# GENETIC VARIATION WITHIN AND AMONG NATURALLY REGENERATING POPULATIONS OF ALDER (*ALNUS GLUTINOSA*)

LEON MEJNARTOWICZ

Polish Academy of Sciences, Institute of Dendrology
Parkowa 5, 62-035 Kórnik, Poland
e-mail: lmejnart@man.poznan.pl

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ABSTRACT

To assess the inter- and intrapopulation genetic variation in the filial generation ($F_1$) of alder (*Alnus glutinosa* (L.) Gaertn.), 11 naturally regenerated populations were analysed. Their parental populations (P), represent the whole Polish territory and belong to three phytosociological associations with alder: typical alder swamp forest Carici elongatae-Alnetum (Ce-A); alder riparian forest Circaeo-Alnetum (C-A); and ash-elm riparian forest Fraxino-Ulmetum (F-U).

$F_1$ populations are grown in a common-garden experiment (provenance trial). Genotyping of individual trees has been carried out by analysis in a bud tissue allele frequency in the 21 isozyme putative loci of 10 enzymes. Differences between populations in respect to the level of genetic diversity were not high. Genetic diversity measured as the number of effective alleles per locus was the highest (Ne = 1.65) in population Wińsko originating from F-U (where also the inbreeding coefficient was the highest, F = 0.429), and the lowest (Ne = 1.48) in population Sławki from Ce-A. In all investigated populations, observed heterozygosity (Ho = 20%) was lower than expected from H-W equilibrium (He = 29%). The highest genetic variation expressed as percentage of polymorphic loci (77.3%) was observed in the offspring populations from Ce-A, and the smallest (69.9%) in the populations originating from F-U. It seems that the low genetic differentiation between populations is probably connected with long-distance seed dispersal via river systems. Alder seed can be transported over long distances thanks to periodical flooding. There is some gene flow between alder populations, with about 2.5 immigrants successfully entering a population per generation (Nm = 2.55). The level of population subdivision within *A. glutinosa* was low ($F_{ST}$ = 0.089). There was no significant genetic differentiation between populations from different phytosociological associations. Mantel test exhibited no significant correlation (r = 0.077) between genetic and geographic distance. In the dendrogram constructed according to Nei (1972) on the basis of interpopulation genetic distances, many small groups can be observed.

KEY WORDS: *Alnus glutinosa*, climax associations, genetic diversity, genetic distance, gene flow, heterozygosity, rare allele, isozymes.

## INTRODUCTION

Alder, named also European black alder (*Alnus glutinosa* (L.) Gaertn.), beside grey alder (*Alnus incana* (L.) Moench.), are ecologically very important European tree species. Alder is wind-pollinated and self-incompatible (McVean 1953). Owing to symbiotic nitrogen-fixing actinobacteria (*Frankia alni*), both alder species are the only native European forest trees having the ability to fix atmospheric nitrogen and simultaneously act as water and air filters (Peters and Peitzmeier 1989). When growing along river and streams, they protect the river banks from excessive erosion. As a pioneer tree species, alder participates in the first forest stage of plant succession on wet, riparian sites, and can be introduced on dryer, impoverished agrarian and degraded soils (Mejnartowicz 2001). Alder is also a forest-forming species, having an ability to compose climax communities on many soil types in Europe.

On the basis of chloroplast DNA (cpDNA) diversity analyses in alder populations, King and Ferris (1998) tried to describe the postglacial history of this species. These authors revealed a high degree of structuring of some cpDNA haplotypes on a European scale, which indicated that most of northern and central Europe (therefore also the Polish territory), was colonized by alders originating from a refuge in the Carpathian Mountains. This is a well-known fact also for other tree species (Breitenbach-Dorfer et al. 1997; Konnert and Bergmann 1995; Petit et al. 2002)

In European countries, in contrast to many other forest tree species, alder forests are composed of predominantly

native populations. There are significant differences between alder stands in quantitative and qualitative morphological leaf characters and tree growth dynamics (Mejnartowicz 1972, 1980, 1981, 1999). The genetic differentiation between investigated alder populations is also quite high (Prat et al. 1992). Due to droughts and other climatic changes in the last decade, ground water and surface water levels declined, and this particularly disturbed the ecological relations in the highly structured alder forests (Alnetum). It could be the reason of epidemics of *Phytophtora sp*. on alders (Cech 1998; Hartmann G. 1995). Therefore, to protect such endangered forests, there is an urgent need to assess the genetic composition of alder populations.

In the early Holocene, around 8000 years ago, the Polish territory was covered with a cool temperate forest and was isolated by the surrounding belt of south taiga forest. As the climate warmed up, south taiga forest withdrew to the north of Europe and the cool temperate forest covered most of Central and West Europe (Adams and Faure 1997). At the end of the early-Holocene isolation, many species started to migrate eastwards and northwards, thus increasing the gene flow from the western European populations of alder and enriching the genetic resources of this species in the area of Poland. This probably applied also to other tree species and can be the reason of the high plasticity of Polish populations of forest tree species observed in international experiments on spruce, pine and fir (Chałupka et al. 2008, Giertych 1978; Gunia and Ilmurzyński 1978; Krutzsch 1992).

The main goal of this study was to answer the following questions:

1) What is the intra- and interpopulation variation of alder populations, studied with isozyme markers?

2) Is there any difference in the genetic structure of populations originating from various natural plant associations when grown in a common-garden experiment?

3) How big is gene flow between alder populations?

4) Is there any connection between the geographic arrangement of populations and their genetic similarity?

## MATERIALS AND METHODS

### *Plant materials*

Live dormant buds were collected from trees grown in a provenance trial established in 1968, as a kind of common-garden experiment. Parental populations (P) for the trial were selected in such a way that they represented the entire natural range of this species in Poland and thereby located in the centre of the whole European black alder range. Seeds (achenes) from eleven P populations aged 80--100 years were collected and the filial generation ($F_1$) has been grown since 1968 in the provenance trial in an Experimental Forest near Kórnik, Poland (Mejnartowicz 1980). The sites of seed material collection for the trial are presented on a map (Fig. 1). Geographic data of the P populations and their geobotanical classification are given in Table 1. The P populations belong to three main phytosociological associations with *Alnus glutinosa* of the phytosociological suballiance Alnenion glutinoso-incanae Oberd., (Mejnartowicz 1972; Wojterski 1981), which represent the climax stage of succession. Four of the studied P populations belong to typical alder swamp forest Carici elongatae-Alnetum (Ce-A), four to alder riparian forest Circaeo-Alnetum (C-A) and three to ash-elm riparian forest Fraxino-Ulmetum (F-U) (Table 1). In Ce-A and C-A, alder is the dominant species, while in F-U it is only an admixed tree species.

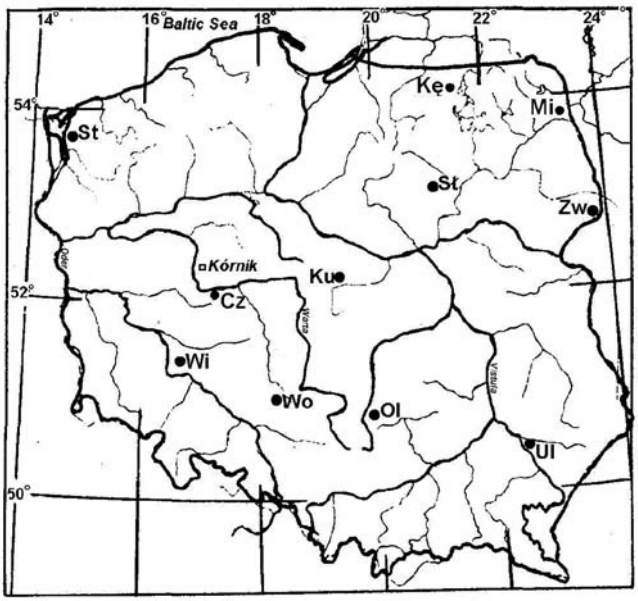


Fig. 1. Location of the studied provenances of *Alnus glutinosa*.

TABLE 1. Phytosociological and geographical data of investigated populations.

| Population | Association | Latitude | Long | Altitude [m] |
|---|---|---|---|---|
| 1. Czeszewo | F-U* | 52°10' | 17°33' | 90 |
| 2. Kętrzyn | Ce-A* | 54°05' | 21°30' | 140 |
| 3. Kutno | C-A* | 52°17' | 19°08' | 125 |
| 4. Mikaszówka | Ce-A | 53°53' | 23°20' | 125 |
| 5. Oleszno | C-A | 50°48' | 20°05' | 225 |
| 6. Sławki | Ce-A | 53°02' | 21°06' | 110 |
| 7. Stepnica | Ce-A | 53°36' | 14°37' | 2 |
| 8. Ulanów | C-A | 51°32' | 22°19' | 200 |
| 9. Wińsko | F-U | 51°26' | 17°55' | 150 |
| 10. Wołczyn | F-U | 51°09' | 18°04' | 180 |
| 11. Zwierzyniec | C-A | 52°45' | 23°48' | 170 |

F-U* – Fraxino-Ulmetum; Ce-A – Carici elongatae-Alnetum; C-A – Circaeo-Alnetum

All $F_1$ trees are aged 38 years and they are grown in 4 replications in similar environmental conditions. Such an experimental design diminished the environmental effects on isozyme activity variation in the buds collected for the analysis. Buds were collected on average from 12 nonadjacent trees per population at the end of February. A total of 130 trees were sampled from 11 populations for the intra- and interpopulation genetic variance analyses.

### *Isozyme analysis*

Collected buds were stored at -70°C for up to 1 week (until analysis). In total 21 putative isozyme loci were studied. Leaf primordia from the inner part of the buds were used for electrophoresis on 11.5% starch gels at +3°C. Homogenization was conducted in 150 µl of 0.1 M TRIS-HCl, pH 7.2, buffer containing 10 mg polyclar AT, 30 mg PVP, 15 µl β-mercaptoethanol and 5 µl non-ionic surfactant Triton X-100. The electrophoretic procedure was similar to those described in an earlier work on mistletoe (Mejnarto-

wicz 2006). Visualization of enzymes was based, with small modifications, on the works of Weeden and Wendel (1989).

Ten enzyme systems were assayed: Fluorescence esterase (E.C. 3.1.1.2; FLE), Glutamate-oxaloacetate-transaminase (E.C. 2.6.1.1; GOT), Isocitrate dehydrogenase (E.C. 1.1.1.42; IDH), Malate dehydrogenase (E.C. 1.1.1.37; MDH), Menadione reductase (E.C. 1.6.4.3; MEN), Phosphoglucomutase (E.C. 5.4.2.2; PGM), Phosphoglucose isomerase (E.C. 5.3.1.9; PGI), 6-Phosphogluconic dehydrogenase (E.C. 1.1.1.44; 6PGD), Shikimate dehydrogenase (E.C. 1.1.1.25; ShDH), and Superoxide dismutase (E.C.1.15.1.1; SOD).

As the offspring from controlled crosses of a single tree was not available, the interpretation of zymograms followed previous alder isozyme studies made by Bosquet et al. (1987; 1988), Konnert et al. (2004), Linares-Bensimon (1984), Murillo and Hattemer (1997), and Prat et al. (1992). As a standard reference, well-known isoenzyme zymograms of European silver fir (*Abies alba*) megagametophytes were used (Bergmann and Mejnartowicz 2002).

Allele frequency was the basis for calculating genetic variation and diversity within populations and genetic distances between populations by using POPGENE software (Yeh and Yang 1999). The statistical Mantel test was used to calculate autocorrelation between matrices of genetic and geographic distances (Mantel 1967).

## RESULTS AND DISCUSSION

In an interesting study of forest communities, Wehenkel et al. (2006) with the help of isoenzyme markers found that pioneer tree species reveal a much higher genetic diversity than the climax tree species. Much earlier, McNaughton and Wolf (1970) discussed a similar question, and concluded that in pioneer species, allelic variation is distributed between individuals while in the climax species allelic variation is distributed within individuals as a high frequency of heterozygous loci. Therefore it was interesting to analyse the genetic structure of populations of alder – the tree species that can be in some circumstances pioneer species but also, as mentioned above, can compose climax communities, i.e. the last stage of plant succession on wetland forest sites.

### *Genetic variation*

In population biology, genetic variation within demes is usually referred to as diversity. Gillet et al. (2005) considered the effective number of diversity types (e.g. the gene in population) as the heterogeneity of demes, and heterozygosity as genetic variation within individuals. There are several genetic parameters to describe genetic diversity. The most frequently used measures of genetic variation in a population are: 1) proportion of polymorphic loci; and 2) average heterozygosity.

### *Proportion of polymorphic loci (%PoL)*

This characteristic was applied for comparing populations from the three phytosociological associations. The analysis of 21 loci of 10 isoenzymes in bud tissues from the 11 populations revealed that 90.5% loci were polymorphic in all studied individuals. Excluding semipolymorphic loci in the studied populations, %PoL amounted to 73.6%. Only both superoxide dismutase loci (SOD1 and SOD2) were homozygous in all populations, while the 6PGD2 locus was semimonomorphic, as it exhibited variation in two alleles in populations Czeszewo and Sławki only (Table 2). In the studied populations, values of %PoL ranged between 85.7% (Sławki, Ce-A) and 61.9% (Oleszno, C-A, and Mikaszówka, Ce-A). Average value of %PoL was the highest in Ce-A populations (77.3%), medium in C-A (72.6%) and the lowest in F-U (66.9%). Those differences between associations (in respect to %PoL) are not high, thus there seems to be no correlation between the level of genetic polymorphism in a population and the phytosociological association to which this population belongs (Mantel test, r = 0.077). Hamrick and al. (1992) quote for plants the range of 23-79 %PoL. It means that alder with the value of 74% is a highly variable tree species, which enables a high level of adaptability to various environmental niches. This result is in good agreement with McNaughton and Wolf's (1970) assumptions that the climax species have individuals with a high frequency of heterozygous loci. The average %PoL for all studied alder populations is slightly higher than for populations of Norway spruce and European silver fir (PoL = 71%) from the Polish territory (Lewandowski and Burczyk 2002; Mejnartowicz 2004).

### *Heterozygosity*

Other analysed measures of genetic variation included the observed heterozygosity (Ho), i.e. the proportion of individuals that possess at a given locus two different alleles, and expected heterozygosity (He) from Hardy-Weinberg (H-W) equilibrium (Nei 1978). The average He was 0.289 and Ho was 0.200. Differences between populations in He were smaller than in Ho. Among the three studied associations, the highest average Ho = 0.211 and He = 0.299 were observed in F-U, and the smallest value (Ho = 0.183) was found in a population originating from C-A (Table 2). Prat et al. (1992) studied *Alnus glutinosa* in France, by analysing 19 isoenzymatic loci in 37 stands. The mentioned authors reported very similar results for expected heterozygosity (He = 240) to those described above for alder populations from Poland. All studied Polish populations have a positive fixation index (F = 0.305), which indicated a homozygote excess relative to H-W equilibrium. A much higher fixation index was calculated by Huh (1999) for Japanese alder (*Alnus japonica*), where it amounted to F = 0.502. Alders are capable of generative reproduction, but they also have an ability to regenerate vegetatively by sprouts or root suckers, which increase the chance of mating between relatives. Steiner and Gregorius (1999) observed in black alder natural population comparatively large amounts of self-pollination that not negatively affected seed production. Inbreeding increases the frequency of homozygotes, but it is very probable that the studied alder populations are subdivided, which may have a similar effect to that of inbreeding. Hattemer et al. (2001) suggests that low efficiency of both pollen and seed transport leads to the clumping of genetically similar trees.

### *Allelic diversity*

Genetic diversity within populations was measured also as mean actual (Na) and effective number (Ne) of alleles per locus, which amounted to 2.62 and 1.59, respectively.

TABLE 2. Summary of genetic variation and heterozygosity in the investigated populations of black alder (*Alnus glutinosa*).

| Population | | N | Na* | Ne | Ho | He | F* | $F_{IS}$ | % PoL | No Ra |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. Czeszewo | (F-U) | 20 | 2.143 | 1.585 | 0.205 | 0.304 | 0.326 | 0.231 | 76.2 | 0 |
| 9. Wińsko | (F-U) | 14 | 2.143 | 1.654 | 0.184 | 0.322 | 0.429 | 0.315 | 66.7 | 0 |
| 10. Wołczyn | (F-U) | 16 | 2.000 | 1.500 | 0.244 | 0.271 | 0.100 | 0.076 | 66.7 | 0 |
| **Average** | **F-U** | **16.7** | **2.095** | **1.581** | **0.211** | **0.299** | **0.285** | **0.207** | **69.9** | **0.0** |
| 2. Kętrzyn | (Ce-A) | 22 | 2.095 | 1.587 | 0.221 | 0.330 | 0.330 | 0.267 | 80.9 | 4 |
| 4. Mikaszów | (Ce-A) | 14 | 1.905 | 1.500 | 0.252 | 0.271 | 0.070 | 0.056 | 61.9 | 0 |
| 6. Sławki | (Ce-A) | 36 | 2.190 | 1.479 | 0.180 | 0.255 | 0.294 | 0.249 | 85.7 | 4 |
| 7. Stepnica | (Ce-A) | 34 | 2.333 | 1.482 | 0.182 | 0.272 | 0.331 | 0.339 | 80.9 | 6 |
| **Average** | **Ce-A** | **27** | **2.131** | **1.513** | **0.209** | **0.282** | **0.256** | **0.228** | **77.3** | **3.5** |
| 8. Ulanów | (C-A) | 42 | 2.286 | 1.534 | 0.170 | 0.285 | 0.404 | 0.338 | 80.9 | 8 |
| 3. Kutno | (C-A) | 34 | 2.381 | 1.563 | 0.176 | 0.299 | 0.411 | 0.407 | 80.9 | 3 |
| 5. Oleszno | (C-A) | 10 | 1.857 | 1.493 | 0.200 | 0.287 | 0.303 | 0.216 | 61.9 | 0 |
| 11. Zwierz | (C-A) | 18 | 2.100 | 1.533 | 0.185 | 0.287 | 0.355 | 0.332 | 66.7 | 0 |
| **Average** | **C-A** | **26** | **2.155** | **1.531** | **0.183** | **0.290** | **0.368** | **0.323** | **72.6** | **2.7** |
| **Grand mean** | | **24** | **2.300** | **1.538** | **0.200** | **0.289** | **0.305** | **0.257** | **73.58** | **2.3** |

Na – mean number of observed alleles; Ne – mean number of effective alleles; Ho – observed heterozygosity; F* – inbreeding coefficient estimated from: 1-(Ho/He); F and $F_{IS}$ – Wright' fixation indices; %PoL – percentage of polymorphic loci; NoRa – number of rare alleles

TABLE 3. Wright's (1965) F-statistics and gene flow in investigated populations of alder (*Alnus glutinosa*).

| Species | Number of populations | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | Nm* |
|---|---|---|---|---|---|
| *Alnus glutinosa* | 11 | 0.271 | 0.336 | 0.089 | 2.554 |

*Nm – gene flow estimated from: $Nm = 0.25\ (1-F_{ST})/F_{ST}$

The smallest Ne = 1.48 was in the populations Sławki and Stepnica (Ce-A) in north Poland. Principal component analysis of six polymorphic allozyme loci by Hamann et al. (1998) in *Alnus rubra* revealed no significant correlations among allozyme frequencies and quantitative traits, but the frequency of the most common allele at most loci decreased with latitude. The average values of Na = 2.13 and Ne = 1.54 for *A. glutinosa* populations from Poland are somewhat higher than those calculated by Huh (1999) for *Alnus* japonica (Na = 1.93 and Ne = 1.39). However, both alder species have a similar percentage of polymorphic loci: 74% for *A. glutinosa* and 76% for *A. japonica*. In the present study, the anemophilous alder has a similar number of effective alleles (Ne = 1.52) to some gymnosperm trees, such as European silver fir (Ne = 1.55), and even higher than that observed for Norway spruce (Ne = 1.29), (Mejnartowicz et al. 2007).

*Number of rare alleles (NoRa)*

In respect to the number of rare allele per locus (NoRa) there are big differences between populations. The greatest NoRa = 8, was found in the Ulanów population (C-A), but on average the highest NoRa = 3.5, was found in the northern populations (Ce-A). Among the eleven studied populations, five had no rare alleles (Table 2).

*Genetic differentiation and gene flow*

Genetic differentiation and gene flow between subdivided populations was estimated with conventional Wright's (1965) F-statistics: $F_{IS}$, $F_{IT}$, and $F_{ST}$. Gene flow was assessed with indirect method ($F_{ST}$), using differences between populations in allele frequency: $Nm = 1/(4N_m+1)$, where: N = population size, m = fraction of N replaced with immigrants. In the studied populations, Nm = 2.55, which means that about 2.5 immigrants reach an average alder population in one generation's duration (Table 3). Slatkin (1987) stated that one migrant per generation is enough to prevent fixation of neutral alleles. Gene flow in the population of wind-pollinated European silver fir is even greater (Nm = 3.29) despite the very heavy pollen of the species (Mejnartowicz 2004). In the dendrogram constructed according to Nei (1972) on the basis of interpopulation genetic distances, many small groups can be observed (Fig. 2). The first one includes only one population: Wińsko (No. 9) from southwestern Poland. The remaining populations form several smaller groups, corresponding neither to phytosociological associations nor to geographic distances. Gene flow among alder populations, mediated by pollen and seeds, can be an evolutionary factor, and is likely to have blurred the existing genetic differentiation between populations from different phytosociological associations. A gene flow was observed, even though infrequently, between *A. glutinosa* and *A. incana*, resulting in hybrids trees, which can also affect the genetic structure of the population of both species (Prus-Głowacki and Mejnartowicz 1992).

Woody species like alder – with large geographic ranges, outcrossing breeding systems, and seed dispersal by wind – have a higher genetic diversity within species and populations but lower variation among populations than woody species with other combinations of traits (Hamrick 1992). Alder seeds have no wings, so they can be spread by wind only over a distance of 30-60 m around the mother tree. However, alder seeds have air bladders, and thus can be transported over large distances by rivers thanks to periodical flooding. In this way alders can colonize new territories and migrate into other natural alder populations growing on river banks.

The genetic differentiation between populations calculated for 21 investigated loci was low and reached merely the value of $F_{ST} = 0.089$. This means that 9% of total genetic variation is due to interpopulation variation, and 91% is lo-

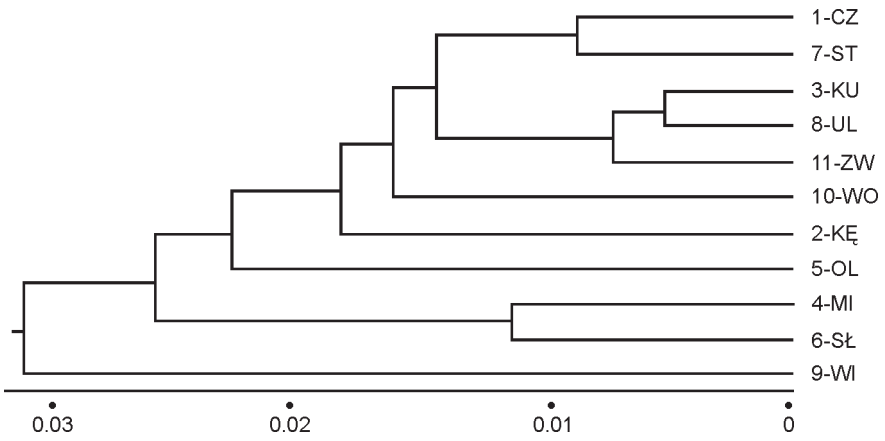


Fig. 2. UPGMA cluster analysis based on Nei's genetic distances among investigated populations of *Alnus glutinosa*.

cated within populations (Table 3). This is much less than in the study of Prat et al. (1992), who found $F_{ST}$ = 0.204, but much more than in the study of *A. glutinosa* from central Slovakia investigated by Gömöry and Paule (2002), who found $F_{ST}$ = 0.022. The population differentiation of *A. glutinosa* in Poland is similar to that of *A. rugosa* in Canada ($F_{ST}$ = 0.052) and *A. japonica* in Korea ($F_{ST}$ = 0.095), (Huh 1999).

The vast majority of investigated loci have intermediate $F_{ST}$ values, indicating a neutral character of allelic variation in these loci. On the scale of Poland, no clear genetic pattern of similarity between populations was recorded, perhaps due to gene flow between populations, more by seeds than by the pollen. Differentiation within regional groups ($F_{IS}$ = 0.271) suggested that mating was mainly random in the populations (Table 3).

## CONCLUSIONS

Alder populations are found usually in the vicinity of watercourses. Alder seeds are not spread far away by wind, but can be spread by running water over large distances. This study revealed no significant correlation between genetic and geographic distance between populations, and between genetic distance and phytosociological classification. It seems that the spatial genetic structure of alder populations was formed in connection to the hydrological system of the country.

The value of $F_{ST}$ = 0.089 indicates small differences between populations. Only 8.9% of the total variation is due to interpopulation differences. Most of the analysed loci have intermediate $F_{ST}$ values, attesting to the neutral character of allelic variation at these loci. Gene flow estimates (Nm = 2.55) showed that reproductive barriers do not separate populations of *A. glutinosa*.

In 10 studied isozyme systems in 21 putative loci, with altogether 56 alleles, a high level of genetic variation within populations was revealed by the mean number of actual alleles (Na = 2.3) and of effective alleles (Ne = 1.54) per locus. Expected heterozygosity (He) amounted to 28.9%, while observed heterozygosity (Ho) reached 20.0%. All studied Polish populations have a positive fixation index (F = 0.305), which indicated a 30% deficiency of heterozygotes. This could be explained by the fact that alder regenerates generatively but also has an ability to regenerate vegetatively by sprouts or root suckers, and this increases the chance of mating between relatives.

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