Distribution of individual heterozygosity in natural populations of Platanus orientalis L. in Bulgaria

Mira Georgieva[iD]

Forest Research Institute – Bulgarian Academy of Sciences, Department "Forest genetics, physiology and plantations", 132, Kliment Ohridski Blvd., 1756 Sofia, BULGARIA
*Corresponding author: mirageorgieva@abv.bg

Abstract. Distribution of individual heterozygosity was studied in 9 natural populations of *Platanus orientalis* L. in Bulgaria. The heterozygosity was assessed by using isoenzyme genetic markers. The study of distribution of individual heterozygosity in populations is an indirect indicator of the way of crossing and how much actual genetic structure of the populations approaches the theoretically expected one. In the present study, the average number of heterozygous loci per individual of all populations was 2.81 (21.58% of all tested loci). The results show that, in general, in the studied populations of *Platanus orientalis* there is a crossing that is close to the panmixia, corresponding to the model of "ideal population". This means that the studied populations produce seed with high heterozygosity and may, if necessary, be used for harvesting of seed materials for forestation purposes and for conservation of valuable gene pool *ex situ*.

Key words: individual heterozygosity distribution, natural population, *Platanus orientalis,* Oriental plane tree, genetic diversity, conservation.

Introduction

In most population genetic studies, heterozygosity is used as an average measure of genetic diversity in populations (in one locus or on average of all loci) (Frankham et al., 2002). In cross-pollinated plants, hetero-zygosity is believed to be an evolutionary advantage, i.e., heterozygous individuals have higher genetic adaptability (so-called "overdominance") (Aidoo et al., 2002) Therefore, in number of cases, besides the average heterozygosity per locus and per population the distribution of individual heterozygosity is also of interest.

In plants, especially coniferous, the growth of heterozygous individuals is often better than that of homozygotes (Mitton & Grant, 1980, 1984; Ledig et al., 1983; Ledig,

1986; Bush et al., 1987), although inter-relationships are complex and may depend on the age of the population and the presence of competition (Ledig et al., 1983; Linhart & Mitton, 1985). In some species, a positive correlation between heterozygosity and fertility is reported (Linhart & Mitton, 1985; Tomekpe & Lumaret, 1991). Based on an isozyme analysis Schaal & Levin (1976) and Wolff & Haeck (1990) demonstrated positive correlations between the level of heterozygo-sity and the size, fertility and longevity of the plants, respectively, in *Liatris cylindracea* Michx. and *Plantago lanceolata* L. For *Gentiana pneumonanthe* L. Oostermeijer et al. (1995) found that in a number of loci the hetero-zygotes show significantly higher genetic adaptability than homozygotes.

For heterozygous individuals, there is a tendency to have a lower dispersion of morphological traits (Mitton, 1978). A positive correlation between level of individual heterozygosity with growth and growth variation in American aspen (*Populus tremuloides* Michx) has been established (Mitton & Grant, 1980), as long as it has not been established in *Pinus ponderosa* Dougl. ex Laws. and *Pinus contorta* Dougl. (Knowles & Mitton, 1980; Mitton & Grant, 1980). Positive dependence between heterozygosity and biomass production was found in *Salix eriocephala* Michx. (Aravano-poulos & Zsuffa, 1998), but such was not found in *Salix exigua* Nutt. (Aravanopoulos, 2000).

In many cases, including this study the heterozygosity was assessed using isozymes genetic markers. Although isozymes represent a relatively small and apparently non-random sample of the genome (Bergmann, 1991), they are indicative of the average heterozygosity in the populations. The isoenzyme genetic markers were used successfully to assess the individual heterozygosity of Norway spruce (Lundkvist, 1979; Gömöry, 1992), in Bulgaria were applied to the Scots (Doncheva et al., 2003) and black pines (Zhelev et al., 2010).

Lundkvist (1979) uses a theoretical model of binomial distribution of individual heterozygosity and establishes an increase in the number of heterozygous loci per individual with increased altitude and bimodal distri-bution of individual heterozygosity (i.e., two peaks distribution). Gömöry (1988) developed a model for the theoretical distribution of individual heterozygosity, emphasizing that binomial distribution can only be expected if heterozygosity is the same in all loci, which in the majority of populations is not met. Applying the developed model Gömöry (1992) establishes statistically significant deviations from the theoretical distribution for four populations of Norway spruce in Slovakia and bimodal distribution in some of the popu-lations. A similar bimodal distribution was also found by Doncheva et al. (2003) for Scots pine populations in Bulgaria, but statistically significant deviations between the expected and observed distribution of heterozygosity were not found in the 15 studied populations. Zhelev et al. (2010) found that there is no credible deviation of the observed distribution from theoretically anticipated distribution in the natural population and seed orchard of *Pinus nigra* Arn. Such deviation was established only in seed orchard offspring.

The aim of the present study is to track and analyze the distribution of individual hetero-zygosity in some natural populations of *Platanus orientalis* L. in Bulgaria.

Materials and methods

The subject of investigation were nine natural populations of *Platanus orientalis* in Bulgaria (Table 1). Two of them represent the northernmost limit of distribution of the species in our country – Asenovgrad and Topolovo towns. Ivaylovgrad is the most isolated population and that of Mt. Slavyanka is located at the highest altitude.

Establishing the degree of genetic diversity is based on biochemical genetic markers. In present study isoenzyme analyzes were used. Enzymes were extracted from dormant winter buds of Oriental plane tree branches collected from 29 to 70 randomly chosen individuals per population. A starch gel electrophoresis was used to separate the isoenzymes. Electropho-resis was established in 12% starch gel in two buffer systems – Ashton (Ashton & Braden, 1961) and Tris-Citrate (Shaw & Prasad, 1970). Nine enzyme systems coding 13 gene loci were analyzed (Table 2). For the histochemical staining of the enzyme fractions, standard recipes indicated in the literature by Cheliak & Pitel (1984), Conkle et al. (1982), and Wendel & Weeden (1989) were used.

Diploid genotypes were reported directly from the electrophoregrams. The following indi-cators of polymorphism in the populations were determined: allelic frequencies, average number of alleles per locus, percentage of polymorphic loci, expected and observed heterozygosity and inbreeding coefficient. For heterozygosity, the approach of Nei (1978) was applied with correc-tion for the number of individuals. The above parameters were calculated using the program product BIOSYS-1 (Swofford & Selander, 1989), except for the effective number of alleles and the inbreeding coefficient, which were calculated using the program product MSO Excel based on the results obtained from the program BIOSYS-1.

Table 1. Natural populations of *Platanus orientalis* L.

Population	Geographical coordinates	Altitude (m)
Asenovgrad(A)	41º 58' N 24º 52' E	150
Topolovo (T)	41º 54' N 25º 00' E	150
Goce Delchev (GD)	41º 37' N 23º 52' E	300
Petrich (P)	41º 24' N 23º 03' E	400
Melnik (M)	41º 30' N 23º 24' E	250
Sandanski (S)	41º 36' N 23º 20' E	200
Slavyanka (SL)	41º 26' N 23º 33' E	500
Kresna (KR)	41º 44′ N 23º 08′ E	250
Ivaylovgrad (IV)	41º 35' N 26º 06' E	300

Table 2. Analyzed enzyme systems.

Enzyme system (E.C.* code)	Number of loci	Buffer system**
Alcohol dehydrogenase (*ADH*, 1.1.1.1)	2	TC
Glutamate dehydrogenase (*GDH*, 1.4.1.2)	1	A
Glutamate-oxaloacetate transaminase (*GOT*, 2.6.1.1)	2	A
Leucine aminopeptidase (*LAP*, 3.4.11.1)	2	A
Malate dehydrogenase (*MDH*, 1.1.1.37)	1	TC
Menadione reductase (*MNR*, 1.6.99.2)	1	A
Phosphoglucomutase (*PGM*, 5.4.2.2)	1	TC
Phosphoglucose isomerase (*PGI*, 5.3.1.9)	2	A
Shikimate dehydrogenase (*SkDH*,1.1.1.25)	1	TC

* E.C. – Enzyme Commission

** A – Buffer system Ashton (pH 8.1); TC – Buffer system Tris Citrate (pH 7.0)

The heterozygosity is the most commonly used measure of level of genetic diversity in populations (Berg & Hamrick, 1997). Directly established heterozygosity (the frequency of heterozygous genotypes) is called observed heterozygosity (H_o). The theoretical heterozygosity, i.e., that which is expected at the available allelic frequency if the population is in genetic equilibrium, is denoted by H_e and is called expected heterozygosity. For each locus, it is calculated according to the Hardy-Weinberg Law:

$$HWL = p^2 + 2pq + q^2 = 1, \qquad (1)$$

where the heterozygosity is given by $2pq$. The rest of the expression ($p^2 + q^2$) is the homozygosity.

$$H_e = 1 - \sum_{i=1}^{n} p_i^2 , \qquad (2)$$

where p_i – is the frequency of the ith (consecutive) allele in this locus;

n – the number of alleles.

Heterozygosity was calculated as the arithmetic mean of all loci in general for population. Comparing the expected with observed heterozygosity has an expression in the inbreeding coefficient (F), which is defined as:

$$F = \frac{H_e - H_o}{H_e} = 1 - \frac{H_o}{H_e} \qquad (3)$$

where H_e is given by Eqn (2) , and H_o is the observed proportion of heterozygotes.

In order to calculate the expected distribution of individual heterozygosity in populations, the program product INDIV (Gömöry, 1988) was used. The observed distribution was found after counting the number of heterozygous individuals in a corresponding number of loci for each population. The correspondence between the expected and observed distribution was tested by χ^2-test. Since, according to the theory of this test, the number of individuals in expected class should not be less than 5, in cases where this was established, the final classes were merged until an expected class ≤ 5 individuals was obtained.

Results and Discussion

Study of distribution of individual hetero-zygosity in populations is an indirect indication of the way of crossing and how much the actual genetic structure of the populations is approaching the theoretically expected according to the established traditionally used Hardy-Weinberg model (Hosking et al., 2004).

In present study, the average number of heterozygous loci per individual (Table 3) of all populations was 2.81 (21.58% of all tested loci).

This value is highest for population of Goce Delchev – 3.3 (25.4%), and lowest for population of Asenovgrad – 2.44 (18.8%). In four cases (Kresna, Melnik, Asenovgrad and Ivaylovgrad) most individuals are heterozygous by two loci, in three cases (Sandanski, Slavyanka and Topolovo) most individuals are heterozygous by three loci, in one case (Petrich) – in four loci and in one case (Goce Delchev) the individuals, heterozygous at three and four loci, are with equal number (Fig. 1).

Table 3. Indicators of individual heterozygosity in the studied populations.

Populations	Average number of heterozygous loci per individual	% heterozygous loci per individual
Kresna	2.56	19.69
Sandanski	2.67	20.54
Slavyanka	3.02	23.23
Petrich	2.98	22.92
Melnik	2.67	20.54
Goce Delchev	3.3	25.38
Asenovgrad	2.44	18.77
Topolovo	2.67	20.54
Ivaylovgrad	2.94	22.62
Average	**2.81**	**21.58**

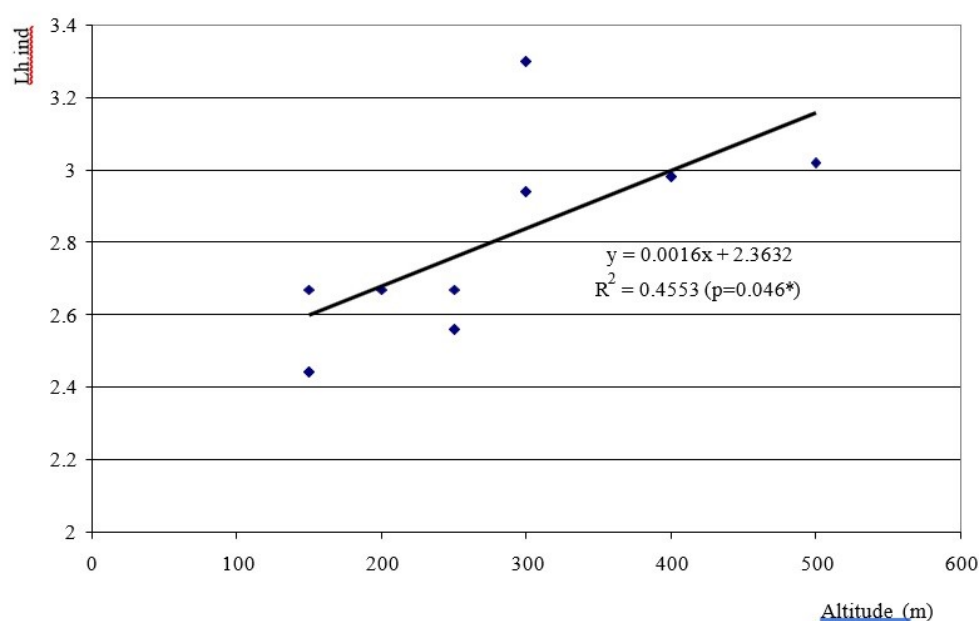$y = 0.0016x + 2.3632$
$R^2 = 0.4553 (p=0.046*)$

Fig. 1. Dependence between the average number of heterozygous loci per individual (Lh.ind^{-1}) and altitude.

The results for distribution of individual heterozygosity (Fig. 2) show that this distribution is different for different populations. In three out of nine cases (Kresna, Slavyanka and Asenovgrad) a statistically significant deviation of the actual distribution from theoretically expected one was found. In the remaining six cases, no significant deviation was observed, which means that the genetic structure of these populations and the nature of pollination in them are close to the theoretical characteristic of a panmict population with free pollination. A trend is observed in the discrepancies – observed values are more than expected at lower values (2 heterozygous loci per individual), while at higher values, theoretically expected are higher than actually found (Fig. 2).
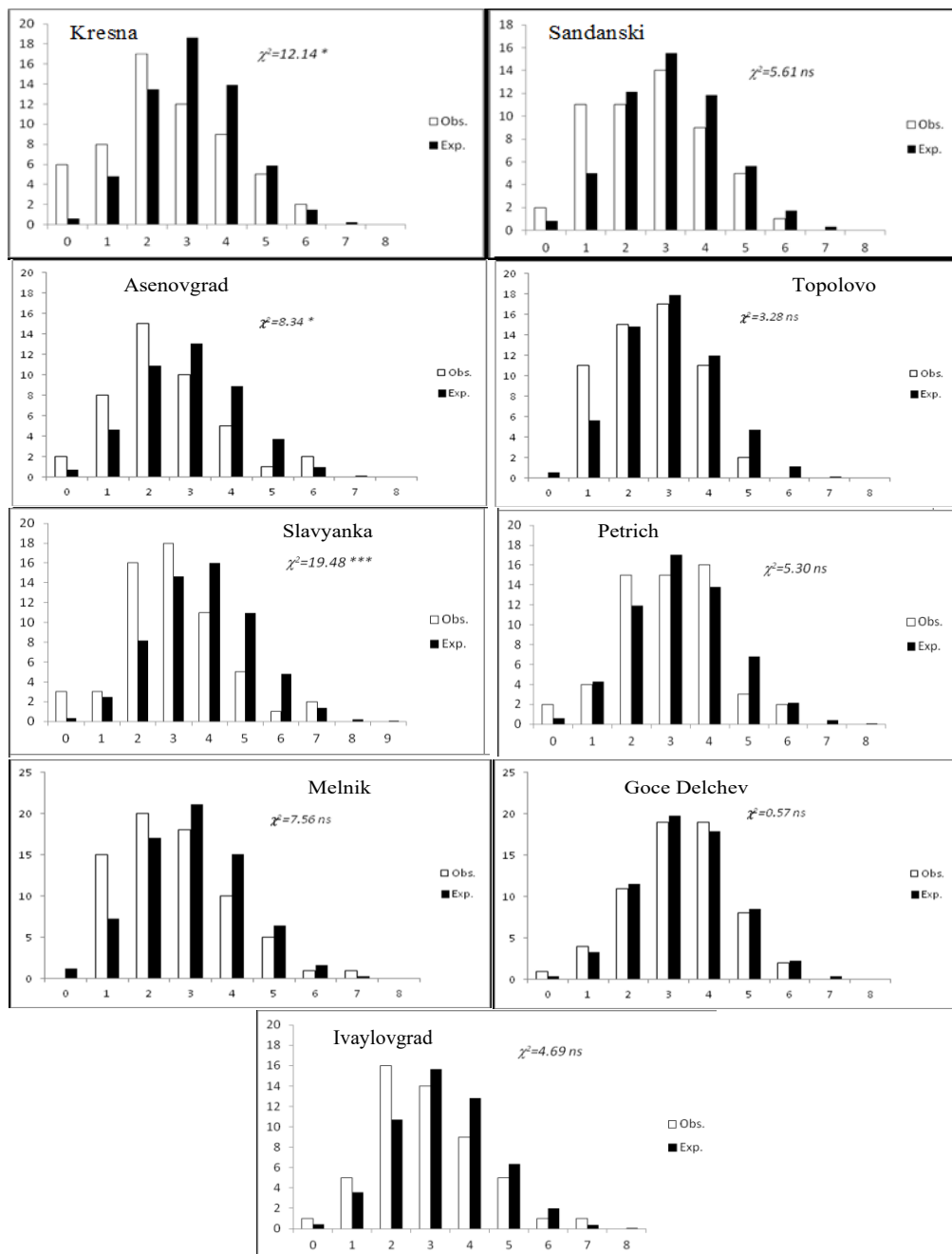
Fig. 2. Distribution of individual hetezygosity in the studied populations.
Legend: on the abscissa – number of loci; on the ordinate – number of individuals heterozygous for the corresponding number of loci. Obs. – observed; Exp. – theoretically expected.
* – statistically significant deviation at p≤0.05; *** – at p≤0.001, ns – statistically insignificant deviation; χ^2 test.

In natural population and orchard of *Pinus nigra* Arn. with adult individuals, Zhelev et al. (2010) found that there was no reliable deviation from the actual theoretically expected distribution, whereas a slight deviation (p = 0.043) was found in the offspring in orchard in Sliven. Statistically significant deviations from the theoretical distribution for four Norway spruce populations in Slovakia and other statistically insignificant deviations in other populations was reported by Gömöry (1992). These results show that deviations from the theoretical distribution of individual heterozygosity have been found in other tree species, with populations characterized by a far more homogeneous spatial structure than those of the Oriental plane. The comparison with the conducted studies shows that the populations of *Platanus orientalis* do not differ in principle from those of the mentioned coniferous species. Although there are significant differences in the peculiarities of the populations – the Oriental plane tree is predominantly linear along the water streams in contrast to coniferous species with numerous populations of large areas, the general characteristic – cross pollinating species with anemophilic pollination appears to be more important as a factor, affecting the distribution of heterozygosity. In the case of the mentioned species as well as Oriental plane tree, in the majority of cases there is no deviation from theoretically expected distribution.

The reasons for deviations from theoretical distribution may be due to different factors – not accidentally and selectively, including inbreeding and self-pollination (at least on separate spots), inhomogeneous spatial structure of populations, pre-zygotic and post-zygotic selection, as well as some other unpredictable factors. Which of these factors have a significant impact is a matter that can be answered after further research.

Conclusions

The results show that, in general, in the studied populations of *Platanus orientalis* there is a crossing that is close to the panmixia, corresponding to the model of the "ideal population". This means that the studied populations produce seeds with high heterozygosity and may, if necessary, be used for harvesting of seeds materials for forestation purposes and for conservation of valuable gene pool *ex situ*. The consistency between the theoretical model and the actual distribution is also an indirect indicator of inheritance of the isoenzyme variants under Mendelian laws.

References

Aidoo, M., Terlouw, D.J., Kolczak, M.S., McElroy, P.D., ter Kuile, F.O, Kariuki, S., Nahlen B.L., Lal, A.A. & Udhayakumar, V. (2002). Protective effects of the sickle cell gene against malaria morbidity and mortality. Lancet 359 (9314), 1311–1312. doi: 10.1016/S0140-6736(02)08273-9.

Aravanopoulos, F.A. (2000). Absence of association between heterozygosity and biomass production in *Salix exigua* Nutt. Theoretical and Applied Genetics, 100(8), 1203–1208. doi: 10.1007/s001220051425.

Aravanopoulos, F.A. & Zsuffa, L. (1998). Heterozygosity and biomass production in *Salix eriocephala*. Heredity, 81, 396–403. doi: 10.1046/j.1365-2540.1998.00409.x.

Ashton, G.C. & Braden, A.W.H. (1961). Serum?-globulin polymorphism in mice. Australian Journal of Biological Sciences 14, 248–253. doi: 10.1071/BI9610248.

Bergmann, F. (1991). Isozyme gene markers. In: Müller-Starck, G., Ziehe, M. (eds.) Genetic Variation in European Populations of Forest Trees. Frankfurt am Main. J.D. Sauerländer's Verlag, 67-78, pp. 67–78.

Berg, E.E. and Hamrick, J.L. (1997). Quantification of genetic diversity at allozyme loci. Canadian Journal of Forest Research, 27, 415-424. doi: 10.1139/x96-195.

Bush, R.M., Smouse, P.E. & Ledig, F.T. (1987). The fitness consequences of multiple-locus heterozygosity: the relationship between heterozygosity and growth rate in pitch pine (*Pinus rigida* Mill.). Evolution, 41, 787–798. doi: 10.1111/j.1558-5646.1987.tb05853.x.

Cheliak, W.M. & Pitel, J.A. (1984). Techniques d'électrophorèse sur gel d'amidon des enzymes d'essences d'arbres forestiers. Service canadien des forêts, Institut forestier national de Petawawa, Chalk River (Ontario). Rapport d'information PI-X-42F. 47 p. ISBN 978-0-662-92784-6.

Conkle, M.T., Hodgskiss, P.D., Nunnally, L.B. & Hunter, S.C. (1982). Starch gel electrophoresis of conifer seeds: Laboratory manual. Tech. Pap. PSW-64, 1–18. doi: 10.2737/PSW-GTR-64.

Doncheva, N., Gagov, V. & Zhelev, P. (2003). Individual heterozygosity distribution in natural Scots pine (*Pinus sylvestris* L.) populations. Genetics and Breeding, 32 (1–2), 61–67. Retrieved from: https://ltu.bg/images/files/file/Zhelev_pubs/Doncheva_et_al_2003.pdf.

Frankham, R., Briscoe, D.A. & Ballou, J.D. (2002). Introduction to Conservation Genetics. New York, NY: Cambridge University Press. doi: 10.1017/CBO9780511808999.

Gömöry, D. (1988). Individuálna heterozygotnosť a jej rozdelenie ako charakteristika populačnej štruktúry. In: Kormuťák A. (ed.). Genofond lesných drevín – jeho zachovanie a využitie, pp. 143–152. SAV, Nitra.

Gömöry, D. (1992) Isoenzyme polymorphism of Norway spruce (*Picea abies* Karst.) in Slovakia. II. Population disequilibrium and genetic diversity in mature Norway spruce stands. Acta Facultatis Forestalis, Zvolen, 34, 87–93.

Hosking, L., Lumsden, S., Lewis, K., Yeo, A., McCarthy, L., Bansal, A., Riley, J., Purvis, I. & Xu, C. (2004). Detection of genotyping errors by Hardy–Weinberg equilibrium testing. European Journal of Human Genetics 12, 395–399. doi: 10.1038/sj.ejhg.5201164.

Knowles, P. & Mitton, J.B. (1980). Genetic heterozygosity and radial growth variability in *Pinus contorta*. Silvae Genetica, 29, 114–117. Retrieved from: https://www.thuenen.de/media/institute/fg/PDF/Silvae_Genetica/1980/Vol._29_Heft_3-4/29_3-4_114.pdf.

Ledig, F.T. (1986). Heterozygosity, heterosis, and fitness in outbreeding plants. In: Soulé, M. E. (ed.), Conservation Biology: The Science of Scarcity and Diversity. Sinauer Associates, Sunderland, MA, ISBN 08-789-37943, pp. 77–104.

Ledig, F.T., Guries, R.P. & Bonefield, B.A. (1983). The relation of growth to heterozygosity in pitch pine. Evolution, 37, 1227–1238. doi: 10.1111/j.1558-5646.1983.tb00237.x.

Linhart, Y.B. & Mitton, J.B. (1985). Relationships among reproduction, growth rate and protein heterozygosity in ponderosa pine. American Journal of Botany, 72, 181–184. doi: 10.2307/2443545.

Loveless, M.D. & Hamrick, J.L. (1984). Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics, 15, 65–95. doi: 10.1146/annurev.es.15.110184.000433.

Lundkvist, K. (1979). Allozyme frequency distributions in four Swedish populations of Norway spruce (*Picea abies* K.). Hereditas, 90, 127–143. doi: 10.1111/j.1601-5223.1979.tb01300.x.

Mitton, J.B. (1978). Relationship between heterozygosity for enzyme loci and variation of morphological characters in natural populations. Nature 273, 661–662. doi: 10.1038/273661a0.

Mitton, J.B. & Grant, M.C. (1980). Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. American Journal of Botany, 67, 1040-1045. Retrieved from: https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=5533&context=aspen_bib.

Mitton, J.B. & Grant, M.C. (1984). Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annual Review of Ecology and Systematics, 15, 479–499. doi: 10.1146/annurev.es.15.110184.002403.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89:583-590, doi: 10.1093/genetics/89.3.583.

Oostermeijer, J.G.B., van Eijck, M.W., van Leeuwen, N.C. & den Nijs, J.C.M. (1995). Analysis of the relationship between allozyme heterozygosity and fitness in the rare *Gentiana pneumo-nanthe* L. Journal of Evolutionary Biology, 8, 739–759. doi: 10.1046/j.1420-9101.1995.8060739.x.

Schaal, B.A. & Levin, D.A. (1976). The demographic genetics of *Liatris cylindracea*. American

Naturalist, 110, 191–206. doi: 10.1086/283059.

Shaw, C.R. & Prasad, R. (1970). Starch gel electrophoresis of enzymes – a compilation of recipes. Biochemical Genetics, 4, 297–320. doi: 10.1007/BF00485780.

Swofford, D L, and Selander, R B. 1989. BIOSYS-1 A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Nat. Hist. Survey, Champaign, Illinois.

Tomekpe, K. & Lumaret, R. (1991). Association between quantitative traits and allozyme heterozygosity in a tetrasomic species: *Dactylis glomerata*. Evolution, 45, 359–370. doi: 10.2307/2409670.

Wendel, J.F. & Weeden, N.F. (1989). Visualization and interpretation of plant isozymes. In: Soltis D. & Soltis P. (eds.) Isozymes in Plant Biology. Dioscorides Press, Portland, OR: 5-45. doi: 10.1007/978-94-009-1840-5_2.

Wolf, K. & Haeck, J. (1990). Genetic analysis of ecologically relevant morphological variation in *Plantago lanceolata* L. VI. The relation between allozyme heterozygosity and some fitness components. Journal of Evolutionary Biology, 3, 243–255. doi: 10.1046/j.1420-9101.1990.3030243.x.

Zhelev, P., Hadzhiyski, V. & Gagov, V. (2010). The reproductive process in a natural stand and in a seed orchard of *Pinus nigra* Arn. Genetics and Breeding, 39(1), 93–101.