Phenogenetic variation pattern in adaptive traits of *Betula pendula*, *Alnus glutinosa* and *Quercus robur* in Lithuania

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The main objective of the study was to estimate and compare genetic parameters in the widespread forest tree species *Betula pendula*, *Alnus glutinosa* and *Quercus robur* in Lithuania. The results were based on assessments of half-sib progeny at juvenile age in field trials located in different eco-geographic regions. Bud flushing and height were measured at ages 4 to 9. Population and family variance components for both traits were the largest in pedunculate oak, whereas genotype × environment interaction estimates were large in black alder. Within a trait, genetic correlations were strong for bud flushing in silver birch and pedunculate oak. The estimates for height were medium to strong only in pedunculate oak, while they were very weak to weak for the other two species.

The strong population effects in pedunculate oak indicate that a larger number of populations needs to be selected for gene conservation than in the other two species. The clinal variation in adaptive traits in black alder means that populations for gene conservation have to be selected along the environmental gradient. Based on our results, silver birch populations from Middle Lithuanian Lowland, showing a good performance and a high genetic diversity for adaptive traits in field trials, would suit best for gene conservation.

Key words: *Betula pendula*, *Alnus glutinosa*, *Quercus robur*, genetic diversity, population structure, genotype × environment interaction, genetic correlation, gene conservation

INTRODUCTION

The prime objective in gene conservation is to safeguard the potential of population for species adaptation [1]. Studies of adaptive traits as well as of numerous 'neutral' molecular markers have shown that genetic variation in temperate forest tree species is highest within populations [2, 3]. It is therefore of great importance for gene conservation to select populations possessing a high intrapopulation genetic variation and, thus, a potential to adapt to the environmental changes. In marginal environments, the interpopulation genetic variation may be more important as a result of a possible specific adaptation achieved by natural selection.

Uncertainty about the rate of climate change and about the future use of forest resources calls for dynamic gene conservation [4]. The *in situ* gene conservation method of MPBS (multiple population breeding system), developed by Namkoong (1984), could be successfully applied to commonly occurring noble hardwood species [1]. This system can be used jointly with a long-term tree breeding programme. At present, such programme is in progress in Lithuania. Some associated species may also benefit from gene conservation of keystone species [5].

The potential to identify populations for gene conservation using molecular markers methods has been limited until now [6]. More comparative studies of species with different life history traits are needed to correct gene conservation strategies for various broadleaved tree species by comparing the differences in species genetic structures in adaptive traits. Above all, the criteria for population selection for gene conservation in a species should ensure a sufficient intrapopulation genetic variation in adaptive traits. Pioneer or intermediate hardwood species exhibit a more pronounced inter-population differentiation compared with climax species with which they share other life history traits [7]. Heuertz et al. [8] reported that interpopulation differentiation is higher in *Fraxinus excelsior* than in oak and beech species. A high genetic differentiation was found in Finnish *Acer platanoides* populations [9]. These results are of importance for sampling strategies for genetic resource conservation and are also significant for long-term breeding strategies.

The main objective of this study was to estimate and compare genetic parameters in the widespread forest tree species *Betula pendula*, *Alnus glutinosa* and *Quercus robur* in Lithuania, genotype × environment interaction and genetic correlations within a trait for adaptive and production traits. Implications of the results for gene conservation will be discussed.

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MATERIALS AND METHODS

Three progeny test plantations or field trials for each species were established in the period 1998–2000. Each experiment was established by replanting seedlings from the nursery to the sites in different ecoclimatic regions (Table 1). The age of replanting for silver birch was 1 year, for black alder 1 year, for pedunculate oak 4 years. The design of silver birch and black alder field trials was 10 tree-rows per family, replicated in 5 complete blocks. The design for pedunculate oak was 10–15 single tree-plots per family, randomly distributed in 4 complete blocks. The spacing in all nine trials was approximately 2.0 m between rows and 1.5 m within rows.

Bud flushing and height were recorded at ages 7 and 8 in silver birch trials, 4 and 9 in black alder trials, 6 and 6 in pedunculate oak trials, respectively. Bud flushing recordings for silver birch and black alder were done in classes from 1 to 5 and for pedunculate oak from 0 to 6.

Analysis of variance was done using the MIXED procedure in the SAS Software 8.0 (SAS Institute Inc. SAS/STAT® software.). The restricted maximum likelihood (REML) method was used for computing variance components.

The following mixed linear model was used for joint analyses of variance of the three progeny trials together (as shown in Falconer [11]):

$$y_{ijlmn} = \mu + b(t)_{ij} + t_j + f(p)_{lm} + p_m + f(p)t_{lmj} + pt_{mj} + \varepsilon_{ijlmn},$$

where y_{ijlmn} is the value of a single observation, μ is the grand mean, t_j is the fixed effect of trial j, $b(t)_{ij}$ is the fixed effect of block i within trial j, $f(p)_{lm}$ is the random effect of family l within population m, p_m is the fixed effect of population m, $f(p)t_{lmj}$ is the random effect of interaction between family l and trial j, pt_{mj} is the fixed effect of interaction between population m and trial j, ε_{ijlmn} is a random error term.

The additive genetic coefficient of variation for population was calculated using the formula:

$$CV_A = \frac{\sqrt{4 \cdot \sigma_f^2}}{\overline{X}} \cdot 100,$$

where σ_f^2 is the family variance component and $\overline{X}$ is the phenotypic mean of the trait.

The mean estimate of CV_A was calculated from the population estimates.

Type B genetic correlations between the same traits assessed at two different trials [12] were calculated at an individual observation level using DFREML software [13]. Instead of genetic correlation, Pearson's correlations between breeding values were calculated for height in silver birch and black alder. This is due to the very large differences between the means of trials for height. As a result, very different amplitudes of variation that cause imprecise estimates. One of the black alder trials was lost after repeated severe spring frosts. The breeding values were obtained from a mixed linear model of the SAS procedure MIXED (BLUP) for individual families within each trial.

Bud flushing mean values of populations of all three trials for the species were calculated by standardising the trait values (mean = 0 and standard deviation = 1) of populations at each trial and then calculating the mean value of each population for all the trials. Standardised population mean values of bud flushing were converted into percentage (the maximum value was taken as 100% and the minimum value as 0%) when drawing the pies for illustration on the map of Lithuania.

The mean values of height for populations of all the trials were calculated as the percentage deviation from the mean of a separate trial and calculating the mean value of each population for all the trials.

Table 1. **Characteristic of progeny test plantations of different forest tree species**

Test plantations	Year of establishment	Area, ha	N. Latitude	E. Longitude	Altitude a.s.l., m	Climate type	Effective temperature sum (>+5 °C)	Amount of precipitation during vegetation season, mm	Starting and ending date of vegetation season	Forest site index[a]	Forest soil[c]	Number of populations / families
Betula pendula												
Šiauliai	1999	1.4	55°58′	23°09′	120	transitional	2450	370	15.4–21.10	Nb	Podzols	24/111
Šilutė	1999	1.5	55°13′	21°33′	12	maritime	2600	439	12.4–26.10	Lc	Luvisols	24/107
Dubrava	1999	1.5	54°55′	23°27′	75	transitional	2600	437	12.4–24.10	Ld[b]	Histosols	24/109
Alnus glutinosa												
Šiauliai	1998	1.6	55°58′	23°09′	120	transitional	2450	370	15.4–21.10	Nd	Luvisols	17/85
Šilutė	1998	1.5	55°13′	21°33′	12	maritime	2600	439	12.4–26.10	Ld[b]	Gleysols	17/58
Kaunas	1998	1.2	55°06′	23°55′	70	transitional	2600	437	12.4–24.10	Nc	Luvisols	17/74
Quercus robur												
Šilutė	2000	1.1	55°33′	21°47′	82	maritime	2600	439	12.4–26.10	Nd	Luvisols	12/68
Panevėžys	2000	1.0	55°25′	24°19′	69	transitional	2500	388	13.4–23.10	Ld	Luvisols	8/50
Dubrava	2000	1.1	54°49′	24°03′	72	transitional	2600	437	12.4–24.10	Nc	Luvisols	15/103

[a] Humidity index: N – normal, L – temporary moisture or overmoistured; fertility index (according to Lithuanian soil classification): b – oligotrophic, c – mesoeutrofic, d – eutrofic;

[b] Organic soil;

[c] Forest soil classification based on ISSS-ISRIC-FAO, 1998 [10].

RESULTS

The results from the mixed linear model analysis of variance of joint data from three field trials (sites) of studied species are presented in Table 2. All three species have shown a strongly significant family × site interaction effect in both traits, except bud flushing in pedunculate oak. Surprisingly, family variance components for bud flushing and height in black alder were close to zero. The family component for bud flushing was much larger than for height in silver birch and pedunculate oak. The opposite trend was found for family × site interaction variance components when comparing two traits. The effect of population was significant only for bud flushing in all species. Oak and alder showed also a significant population × site interaction effect for that trait. Site and block effects were significant in all except one case. CV_A estimates for bud flushing mostly exceeded those for height (Table 2).

Genetic correlations within the trait and among the trials were stronger for bud flushing compared to height in all species studied. Very strong trial–trial correlations were obtained for pedunculate oak, even for height.

Bud flushing of silver birch populations is shown in Fig. 1. No trend can be seen across forest ecoclimatic zones. The percentage of the mean height deviation of populations shows that the fastest growing populations originate from Middle Lithuanian Lowland. Populations of black alder show some southeast-northwest clinal trend in bud flushing time. Among populations from the western part of Lithuania, late-flushing ones are prevailing (Fig. 2). The late flushing populations are among those best growing at a juvenile age. Pedunculate oak populations show a very diverse pattern with late and early flushing populations growing in the neighbourhood (Fig. 3). Besides, growth at a juvenile age is not connected to bud flushing.

DISCUSSION

ANOVA results with a significant population effect for bud flushing (Table 2) show that population structuring might exist in all the three species in Lithuania. The limiting factor in this study could be the small number of families that represent populations in the trials, at least in silver birch and black alder. Population effect would be expected to be more pronounced in pioneer species

Table 2. ANOVA results of data from three progeny trials of silver birch, black alder and pedunculate oak: the estimates of variance components and F-criteria, also significance of effects. Level of significance is denoted by *: 0.05 > P > 0.01, **: 0.01 > P > 0.001, ***: P < 0.001

Trait	Variance components, %		F-criteria and significance of fixed effects				CV$_A$, %
	Family	Family × site	Site	Block	Population	Population × site	
Betula pendula							
Bud flushing at age 7	12.6***	6.3***	291.6***	12.5***	1.9**	1.1	18.9
Height at age 8	2.6*	11.4***	897.6***	65.8***	1.5	1.0	10.2
Alnus glutinosa							
Bud flushing at age 4	0.00	8.34***	3.8	35.9***	4.3***	2.3**	16.9
Height at age 9	0.74	15.07***	278.6***	94.0***	1.6	1.0	26.0
Quercus robur							
Bud flushing at age 6	15.8***	1.1	133.9***	4.7***	16.5***	3.8***	38.4
Height at age 6	6.7**	6.0***	54.1***	7.7***	1.0	1.5	20.2

Fig. 1. Silver birch population mean values of in bud flushing and height deviation from the overall mean in three progeny field trials. The larger the sector of a circle the more advanced stage in bud or leaf development. Different grey tones indicate provenance regions
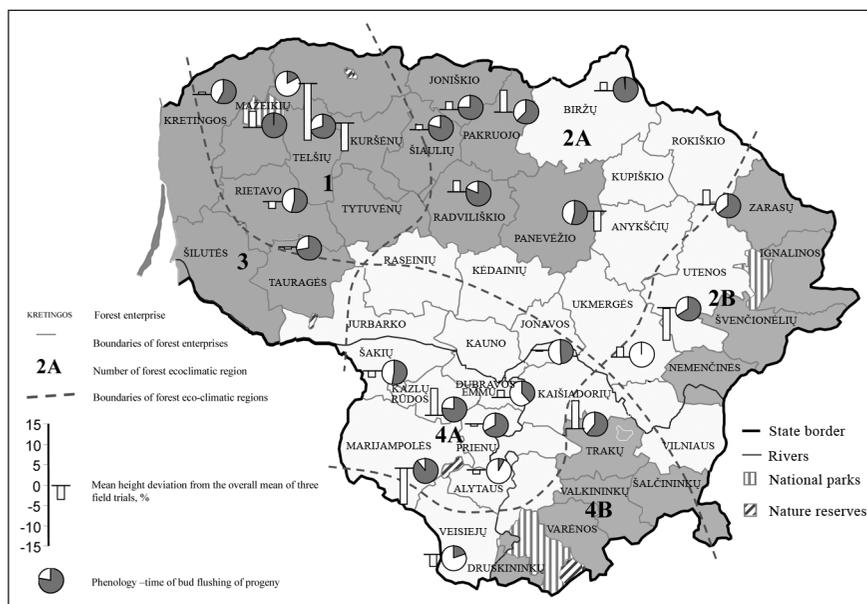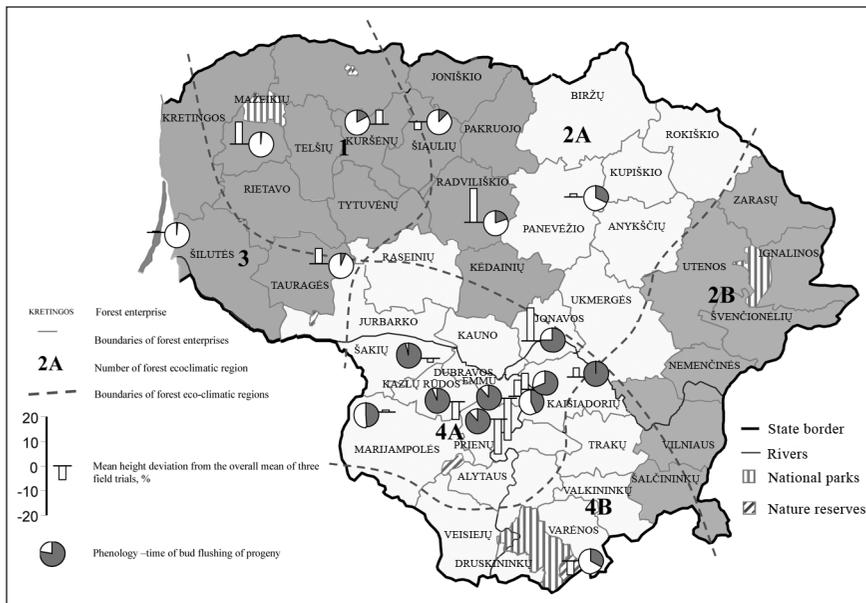
Fig. 2. Black alder population mean values of bud flushing and height deviation from the overall mean in three progeny field trials. The larger the sector of a circle the more advanced stage in bud or leaf development. Different grey tones indicate provenance regions
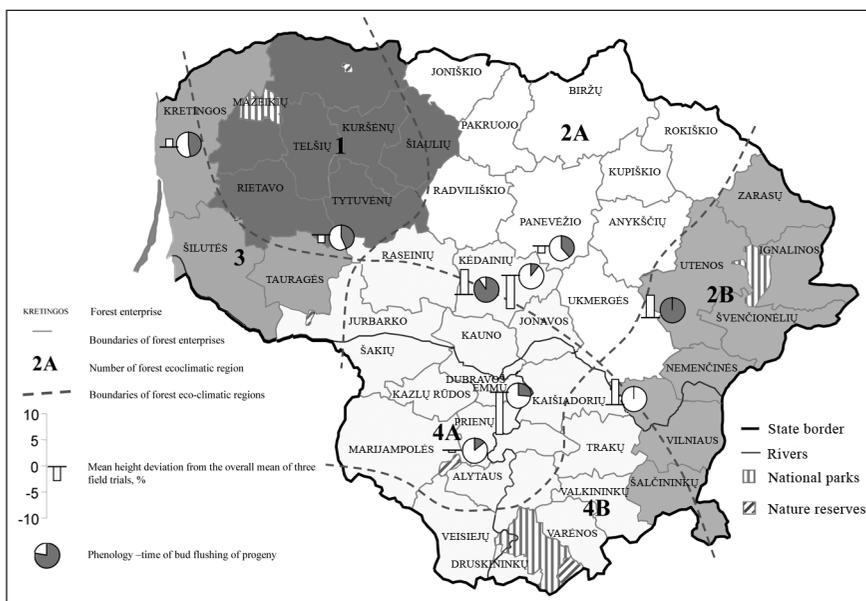
Fig. 3. Pedunculate oak population mean values of bud flushing and height deviation from the overall mean in three progeny field trials. The larger the sector of a circle the more advanced stage in bud or leaf development. Different grey tones indicate provenance regions

compared to climax species, such as pedunculate oak, but in our study this tendency was not evident. The reported estimates of the population variance component for pedunculate oak bud flushing in a nursery (with more populations and families, and repeated measurements in two succeeding years) exceeded twice the family component [14]. The itrapopulation variation for both traits is somewhat higher in oak. Similar results were obtained in our earlier studies [15]. Black alder is very susceptible to spring frost in typical field trials. The family variance component estimate (Table 2) at age 4 was almost zero and was unexpectedly low, because there was a severe spring frost at the time of measurements. The other year records of bud flushing on this species resulted in the family variance component estimate up to 11%, but the effect of population was close to zero (data not shown).

Inter-trial genetic correlations within the traits studied were high for bud flushing, except for black alder, which was due to differences in the leaf spreading process in separate trials, caused by spring frost. The most severe repeated frost injuries happened in the Kaunas and Šilutė black alder trials, which

resulted in resprouting at the ground level and formation of several stems in the majority of plants in the Kaunas trial and a complete dieback in the Šilutė trial (frost damage happened not in the same year as in the Kaunas trial). The frost damage effect was long lasting and can be seen in the inter-trial type B genetic correlation for height (Table 3). Similar results were shown in other studies [16]. The correlations for height in silver birch were also very low. There were two main reasons for that: the Dubrava trial was established on organic soil, and the overall height of plants was more than twice lower in the Šiauliai trial (due to the thin humous layer of soil). For these reasons, bud flushing of families is more stable than height over different sites. This is in agreement with the results of other studies [15, 17]. Kleinschmit and Svolba [18] in their study of *Betula pendula* populations' progenies at juvenile age noted that the genotype × environment interaction increased with decreasing the genetic variability. The rather large family × site interaction variance component in tree height (Table 2) indicates the existence of a genetic variation in phenotypic plasticity and reaction norms of the families. These

Table 3. **Genetic correlations for separate traits among the trials (type B). Traits were recorded at the same age as presented in Table 2**

Species	Trial-trial	Trait	
		Bud flushing	**Height**
Betula pendula	Šilutė – Dubrava	0.76 ± 0.10	0.09 [a]
	Šilutė – Šiauliai	0.68 ± 0.09	0.22* [a]
	Šiauliai – Dubrava	0.73 ± 0.10	0.09 [a]
Alnus glutinosa	Šiauliai – Kaunas	0.14 ± 0.14	0.07 [a]
	Šilutė – Kaunas	0.10 ± 0.11	.
	Šilutė – Šiauliai	0.57 ± 0.07	.
Quercus robur	Panevėžys – Dubrava	0.93 ± 0.04	0.76 ± 0.12
	Šilutė – Dubrava	0.93 ± 0.04	0.37 ± 0.15
	Panevėžys – Šilutė	1.00 ± 0.03	0.52 ± 0.17

[a] Pearson's correlation was calculated among the breeding values of families in different sites (trials).

differences in the environmental behaviour of the families call for the need of environment-specific selection of families in order to increase the genetic gain in tree breeding.

The average CV_A estimates were much larger for bud flushing than for tree height (Table 2). Only a specific year estimates for black alder showed the opposite tendency, but this was a year depended results as the other year estimates were much similar to the ones obtained for silver birch and pedunculate oak (Table 2). Clinal and ecotypic patterns may exist side by side in *Quercus robur* and *Fraxinus excelsior* [19]. Some traits (e. g., growth rhythm) demonstrate a clinal pattern, while others (e. g., productive traits) show an ecotypic one. Bud set in most cases reveals a clearer clinal variation pattern than bud flushing [19]. This was confirmed in numerous studies of *Betula pendula* progenies and well overviewed by Eriksson and Jonsson [20]. Closely located populations of European *Q. robur* were found to be genetically different by an isozyme study [21]. The proportion of intrapopulation to interrepopulation variation is usually higher in wind-pollinated species. Larger CV_A estimates were observed for juvenile growth and phenology traits in *Acer platanoides* than *Betula pendula* [22]. Gene flow in the two species was estimated at 2.3 and 7.6 for *Acer platanoides* and *Betula pendula*, respectively.

Populations of silver birch differ by their sensitivity to stressors, such as a response to ultraviolet-B radiation and ozone exposure [23]. The genetic adaptation of a population depends on the genetic intrapopulation variation. Genetic variation tends to be higher in populations tolerant to different environmental stresses [24]. Stern and Roche [25] described the crucial influence of environmental conditions in formation of separate silver birch populations with predominance in each of individuals differing by adaptive and production traits. Natural selection in frequently disturbed environments acted on directions when selection favoured individuals characterised by early flushing, fast growth at early age (very bushy) and also by early maturation with prolific flowering, while just in the neighbourhood, in an undisturbed environment, the majority were late flushing with tall and straight stems and late maturation. This may also be true of black alder as it is also a pioneer species. For pedunculate oak, the other traits might be of importance when increasing competitive ability in disturbed environments.

The chilling requirement in birch populations is highest in maritime populations and lowest in the most continental ones [26]. This is probably valid for alder as well. This is of importance under global warming when populations from maritime or transitional climates could be advantageous for growing in other localities. Some results indicate that populations of pioneer species from the optimal growing conditions are able to adapt better to a broader spectrum of site conditions than those from the margins [27].

The results of numerous provenance trials prove that some adaptedness to local environmental conditions has been reached by broadleaved tree species. The growth rates of continental provenances of *Betula pendula*, *Quercus petraea* and *Alnus glutinosa* were inferior to British populations in 90% of the cases when grown in Britain [28]. Continental populations of these species also exhibited a lower survival. While provenance trials are useful for testing the adaptedness of local populations, quite often conditions in field trials are not the same as regards the imitations of the conditions in a natural forest environment.

The robustness of the genetic structure of most temperate tree species proves that there is no need to try to preserve individual multilocus genotypes, but it is more reasonable to create good prospects for dynamic processes in gene resource populations [29]. Relatively more populations of pioneer tree species are needed for gene conservation and / or tree breeding compared to climax ones. Similarly, a smaller number of populations have to be selected for gene conservation in species with a large gene flow than in species with a restricted extensive gene flow [30]. Classification of species by their abundance would be useful in practical gene conservation [1].

The strong effects of population in pedunculate oak indicate that a larger number of populations needs to be selected for gene conservation than in the other two species. The clinal variation in adaptive traits in black alder means that populations for gene conservation should be selected along the environmental gradient. According to our results, silver birch populations from the Middle Lithuanian Lowland would be best for gene conservation.

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TRIJŲ LAPUOČIŲ MEDŽIŲ RŪŠIŲ FENOGENETINIS PASISKIRSTYMAS LIETUVOJE

Santrauka

Tyrimais siekta palyginti tarpusavyje plačiai paplitusių Lietuvoje miško medžių rūšių – *Betula pendula*, *Alnus glutinosa* ir *Quercus robur* – genetinius parametrus. Rezultatai gauti įvertinus šių medžių rūšių palikuonis bandomuosiuose želdiniuose. Pumpurų sprogimas ir medelių aukštis buvo vertinamas 4–9 augimo metais. Abiejų požymių populiacijos ir šeimos variacijos komponentės buvo didžiausios paprastojo ąžuolo, tuo tarpu × genotipo aplinkos sąveikos komponentės buvo didesnės juodalksnio. Apskaičiuoti karpotojo beržo ir paprastojo ąžuolo pumpurų sprogimo tarp skirtingų želdinių genetinės koreliacijos koeficientai buvo vidutiniai arba dideli. Vidutiniai aukščio koeficientai nustatyti tik paprastajam ąžuolui, o kitų rūšių jie buvo artimi nuliui.

Patikimas ir stiprus paprastojo ąžuolo populiacijos efektas rodo, kad sąlyginai didesnis nei kitų dviejų rūšių populiacijų skaičius turėtų būti atrinktas genetinių išteklių išsaugojimui. Adaptyvių požymių kintamumas juodalksnio populiacijose patvirtina, kad populiacijos genetiniams ištekliams turėtų būti atrenkamos atsižvelgiant į geografinį veiksnį. Mūsų tyrimo duomenimis, gerai augančios eksperimentiniuose želdiniuose didelės genetinės įvairovės karpotojo beržo populiacijos iš Lietuvos Vidurio žemumos labiausiai tiktų formuojant šios rūšies genofondą.