESULTS

A few from 30 random sequence primers did not show any DNA amplification, but the majority of primers showed only monomorphic band patterns, or differences among patterns were uncertain. Only eight primers yielded clear, reproducible, polymorphic patterns (Table 2). These primers were used for further analysis. Nine to fifteen DNA bands per primer were yielded. The size of the bands ranged from 400 bp to 2400 bp. Eighty-two loci were detected, while the part of polymorphic loci comprised 21.9% (18 loci) (Table 2). Part of the polymorphic loci estimated in each plant group ranged from 9.7% in group 5 to 18.3% in group 1 (Table 3). Only one private band (1200bp) produced by the primer 270-2 was detected in group 1. Other plant groups had not private bands.

The proportion of distinguishable phenotypes in the whole group of plants was 0.68. In different groups it ranged from 0.37 (group 1) to 1 (group 2). The lowest proportion of distinguishable phenotypes was calculated in group 1, which was located in mown grassland and separated from the other groups by a sand road and a spring. Each plant in group 2 had its own genotype; this group was located on an unmown hill.

The UPGMA dendrogram based on Nei's genetic distances among individual plants is shown in Fig. 2. One can see that plants separated by natural barriers did not make individual clusters. Genetic distance among the plants from one group was often bigger than the distance between the plants collected from different groups. Among the individuals in different groups it ranged from 0 to 0.85. The mean genetic distance among the pairs of plants within group 1 was 0.24 (range: 0.00–0.50). The highest mean genetic distance among the pairs of plants within a group was estimated in group 3 (0.32; range: 0.07–0.6). The highest genetic distance was found between the most distant plants. No clonal plants were detected in this group. The lowest mean genetic distance among pairs of individuals within a group was estimated within group 5 (0.05; range: 0.00–0.3). Genetic distance among the pairs of plants within the other two groups ranged from 0.00 to 0.625 (mean 0.28) in group 2 and from 0.00 to 0.25 (mean 0.09) in group 4.

To estimate relationship between five plant groups separated by natural barriers, an UPGMA dendrogram based on Nei's genetic distance among the groups was generated (Table 4, Fig. 3). The dendrogram showed quite a good correspondence between genetic distance and isolation degree of the plant groups. The lowest genetic distance (0.083) was calculated between groups 2 and 3. These groups were located close to each other and not separated by any barriers. The highest genetic distance was calculated between groups 1 and 5 (0.281). It is reflected by a separate branch on the UPGMA dendrogram.

The mean number of observed alleles per locus ranged from 1.44 (group 5) to 1.78 (groups 1–3). The observed number of alleles in group 4 was 1.50. Due to exclusion from the analysis of monomorphic bands (which were shared by 95% or more of the individuals), all loci had two alleles in total.

The values of Shannon's Information Index (I) and Nei's gene diversity (h) based on the group frequency of alleles varied from I = 0.5, h = 0.33 (group 2) to I = 0.57, h = 0.39 (group 1). Differences among the groups reflect a distinct proportion of monomorphic bands and frequency of alleles in the groups: groups 4 and 5 had markedly more monomorphic bands than did other groups.

The total gene diversity (Ht) varied from 0.18 to 0.5, the mean value being 0.33. Gene diversity within the groups (Hs) ranged from 0.10 to 0.49, mean 0.24. The proportion of diversity among the groups (Gst) varied from 0.02 to 0.52, mean 0.28. Gene flow (Nm) varied from 0.46 to 24.48, mean 1.31.

AMOVA analyses revealed 76% of the overall genetic variation within plant groups and 24% among plant groups (Φ_{PT} = 0.23, p = 0.001).

DISCUSSION

Many studies of plants confirm the influence of plants' mating mode on the population genetic structure. Populations of clonal plants are expected to be dominated by one or a few locally adapted genets [24] as distinct from plant population with predominant sexual propagation.

Our study revealed a comparably low DNA polymorphism level (21.9%) in *Paris quadrifolia*. It was substantially lower than in many outcrossing plant species such as *Iris aphylla* (91.5%) [25], *Lilium martagon* (87.3%) [26], *Primula farinosa* (82.3%) [6], *Trillium camschatcense* (90.9%) (allozyme electrophoresis data) [27] as well as in outcrossing long-lived perennial species (65.5%) [28]. The observed DNA polymorphism level was more similar to that of wild autotetraploids such as *Turnera intermedia* (20.1%) [29], *Aster kantoensis* (36.9%) [30], predominantly selfing *Senetio vulgaris* (35.0%) [31] and *Phragmites australis* (49.0%) (allozyme electrophoresis data) [32]. These autotetraploids propagate by rhizome as well

as by the 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 *Paris quadrifolia* to spread clonally. On the other hand, in our polymorphism study we used only one *Paris quadrifolia* population, which could not represent the actual level of *Paris quadrifolia* DNA polymorphism in general.

Thirty-four unique RAPD profiles were observed in the Kairënai *Paris quadrifolia* population. In group 3, all seven plants had distinct RAPD profiles. This group was stretched out for about 60 meters. The relatively big distance among the plants decreased possibility to study genetically identical plants arisen from one rhizome. This factor could increase genetic diversity within this group.

The lowest proportion of distinguishable RAPD profiles was observed in group 1 (0.37). It was more than two times lower than the part of distinguishable RAPD profiles in other groups of *Paris quadrifolia* population (0.85, P = 0.0019, Fisher's exact probability test). Group 1 was located next to a spring, which is a favourite place of the visitors of Kairënai Botanical Garden. At least once a year (in the springtime or / and summer) grassland in this place is mown. It is well known that meadow plants are evolutionary adapted to haymaking [33]. However, *Paris quadrifolia* is an ancient-forest plant [12] and may not be adapted to such drastic environmental changes. Thus, it may be suggested that plants from the mown part of the population often cannot mature and spread the seeds. Interestingly, the portion part of distinguishable RAPD profiles in group 1 (0.27, range: 0.002–1.0) was very similar to the one established for clonal plants with predominantly vegetative propagation [34].

In the unmown part of the population, the relatively high proportion of distinguishable RAPD phenotypes most likely could be explained by repeated recruitment of *Paris quadrifolia* seedlings within population. Several studies of clonal plant species have reported quite a wide variation in part of distinguishable RAPD profiles: from 0.21 for *Poikilacanthus macranthus* [35] to 0.93 for *Viola riviniana* [36]. Such differences could arise due to a different mode of clonal spread and seed distribution. For example, *Viola riviniana* ripens a lot of small seeds which are explosively dispersed by dehiscent capsules and secondarily by ants [36]. It could be expected that the distance among the genets would be bigger if birds or mammalians disperse the seeds [37]. According to Ridley [17], seeds of *Paris quadrifolia* are dispersed by ants and birds, however, a colonization study did not show any evidence of seed dispersal by birds [12]. On the contrary, another study confirmed dispersal of seeds by ingestion [38]. 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.

Estimation of genetic distances indicated that in most cases plants from the different groups were more closely related to each other than plants from the same group (except plants from the mown group 1). It is reflected in the UPGMA dendrogram: plants that were separated by natural barriers did not make their own clusters. These data indicated quite an intensive gene flow among the groups. On the other hand, the UPGMA dendrogram based on the genetic distance among the groups revealed a less genetic distance among close groups not separated by any natural barriers. Natural barriers seem to restrict the spread of *Paris quadrifolia* by rhizome, thus keeping some uniformity and individuality of a group. Karpisonova [11] has suggested that clonality is the main propagation type of *Paris quadrifolia.* Although UPGMA analyses among the groups confirmed the influence of clonality in the spread of *Paris quadrifolia,* our other findings have indicated that seed dispersal is also an important mode of propagation of herb-Paris. Indeed, in our study 76% of genetic variation was found within the plant groups and only 24% among the plant groups. The amount of genetic variation attributable to differences among the groups of *Paris quadrifolia* was similar to that estimated for other outcrossing plant species such as *Digitalis minor* (26.5%) [39], *Primula farinosa* (20.6%) [6], *Aster tripolium* (17.5%) [40], *Digitalis obscura* (15.2%) [41]. Thus, genetic differentiation of the groups of herb Paris plants analysed in our study is low, as could be expected for outcrossing species [39]

The mean Nei's gene diversity among *Paris quadrifolia* groups ranged from 0.16 to 0.305. Nybom and Bartish [7] summarized numerous RAPD studies and for outcrossing plant species reported the mean gene diversity as equal to 0.26. In our study, a few groups revealed slightly lower and a few slightly higher gene diversity. Most likely these groups belonged to one population and were not isolated from gene flow by space as were real populations in other studies.

The estimated gene diversity among populations (Gst) in our study was 0.28. Despite the possible inaccuracies due to the use of Gst for estimation of diversity among groups in one *Paris quadrifolia* population, the calculated Gst value was similar to the one reported for monocotyledons (0.31), perennial (short-lived perennial 0.30; long-lived perennial 0.23), outcrossing (0.23) plant species [7].

The estimated relatively high gene flow (Nm = 1.31) as well as genetic distance analyses data have confirmed our presumption that sexual propagation is significant for the spread of *Paris quadrifolia*.

Thus, sexual propagation may play an important role in forming new populations of *Paris quadrifolia*.

On the other hand, *Paris quadrifolia* plants come into flower only after they reach 10–15 years of age [11]. Therefore, propagation by rhizome is very important for the survival and spread of their populations under stabile environmental conditions as well.

ACKNOWLEDGEMENTS

This work was partially supported by the Norwegian Centre for International Cooperation in Higher Education (grant CCP 03/02: ENLINO Master programnetwork) and the Lithuanian State Science and Studies Foundation (grants No. 34139 and No. 35155 to V. J.)

Received 12 April 2005
Accepted 10 June 2005

References

1. Klimeš L, Klimešová J, Hendriks R, van Groenendael J. In: The Ecology and Evolution of Clonal Plants (eds de Kroon H, van Groenendael J.). Leiden, Backhuys Publishers 1997: 1–29.
2. McLellan A, Prati D, Kaltz O, Schmid B. In: The Ecology and Evolution of Clonal Plants (eds de Kroon H, van Groenendael J.). Leiden, Backhuys Publishers, 1997: 185–210.
3. Marshall C. Vegetatio 1996; 127: 9–16.
4. Chapin FS. Ann Rev Ecol Syst 1980; 11: 233–60.
5. Williams GS. Sex and Evolution. Princeton, Princeton University Press, 1975.
6. Reisch CH, Anke A, Röhl M. Basic Appl Ecol 2005; 6: 35–45.
7. Nybom H, Bartish IV. Perspect Plant Ecol 2000; 3/2: 93–114.
8. Warburton CL, James EA, Fripp YJ et al. Biol Conserv 2000; 96: 45–54.
9. Williams GK, Kubelik AR, Livak J et al. Nucleic Acids Res 1990; 18: 6531–5.
10. Darlington CD, Ann Bot 1941; 18: 203–16.
11. Êàðïåíîâà ÐÀ, Áèîëîãè÷åñêàÿ ôëîðà Ìîñêîâñêîé îáëàñòè. Ìîñêâà, Èçäàòåëüñòâî Ìîñêîâñêîãî óíèâåðñèòåòà, 1974: 34–40.
12. Honnay O, Hermy M, Coppin P. Forest Ecol and Manag 1999; 115: 157–70.
13. Bagdonaitë A, Galinis V, Jankevièienë R et al. Lietuvos TSR flora. Vilnius, Valstybinë politinës ir mokslinës literatûros leidykla, 1963: 548–9.
14. Ñåðåáðÿêîâ ÈÃ. Ìîðôîëîãèÿ âåãåòàòèâíûõ îðãàíîâ âûñøèõ ðàñòåíèé. Ìîñêâà, Coâåòñêàÿ íàóêà, 1952.
15. Daumann E. Preslia 1959; 31(3).
16. Online Encyclopedia: http://encyclopedia.jrank.org/LEO_LOB/LILIACEAE.html
17. Ridley HN. The Dispersal of Plants Throughout the World. Ashford, Reeve and Co, 1930: 744.
18. Þvingila D, Verbylaitë R, Abraitis R et al. Balt Forest 2002; 8: 2–7.
19. Nei M, Li W-H. Proc Natl Acad Sci USA 1979; 76: 5269–73.
20. Van de Peer Y, De Wachter R. Comput Appl Biosci 1994; 10: 569–70.
21. Peakall R, Smouse PE. (2001) GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia, 2001. http://www.anu.edu.au/BoZo/GenAlEx/
22. Nei M. P Natl Acad Sci USA 1973; 70: 3321–3.
23. Yeh FC, Yang RC. POPGENE (Version 1.31): Population Genetic Analysis Software. Alberta, University of Alberta and Tim Boyle Center for International Forestry Research, 1999.
24. Janzen DH. Am Nat 1977; 111: 586–9.
25. Wróblewska A, Brzosko E, Czarnecka B et al. Bot J Linn Soc 2003; 142: 65–72.
26. Persson HA, Lundquist K, Nybom H. Hereditas 1998; 128: 213–20.
27. Tomimatsu H, Ohara M. Biol Conserv 2003; 109: 249–58.
28. Hamrick JL, Godt MJW. London, Philosophical Transactions of the Royal Society of London, 1996: Series B 1291–8.
29. Shore J. Heredity 66; 305–12.
30. Maki M, Masuda M, Inoue K. Am J Bot 1996; 83: 296–303.
31. Müller-Schärer H, Fischer M. Mol Ecol 2001; 10: 17–28.
32. Guo W, Wang R, Zhou S et al. Biochem Syst Ecol 2003; 31: 1093–109.
33. Ðàáîòíîâ TA. Ëóãîâåäåíèå. Mocêâà, 1984: 320.
34. Widén B, Cronberg N, Widén M. Folia Geobot Phytotx 1994; 29: 245–63.
35. Bush SP, Mulcahy DL. Mol Ecol 1999; 8: 865–70.
36. Auge H, Neuffer B, Erlinghagen F et al. Mol Ecol 2001; 10: 1811–9.
37. Grime JP, Hodgson JG, Hunt R. Comparative Plant Ecology. London, Unwin Hyman, 1988.
38. Brunet J, Von Oheimb G. J Ecol 1998; 86: 429–38.
39. Sales E, Nebauer SG, Mus M et al. Am J Bot 2001; 88(10): 1750–9.
40. Krüger AM, Hellwing FH, Oberprieler C. Mol Ecol 2002; 11: 1647–55.
41. Nebauer SGL, Del Castillo Agudo, Seguda J. Theor Appl Genet 1999; 98: 985–94.

Vaida Jogaitë, Jonas R. Naujalis, Juozas R. Lazutka

GENETINË ÁVAIROVË FRAGMENTUOTOJE KETURLAPËS VILKAUOGËS (*PARIS QUADRIFOLIA* L., TRILLIACEAE) POPULIACIJOJE

Santrauka

Keturlapës vilkauogës (*Paris quadrifolia* L.) polimorfizmo tyrimai atlikti panaudojus atsitiktinai amplifikuotos poli-

morfinës DNR (RAPD) metodà. Iðtirta Vilniaus universiteto Kairënø botanikos sodo keturlapës vilkauogës populiacija, nedideliais gamtiniais barjerais padalyta á grupes. Panaudojus aðtuonis atsitiktinius pradmenis nustatyta, kad 21,9% lokusø buvo polimorfiniai, o likæ – monomorfiniai. Unikaliø RAPD fenotipø dalis skirtingose grupëse svyravo nuo 0,37 iki 1 (vidurkis – 0,68). Maþiausia unikaliø RAPD fenotipø dalis nustatyta ðienaujamoje keturlapës vilkauogës populiacijos teritorijoje. Didþiausià molekulinës variacijos dalá (76%) sudaro variacija grupiø viduje, o likusià dalá (24%) – variacija tarp grupiø. Tai rodo, jog dauginimasis sëklomis yra svarbus ðio, kaip manoma, daugiausiai vegetatyviniu bûdu besidauginanèio augalo plitimui. Genetiniø atstumø analizë rodo, jog esama ryðio tarp genetinio atstumo ir grupës izoliacijos lygio – maþiausias genetinis atstumas (0,083) buvo nustatytas tarp greta esanèiø ir gamtiniais atstumais neatskirtø keturlapës vilkauogës grupiø.