

GENETIC STRUCTURE OF ISOLATED *Vaccinium oxycoccus* POPULATIONS IN LITHUANIA

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The genetic population structure of the wild Cranberry *Vaccinium oxycoccus* was studied using RAPDs (random amplified polymorphic DNA). During the last century, intensive peat bogs drainage, regulation of water levels, and intensive cranberry picking has caused a risk for survival of wild cranberry *Vaccinium oxycoccus* populations in Lithuania. Genetic variation among and within isolated *V. oxycoccus* populations was investigated with RAPD profiles. Fifty-six clones were sampled in four populations at the Čepkeliai, Žuvintas, Kamanos Reserves and Aukštaitijos National Park. RAPD analyses of nine primers showed 213 polymorphic loci in the samples. The polymorphism level in the Čepkeliai reserve population was 56.34%, in Žuvintas 49.77%, in Kamanos 46.95% and in Aukštaitija 43.19%. Polymorphism among populations was 100%. For the total sample group, Shannon's Information Index was 0.2 and Nei's gene diversity 0.12. The estimated total proportion of diversity among populations (GST) and gene flow (Nm) were 0.14 and 3.1, respectively. The UPGMA analyses have revealed that populations of *V. oxycoccus* are clearly separated into four lineages and only one Čepkeliai lineage had a homogenous haplotype. Others Žuvintas, Kamanos and Aukštaitijos lineages differed from each other. One clone sampled from Aukštaitija National Park (a8) had a unique haplotype.

Key words: genetic diversity, RAPD, isolated populations, *Vaccinium oxycoccus*.

INTRODUCTION

Loss of natural habitat due to human activities is widely recognised as the most important factor causing species decline world-wide (Sih *et al.*, 2000). During the last century intensive peat bogs drainage, regulation of water levels, intensive cranberry picking has caused survival risk for wild cranberry *Vaccinium oxycoccus* populations in Lithuania. The amount and partitioning of genetic variation among and within populations results from the dynamic processes of gene flow, selection, inbreeding, genetic drift, and mutation (Harlt and Clark, 1994). In isolated populations genetic drift may eventually reduce genetic variation (Lacy, 1987; Ellstrand and Elam 1993; Frankham, 1996), especially because effective population sizes are usually much smaller than the number of reproductive individuals in a population (Frankham, 1995). At the same time genetic variation represents the starting point for further evolution and is an important prerequisite for the prediction of evolutionary responses. This is of practical significance in light of human-caused habitat fragmentation, alteration, or destruction (Fisher *et al.*, 2000). Genetic structure among and within populations

also depends on the life history of a species. Out crossing species tend to have higher levels of variability within populations but smaller degrees of differentiation among populations than selfing species (Brown *et al.*, 1990; Schoen and Brown, 1991).

By definition genetic drift is selectively neutral. Therefore, it is best measured using selectively neutral genetic markers such as RAPD (random amplified polymorphic DNA) (Williams *et al.*, 1990). Once established, RAPD-PCR (polymerase chain reaction) has the advantage of being quick and easy, requiring little plant material, and having a high resolution (Steinger *et al.*, 1996; Gugerli *et al.*, 1999). The aim of our study was to evaluate the genetic variability of *V. oxycoccus* from the Čepkeliai, Žuvintas, Kamanos Reserves and Aukštaitija National Park by the RAPD method.

MATERIALS AND METHODS

The *V. oxycoccus* is a perennial trailing woody vine that is native to bogs and swamps. Cranberry blooms during June and July, bearing ranks of solitary flowers along short up-

right shoots that grow above the mat of vines (Butkus *et al.*, 1987).

Fifty-six clones with clearly differing morphological features (colour, size, shape of berry and productivity) (Daubaras and Česonienė, 2004) were collected from three populations in Lithuania bogs (Čepkeliai, Žuvintas/Kamanos and Aukštatija) during 1995–1999. Populations were geographically isolated: Čepkeliai ($54^{\circ}23' - 54^{\circ}30'N$, $24^{\circ}25' - 24^{\circ}35'E$), Žuvintas ($54^{\circ}23' - 54^{\circ}30'N$, $23^{\circ}25' - 23^{\circ}40'E$), Kamanos ($56^{\circ}15' - 56^{\circ}20'N$, $21^{\circ}35' - 22^{\circ}45'E$) and Aukštatija ($55^{\circ}40' - 55^{\circ}00'N$, $25^{\circ}80' - 26^{\circ}25'E$).

DNA extraction and RAPD analysis were conducted as previously described (Nei and Li, 1997). Nine (OPA-1, OPA-2, OPA-5, OPA-9, OPA-10, OPB-11, ROTH-6, ROTH-8, ROTH-10) (Table 1). 10 nt long primers of random sequence (Fermentas, Lithuania; Roth, Germany) were used. DNA amplification was performed in a thermocycler (Mastercycler, Eppendorf, Germany) under the following conditions: initial denaturation for 4 min at $94^{\circ}C$, 44 cycles of denaturation for 1 min at $94^{\circ}C$, primers annealing for 1 min at $35^{\circ}C$, extension for 2 min at $72^{\circ}C$ followed by a final extension for 5 min at $72^{\circ}C$.

Relationships among *V. oxycoccus* individuals were evaluated using a dendrogram based on Nei and Li's genetic distances (Areškevičiūtė *et al.*, 2006) using the UPGMA (unweighted pair group method) cluster analysis. Calculation of genetic distances and UPGMA cluster analyses were performed with the TREECON programme for Windows V 1.3b (Nei and Li, 1979). Calculation of the observed num-

ber of alleles, Nei's (Peer *et al.*, 1994; Nei and Nalt, 1973) gene diversity (H), Shannon's Information Index (I), total gene diversity (H_t), gene diversity within populations (H_s), gene diversity among populations (G_{st} = (H_t - H_s)/H_t), gene flow (N_m = 0.5 (1 - G_{st})/G_{st}) and generation of a Nei's genetic distance based dendrogram were carried out with POPGENE V 1.31 software (Yeh and Yang, 1999).

RESULTS

In our study, RAPD markers proved to be a powerful method for the detection of spatial genetic variation. Based on the literature (Nei and Li, 1979) we chose nine OPA and ROTH primers. Using nine primers (Table 1) we obtained 213 fragments and could differentiate the 56 *V. oxycoccus* individuals, reflecting a rich allelic diversity among the populations.

The size of the amplified fragments ranged from 80 to 2750 bp, all loci were polymorphic. The number of bands per primer ranged from 15 (RAPD OPB-11) to 31 (RAPD ROTH-180-09). The polymorphism level in the Čepkeliai reserve population was 56.34%, in Žuvintas 49.77%, in Kamanos 46.95% and in Aukštatija 43.19%.

To estimate genetic variation between populations, the Shannon's Information Index (I), Nei's gene diversity (H) and the observed number of alleles per locus (Na), number and percentage of polymorphic loci were calculated (Table 2). For the total sample, Shannon's Information Index was 0.2 and Nei's gene diversity 0.12. The observed number of alleles per locus ranged from 0.16 in the Čepkeliai population to 1.5 in Kamanos and Žuvintas and 1.4 in Aukštatija population. The estimated total proportion of diversity among populations (G_{ST}) and gene flow (N_m) were 0.14 and 3.1, respectively.

Shannon's index estimates of intraspecific genetic diversity within *V. oxycoccus* were slightly higher. The Shannon information index has general applications in ecology and is relatively insensitive to skewing effects caused by the inability to detect heterozygous loci (Dawson *et al.*, 1995).

To estimate the relationship between *V. oxycoccus* 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. It is noteworthy that from the same locality al-

Table 1
USED PRIMERS, THE NUMBER OF BANDS PER PRIMER, AND THE RANGE OF MOLECULAR WEIGHT IN BASE PAIRS (BP) AMPLIFIED BY PCR FOR INDIVIDUALS OF *V. oxycoccus*

Primer	Sequence (5' to 3')	Range of molecular weight (bp)	Number of bands
OPA-01	CAGGCCCTT	125–2200	30
OPA-04	AATCGGGCT	225–2250	21
OPA-05	AGGGGTCTT	90–2000	28
OPA-09	GGGTAACGC	280–2750	25
OPA-10	GTGATCGCA	80–2000	27
OPB-11	GTAGACCCG	290–1900	15
180-6	GCACGCCGGA	80–1235	19
180-8	CGCCCTCAGC	300–1750	17
180-9	GCACGGTGGG	110–2400	31

GENETIC DIVERSITY OF LITHUANIAN POPULATIONS OF *V. oxycoccus*

Population	Shanon's Information Index (I)	Nei's gene diversity (H)	Observed mean number of alleles (Na)	The number of polymorphic loci	The percentage of polymorphic loci % (P)
Čepkeliai	0.2357	0.1486	0.1563	120	56.34
Žuvintas	0.1538	0.0896	1.4977	106	49.77
Kamanos	0.1231	0.0674	1.4695	100	46.95
Aukštatija	0.1914	0.1216	1.4319	92	43.19
Total	0.2181	0.1166	2.0000	213	100.00

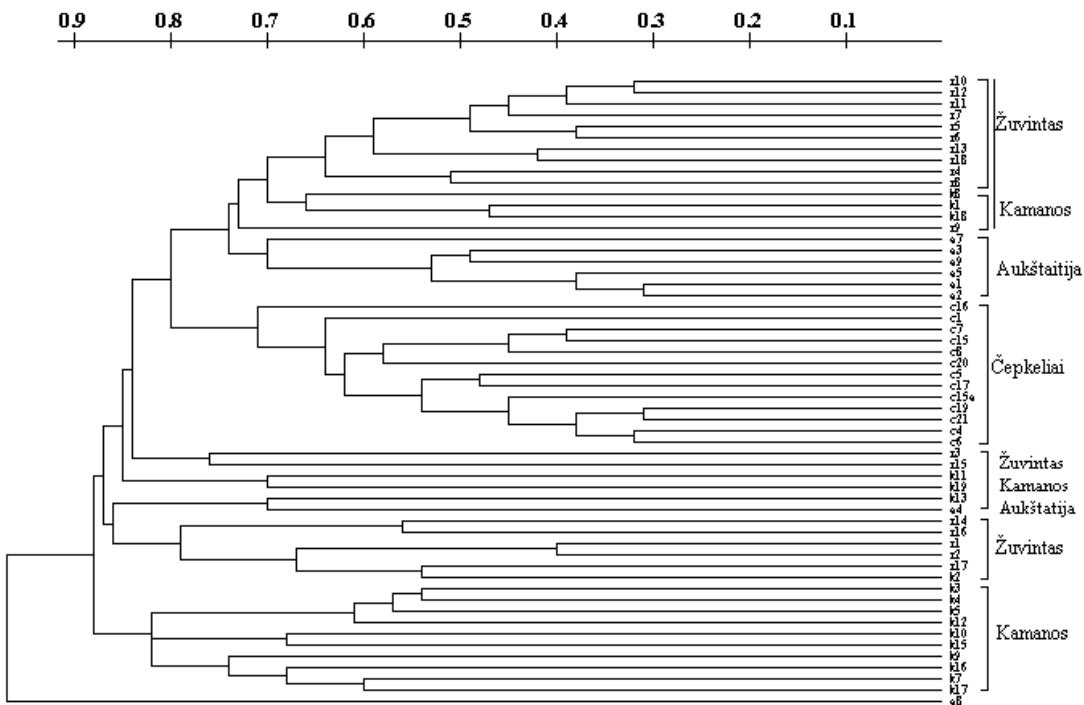


Fig. 1. UPGMA dendrogram based on Nei's genetic distances between individuals of *V. oxycoccus* from Lithuania.

cality always had the same haplotype while elsewhere in the distribution range clones differed (Fig. 1).

DISCUSSION

UPGMA analysis showed that populations of *V. oxycoccus* were clearly separated into four lineages and only one lineage (Čepkeliai) had a homogenous haplotype. The other lineages (Kamanos, Žuvintas and Aukštaitija) differed from each other. One clone sampled from the Aukštaitija National Park (a8) had a unique haplotype. The observed different and mixed lineages confirm the prediction that these two Lithuanian *V. oxycoccus* populations are ancestral to one population before glaciation and that the Čepkeliai population differed from them. One more factor that could have influenced the differences postglacial retreat in the populations of Čepkeliai, Žuvintas, Kamanos and Aukštaitija (Webb and Bartlein, 1992) (Fig. 2). In the Lithuanian glaciation stage of pomerania only a small part of Lithuania was not covered by ice, which includes Čepkeliai Reserve. Thus, the Čepkeliai Bog represents the first bog uncovered by ice. In the next stage of ice retreat the Žuvintas Reserve Bog was uncovered (South Lithuanian ice retreat stage) later Aukštaitija National Park (Midle Lithuania ice retreat stage) and followed by the Kamanos Reserve Bog (Fig. 2). According to Lithuania deglaciation periods, these four isolated populations genetically separated into different lineages (Fig. 1). The differences in the Čepkeliai population distinction could also be due to the river Nemunas. Many years ago this river was very wide restricting gene flow among populations.

The low genetic diversity within but high diversity among clonal plant populations is expected. On the other hand, in about 40% of clonal plant species reproduction by seeds is

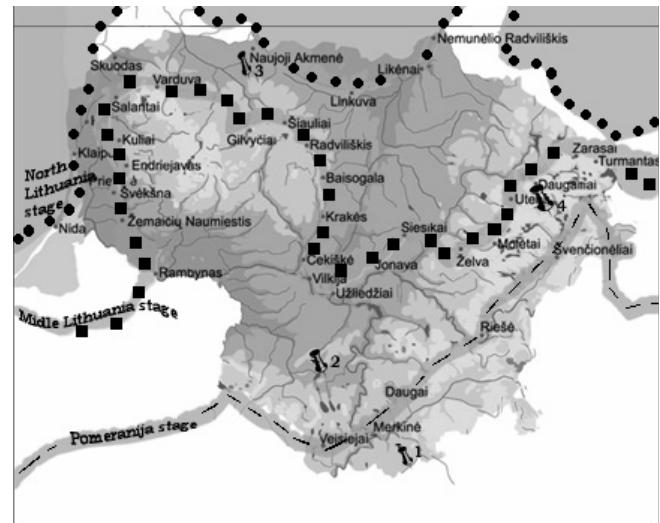


Fig. 2. Locations of sampling of isolated populations of *V. oxycoccus* from Lithuania (1 – Čepkeliai, 2 – Žuvintas, 3 – Kamanos reserves, 4 – Aukštaitija national park). Different lines on Lithuania map show different Lithuania glaciation stages. Line with dots – North Lithuania, line with square – middle Lithuania, line with dashes – Pomerania glaciation stage.

observed (Kreher *et al.*, 2000). Studies of genetic variation in *V. Statimeneum*, *Viola riviniana* and other clonal plant species reported high levels of genetic diversity within populations (Kreher *et al.*, 2000).

Our study showed a comparatively high level of DNA polymorphism in *V. oxycoccus*. RAPD indicates that Lithuanian *V. oxycoccus* appears to maintain a very high level of the total genotypic variance among populations (100%) comparing to American *V. macrocarpon* (61%) (Stewart and Excoffier, 1996). However, low total genotypic variance (41–57%) was found within Lithuanian *V. oxycoccus* popu-

lations (Table 2) comparing to American *V. macrocarpon* (more than 91%). This could be due to postglacial retreat and isolation of populations (Webb and Bartlein, 1992). One more reason that could reduce genetic variability of *V. oxycoccus* is intensive cranberry picking in autumn. Reduction of seeds leads to one-way cranberries reproduction—clonal reproduction and this can cause small genotypic variance within populations.

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LIETUVAS *Vaccinium oxycoccus* IZOLĒTO POPULĀCIJU GENĒTISKĀ STRUKTŪRA

Ar RAPD molekulāriem markieriem tika pētīts ģenētiskais polimorfisms vairākās Lietuvos izolētās savvaļas dzērveņu populācijās. Atrastas ļoti būtiskas ģenētiskās atšķirības starp pētītām populācijām, tiek apsprests šo atšķirību cēlonis.