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GENETIC STRUCTURE OF AN INVASIVE BIVALVE *DREISSENA POLYMORPHA* (PALLAS) FROM POLAND – I. GEOGRAPHICAL AND INTRA-POPULATION VARIATION

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ABSTRACT: Thirty two populations of *Dreissena polymorpha* (Pall.) from Poland were electrophoretically studied with respect to seven enzymatic loci. The zebra mussel was found to be genetically highly variable, as indicated by high polymorphism of particular loci and wide individual genotypic variation. The parameters of genetic variation of *D. polymorpha* from Poland were: 100% polymorphic loci, mean number of alleles per locus 4.7, mean number of genotypes per locus 10.3, mean expected heterozygosity per locus 0.473. The mean percentage of polymorphic loci per population was 89.3, mean number of alleles per locus per population 3.5, mean number of alleles per polymorphic locus per population 3.7, mean expected heterozygosity per locus per population 0.447, mean number of genotypes per population 5.3. Colonies of *D. polymorpha* proved to be genetically much differentiated: the mean percentage of polymorphic loci per colony was 86.2, number of alleles per locus 2.62, genotypic differentiation 95%. The genetic similarity between the populations was high which indicates a large uniformity of the species in Poland. The genetic similarity between the populations was 0.828–0.999, the genetic distance 0.001–0.189. No genetic differences were found between populations from the regions of Pomerania, Mazurian Lakeland and Konin. The genetic similarity between the three groups of populations ranged from 0.975 to 0.986, the genetic distance from 0.014 to 0.025. The Polish populations of the zebra mussel showed a slightly higher level of genetic variation compared to W European and N American populations. Colonisation is not accompanied by a decrease in the gene pool resulting from genetic drift; the species seems to expand its range using all its genetic potential.

KEY WORDS: *Dreissena polymorpha*, enzymatic loci, genetic variability, population, colonies, geographical variation

CONTENTS

INTRODUCTION

The zebra mussel (*Dreissena polymorpha*) is a typically invasive species whose distribution range has been gradually expanding for ca. 200 years, from the regions of the Black, Caspian and Azov Seas, the expansion still being in progress (WIKTOR 1969, STAŃCZYKOWSKA 1977).

The expansion of the zebra mussel is favoured by its numerous biological properties, among others high fertility of females (BORCHERDING 1991), internal fertilisation and the veliger larva which is capable of long-distance dispersal within water bodies (LEWANDOWSKI 1982a). The expansion is also aided by transport of adult individuals attached with their byssus threads to boats, barges, etc. (LEWANDOWSKI 1982b, BORCHERDING 1991) and migration of adult individuals after dissolving their byssus threads (ACKERMAN et al. 1994). Besides, the process is facilitated by the ability to survive a few days outside water (WIKTOR 1969, GRIFFITHS et al. 1991), to colonise waters of various trophic conditions (WIŚNIEWSKI & DUSOGE 1983, LEWANDOWSKI 1991), as well as polluted (STAŃCZYKOWSKA et al. 1983, PIECHOCKI & DYDUCH-FALNIOWSKA 1993), heated and brackish waters (WIKTOR 1969, KORNOBIS 1977).

Fossil record indicates that *D. polymorpha* was present on the continent before the Ice Ages (NOWAK 1974). Its Tertiary range included the area between the Atlantic Ocean in the west and the Aral Sea in the east, the White Sea in the north and the Black and Caspian Seas in the south. During the Ice Ages the bivalve became almost completely extinct, its distribution being limited to the shores of the Black and Caspian Seas, from where later its expansion started (PIECHOCKI & DYDUCH-FALNIOWSKA 1993). The expansion proceeded mainly along rivers and was associated with inland shipping (NOWAK 1974). Its first stage, leading from the northern part of the Caspian Sea and the delta of the Volga River up this river and into its tributaries, included the European part of Russia. Another and stronger expansion started from the shores of the Black Sea and proceeded northward along the Dnieper River and its tributaries. Having invaded Eastern Europe, the zebra mussel penetrated Central Europe by two main routes: along the coast of Northern Europe and along the Danube River valley (NOWAK 1974).

In 1800–1960 the zebra mussel invaded the total area of ca. 1.25 mln km^2 in Europe, which constituted 35% its present distribution range. The mean expansion rate was ca. 7,800 km^2 per year (NOWAK 1974).

According to some authors, during the Ice Ages *D. polymorpha* did not become extinct in entire Europe, but survived in at least a few isolated areas: lakes of Schlezwig-Holstein in Thuringia (WALZ 1974), Kuronian Bay, Ohrida Lake in the former Yugoslavia, some water bodies of the Hungarian Lowland (NOWAK 1974) and in the Balkans (PIECHOCKI & DYDUCH-FALNIOWSKA 1993). Besides, the proponents of this view are of opinion that the over 200 year expansion in entire Europe was fast due to the presence of isolated localities on the whole continent, and the present distribution range is a reconstruction of the earlier range (NOWAK 1974, STAŃCZYKOWSKA 1977).

Besides the invasion in Europe, the zebra mussel spread also southward and eastward of its endemic distribution range, though the details of this process are unclear (NOWAK 1974). In 1986, *D. polymorpha* invaded also the Great Lakes of North America from where it is expected to expand its range to the whole American continent (HEBERT et al. 1989, BORCHERDING 1991, CLAXTON et al. 1997).

In Poland, the earliest records of *D. polymorpha* date from 1824, from the former Eastern Prussia, while in Western Pomerania the species was observed only in 1896 (BRANDT 1896, PIECHOCKI & DYDUCH-FALNIOWSKA 1993). It is suspected that the zebra mussel reached the Baltic coast through the Neman River which, at the end of the 18th c., was connected with the Dnieper River by the Ogiński Canal (NOWAK 1974).

At present in Poland the species occurs mainly in the Mazurian Lakeland, Pomerania and Wielkopolska. It inhabits fresh and brackish waters: slow-flowing rivers, canals, harbours, lakes, ponds, estuaries and dam reservoirs (WIKTOR 1969, STAŃCZYKOWSKA 1972, PIOTROWSKI & OCHMAN 1993, STAŃCZYKOWSKA et al. 1997). Such diverse conditions testify to a great adaptive potential of the bivalve. The zebra mussel tolerates high loads of chemical pollution, and changes in habitat conditions in water bodies affect it to a lesser degree compared to other molluscs (MOUTHON 1981, STAŃCZYKOWSKA et al. 1983). These properties have no doubt played a considerable part in the expansion of the species.

D. polymorpha plays an important ecological role in water bodies, due to biofiltration, biosedimentation and bioaccumulation (WIKTOR 1969, PIESIK 1983, STAŃCZYKOWSKA et al. 1983, LEACH 1993, REEDERS et al. 1993). It considerably affects the matter circulation in water bodies, uses excessively developing phytoplankton as food, and constitutes food basis for crayfish, crabs, fish and birds (WIKTOR 1969, PIESIK 1974, SZLAUER 1974, STAŃCZYKOWSKA 1977, STAŃCZYKOWSKA & PLANTER 1985, FRENCH 1993, STOCZKOWSKI & STAŃCZYKOWSKA 1995).

Many mollusc species of wide geographical distribution show a high genetic variation. For example, a wide variation (expected heterozygosity H_0=0.61–0.66) was found in two species of the genus *Cerithium* (RITTE & PASHTAN 1982), four species of *Macoma*

(H_o=0.21–0.44; WENNE 1993) and *Brachidontes variabilis* (H_o=0.62–0.66; SAFRIEL & RITTE 1986).

Like other expanding species, the zebra mussel is characterised by a wide genetic variation which enables it to spread over large areas and occupy a variety of habitats (WIKTOR 1969, STAŃCZYKOWSKA 1977, HEBERT et al. 1989, GARTON & HAAG 1991, MAY & MARSDEN 1992, BOILEAU & HEBERT 1993, PIECHOCKI & DYDUCH-FALNIOWSKA 1993, SPIDLE et al. 1994).

First estimates of the allozyme variation in *D. polymorpha* were made in America (HEBERT et al. 1989, GARTON & HAAG 1991, ROSE & ECKROAT 1991, MAY & MARSDEN 1992, BOILEAU & HEBERT 1993, SPIDLE et al. 1994). The mean expected heterozygosity per locus (H) for the analysed populations was 0.35. The wide variation of American populations indicates that the invasion included abundant founder populations, and thus no genetic drift was observed (HEBERT et al. 1989, GARTON & HAAG 1991).

A high level of variation, comparable to that found in American populations (H=0.40), was observed in Western European populations of *D. polymorpha* (BOILEAU & HEBERT 1993, SPIDLE et al. 1994). A population of *D. polymorpha* from a Lithuanian lake Dringis, analysed by ZAPKUVIENNE (1992), also showed a high polymorphism (H=0.44). BOILEAU & HEBERT (1993) classified *D. polymorpha* in a group of 10 animal species with the highest level of genetic variation.

Previous genetic studies on the variation of Polish populations of the zebra mussel were of limited range and pertained to single populations from Western Pomerania and Mazurian Lakeland (ZIELIŃSKI et al. 1995, 1996, 2000, SOROKA et al. 1997, PIESIK et al. 1998, SOROKA 1999). The studied populations showed a high allozyme variation of 7–8 analysed loci. The basic variation parameters were: 75–100% polymorphic loci, mean number of alleles per locus 2.8–4.5, per polymorphic locus 3.5–3.9, mean expected heterozygosity per locus per population 0.338–0.455. These preliminary data indicated that the level of genetic variation in Polish populations of *D. polymorpha* was similar to or even higher than that found in W European or even American populations.

The main objective of this study was an estimate of genetic variation and genetic structure of Polish populations of *D. polymorpha* in view of the effect of the geographical location.

The technique used was isoenzyme electrophoresis on starch gel. It is commonly applied in genetic-population and genetic-evolutionary studies (NEI 1972, 1987, HEDRICK 1975, HAMRICK & GODT 1990).

MATERIAL AND METHODS

2.1. MATERIAL

D. polymorpha was collected from 32 water bodies in Poland. The sites were selected in such a way as to represent the areas of Poland where *D. polymorpha* was the most common i.e. Pomeranian, Wielkopolskie, Mazurian and Suwalskie Lakelands, and lakes of S Baltic coast. The location and list of the sites are presented in Fig. 1 and Table 1, respectively.

It was assumed that *D. polymorpha* from one water body constituted one population. Such an assumption was justified by earlier detailed studies on *D. polymorpha* from the lakes Ińsko (SOROKA et al. 1997) and Dąbie (PIESIK et al. 1998).

Each sample included material randomly collected from many parts of the lake and depths ranging from 0.1 to 20 m. The material was collected in such a way that each sampling point constituted a part of a compact colony of *D. polymorpha*. From 20 to 50 individuals were collected at each point. The number of sampling points depended on the surface area of the lake, the length of its shoreline and the size of the zebra mussel population. The number of sampling points per population ranged from 2 to 31, the mean being 12 (Table 1). Only in lake Sitno, where individuals of *D. polymorpha* were scattered over the bottom, 10 specimens were collected from a small area. The material was collected by a diver, Mr. MAREK ŚWIERCZYŃSKI, M. Sc., from the Chair of Animal Ecology, University of Szczecin.

The samples were placed in separate containers and transported, live or deep-frozen, depending on the distance, to the laboratory. The material from Western Pomerania (population samples 1–19) was maintained in laboratory culture. Water in the aquaria was aerated and lit, and the mussels were fed

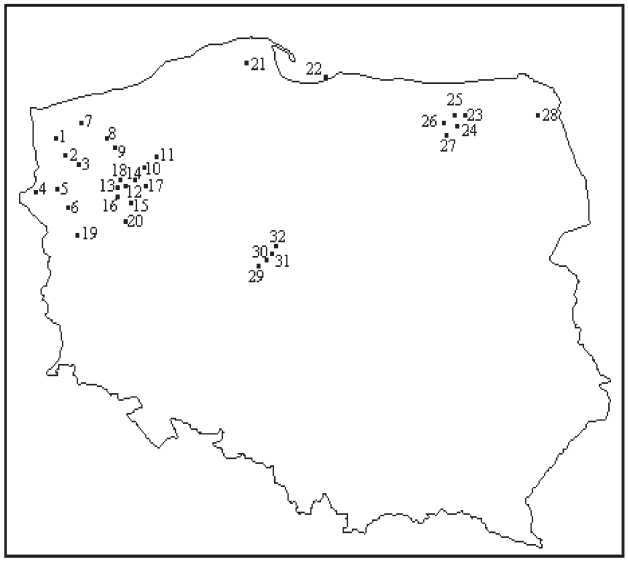

Fig. 1. Sampling sites of *D. polymorpha*

with algal suspension. The remaining samples (20–32) were frozen and stored at –20°C. Thus protected samples were stored for ca. 1 month during which electrophoretic analyses were carried out. The material preserved its enzymatic activity during the whole period of analysis.

The total number of analysed specimens was 3,870 from 32 populations (Fig. 1, Table 5). The number of analysed specimens per population ranged from 20 to 310, the mean being 121. Ten specimens from each colony were analysed.

Table 1. List of sampling sites of *D. polymorpha*

Number	Lake	Number of sampling points	Date of collection
1	Dąbie	19	20.04.91, 5.07.91
2	Miedwie	30	28.03.94, 2.04.94, 5.05.94
3	Gardzko	4	12.06.94
4	Orzechów	2	10.01.94
5	Chłop	11	25.09.93
6	Marwicko	11	15.04.93
7	Czarnogłowy	11	8.06.93, 4.02.94
8	Woświn	20	23.09.92, 6.10.92, 16.06.93
9	Ińsko	31	15.09.92, 5.11.92, 7.05.93
10	Lubianka	15	17.04.93
11	Duże	11	11.12.92
12	Raduń	10	28.09.93
13	Adamowo	22	15.04.94
14	Sitno	4	16.04.94
15	Ostrowiec	12	20.04.94
16	Płociowe	10	20.01.94
17	Marta	10	21.04.94
18	Krzywe	16	1.06.93
19	Chycina	11	30.04.94
20	Jaroszewskie	14	9.05.93
21	Łeby-Redy	9	29.09.93
22	Vistula Bay	8	15.05.94
23	Śniardwy	10	16.05.94
24	Mikołajskie	4	16.05.94
25	Wersminia	8	17.05.94
26	Inulec	10	17.05.94
27	Majcz	14	18.05.94
28	Necko	10	19.05.94
29	Gosławskie	10	4.03.95
30	Pątnowskie	10	4.03.95
31	Mikorzyńskie	10	7.03.95
32	Ślesińskie	10	8.03.95

Because of the distribution of *D. polymorpha* in Poland, three regions were distinguished: Pomerania, Mazurian Lakeland and Konin. Pomerania (N=2,830) was represented by populations 1–21, Mazurian Lakeland (N=560) by 23–28, Konin (N=400) by 29–32. Population 22 from the Vistula Bay was not included in any region.

Some of the lakes were naturally or artificially connected, or belonged to the same catchment area which was considered when interpreting the results. Such lakes included: Woświn and Ińsko (Ina River catchment area), Sitno, Adamowo and Ostrowiec (Drawa River catchment area), Śniardwy and Mikołajskie (naturally connected), Gosławskie, Pątnowskie, Mikorzyńskie and Ślesińskie (parts of the cooling system of the Konin and Pątnów power plants). The lakes Orzechów, Czarnogłowy, Duże, Płociowe and Marta are isolated water bodies located among forests or fields, fed exclusively by atmospheric precipitation and underground waters. It is noteworthy that lake Czarnogłowy (no. 7), contrary to all the other lakes which are post-glacial, is a young reservoir. It was formed in the 50s of the 20th c. as a result of limestone excavation.

2.2. BIOCHEMICAL METHODS

Seven enzymes were analysed with starch gel electrophoresis. The electrophoresis followed standard procedures (PASTEUR et al. 1988, SOLTIS & SOLTIS 1989), with some modifications (SOROKA et al. 1997).

2.2.1. Enzyme protein extraction

A 20 mg fragment of muscle tissue was taken from each live or frozen individual, pulverised in a cooled mortar with 200 µl extraction buffer and a pinch of quartz sand.

Extraction buffer 0.1 M Tris-HCl, pH 7.5: Tris 1.21 mg, KCl 75 mg, EDTA $Na_2 \times 6\ H_2O$ 38 mg, $MgCl_2 \times 6\ H_2O$ 203 mg, Triton X–100 20 ml 0.5%, distilled water added till 100 ml.

Immediately before use 20 µl 2-mercaptoethanol were added to the extraction buffer. The resulting tissue extract was placed in a refrigerator at +4°C for 15 minutes, then three Whatman 3 MM blots of 5 × 8 mm size were soaked in it. The blots were spread on the slots of three different starch gels (Sigma electrostarch) and electrophoresis was run in three different buffer systems.

2.2.2. Gels and separating buffers

In order to ensure the optimum separation, three different kinds of gels and separating buffers were used: lithium-borate, pH 8.0, morpholine-citrate, pH 6.1 and Tris-citrate (electrode buffer, pH 8.0 and gel buffer, pH 9.1).

Aspartate aminotrasferase (GOT, AAT, E.C.2.6.1.1), esterase (EST, E.C.3.1.1.2) and phosphoglucoisomerase (PGI, E.C.5.3.1.9) were separated in lithium-borate buffer, NAD-dependent malate dehydrogenase (MDH, E.C.1.1.1.37), NADP-dependent malate dehydrogenase (ME, E.C. 1.1.1.40) and isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) in morpholine-citrate buffer, and phosphoglucomutase (PGM, E.C.2.7.5.1) in Tris-citrate buffer.

Lithium-borate buffer: electrode buffer, pH 8.0: lithium hydroxide 1.2 g, boric acid 11.89 g, H_2O till 1 l; gel buffer, basic solution, pH 8.0: Tris 6.2 g, citric acid 1.6 g, H_2O till 1 l. The gel buffer was prepared by combining 9 parts of basic gel buffer and one part of electrode buffer.

Morpholine-citrate buffer: electrode buffer, pH 6.1: citric acid 8.4 g, N-3 aminopropylomorpholine 9.1 ml, H_2O till 1 l; gel buffer, pH 6.1: electrode buffer 36 ml, H_2O 964 ml.

Tris-citrate buffer: electrode buffer, pH 8.0: boric acid 18.6 g, sodium hydroxide 2 g, H_2O till 1 l; gel buffer, pH 9.1: Tris 9.2 g, citric acid 1.1 g, H_2O till 1 l.

2.2.3. Electrophoretic separation of enzyme proteins

Electrophorectic separation was run horizontally in perspex apparatus. Starch gels of 190 × 105 mm size were prepared the day before the electrophoresis. Prior to the analysis the gels were cleaned, and in their slots ca. 23 blots soaked in extract were placed. The gel was covered with foil and a glass plate on which containers with cooling liquid were placed to prevent heating of the gel during electrophoresis. All the proteins migrated in the anodal part of the gel; the distance of their separation was 8 cm. The electrophoresis in the lithium-borate buffer was run for 4.5 hrs, till the front reached the distance of 3–5 mm from the end of the gel. The current used was 220 V and 42 mA. The duration of electrophoresis in the morpholine-citrate buffer was 5 hrs, the current being 110–120 V and 32 mA. The parameters, and the time of separation were followed strictly because of the absence of front in this buffer. In the Tris-citrate buffer the electrophoresis was run for 4 hrs, till the front reached the distance of 3–5 mm from the end of the gel. The current used was 140 V, 34 mA.

2.2.4. Enzyme staining

Enzymes were histochemically stained immediately following electrophoresis, through incubation of the gel in a staining mixture (GOT, EST) or covering it with agar overlay with staining mixture (PGI, MDH, ME, IDH, PGM). Staining was carried out in a thermostat at 37°C and lasted up to 1 hr.

Aspartate aminotransferase (GOT): aspartic acid 130 mg, ketoglutaric acid 50 mg, EDTA 7 mg, FBRR 150 mg, pyridoxal–5-phosphate x H_2O 3 mg, 0.1 M Tris-HCl, pH 7.1 50 ml, 0.4 M phosphate buffer, pH 7.4, 50 ml. The substrates were solved in buffers, while FBRR and pyridoxal-5-phosphate were added immediately before staining the gel.

Esterase (EST): α-naphthyl acetate 100 mg, FBRR 100 mg. Weighed amounts were solved in a mixture of acetone and distilled water as 1:1, then 100 ml 0.1 M phosphate buffer pH 6.0 was added.

In the agar-overlay method, the weighed amounts were solved in 5 ml Tris-HCl buffer pH 8.0; then 5 ml of this buffer were added, with 75 mg agar dissolved in it. The gel surface was covered with agar film. The enzymes listed below were stained with this method:

Phosphoglucoisomerase (PGI): fructose-6-phosphate 5 mg, $MgCl_2$ 10 mg, NADP 2 mg, MTT 2 mg, PMS 0.5 mg, G-6-PDH 5 units.

NAD-dependent malate dehydrogenase (MDH): malic acid 300 mg, neutralised with Na_2CO_3 (ca. 240 mg), NAD 3 mg, MTT 2 mg, PMS 0.5 mg.

NADP-dependent malate dehydrogenase (ME): malic acid 500 mg, neutralised with Na_2CO_3 (ca. 400 mg), $MgCl_2$ 10 mg, NADP 2 mg, MTT 2 mg, PMS 0.5 mg.

Isocitrate dehydrogenase (IDH): sodium salt of DL-isocitrate acid 60 mg, $MgCl_2$ 10 mg, NADP 2 mg, MTT 2 mg, PMS 0.5 mg.

Phosphoglucomutase (PGM): α-D-glucose-1-phosphate $Na_2 \times 4\ H_2O$ 30 mg, $MgCl_2$ 10 mg, NADP 2 mg, MTT 2 mg, PMS 0.5 mg, G-6-PDH 5 units.

2.2.5. Documentation of results

The results were documented for each electrophoretic run; following staining of the enzymes the results were entered in tables, each enzymatic phenotype being assigned a number. Each specimen had a numerical summary record of its phenotype consisting of 7 analysed enzymes. Photographs of selected gels were taken.

2.3. STATISTICAL METHODS

The resulting electrophoretic phenotypes were analysed with respect to their frequency (phenotype analysis); their genetic analysis was also carried out.

In phenotypic analysis each enzyme was statistically analysed with Microsoft Excel and Chi-square test in nominal scale. Coefficients of genetic similarity (I_H) and genetic distances (D_H) between the populations were based on the frequency of phenotypes (according to HEDRICK 1975).

Dendrogram for 32 populations of *D. polymorpha* was constructed with UPGMA method based on coefficients of genetic similarity, according to HEDRICK (1975).

Genetic analysis of genotype records of all loci was performed with programmes BIOSYS (SWOFFORD & SELANDER 1983) and GENESTAT-PC v. 2.1 (WHITKUS 1988). Occurrence of individuals with unique genotypes (UG) considering 7 loci in the

populations was analysed with a programme of Mr. P. KONIECZNY, M. Sc.

The following parameters of genetic structure were calculated:

Degree of genetic variation of the species and populations of *D. polymorpha* (NEI 1978):
1. Percentage of polymorphic loci (PL). According to the adopted criterion of polymorphism 0.99, in a polymorphic locus the frequency of an allele does not exceed the value of 0.99. Alleles of frequencies below 0.01 are called rare alleles.
2. Mean number of alleles per locus and per polymorphic locus, and mean number of genotypes per locus. All the phenotypes and alleles were considered, including rare ones.
3. Expected heterozygosity:
3.1. Coefficient of expected heterozygosity in a locus in population H;
3.2. Mean value of coefficient of expected heterozygosity in locus per population $\overline{H}$;
3.3. Mean value of coefficient of expected heterozygosity per locus in population H_s;
3.4. Mean value of coefficient of expected heterozygosity per locus per population $\overline{H}_s$;
3.5. Coefficient of expected hetrozygosity per locus for species H_T;
3.6. Mean value of coefficient of expected heterozygosity per locus for species $\overline{H}_T$.

Inter-population differences (according to NEI 1973):
1. Coefficients of genetic similarity (I_N) and genetic distance (D_N) (NEI 1972, 1978). UPGMA dendrogram was constructed for 32 populations of *D. polymorpha* based on coefficients of genetic similarity (NEI 1978).
2. D_{ST} coefficient reflecting the degree of variation in a population in relation to the variation in the species for a given locus or as a mean of all loci.
3. G_{ST} coefficient reflecting the proportion of inter-population variation in the whole variation in the species in a given locus or as a mean of all loci.

Intra-population differences:
1. Variation of alleles within colonies.
2. Genotypic composition of colonies.

Genotypic variation of individuals with respect to 7 loci at the population level:
1. Frequencies of genotypes in populations.
2. Analysis of genotype frequency in view of Hardy-Weinberg equilibrium.
3. Coefficient of heterozygote excess (D).
4. Analysis of unique genotypes (UG%) in populations. Unique genotypes are genotypes which were present in a population only once.
5. Analysis of various genotypes in colonies (G_1) in selected populations (Czarnogłowy, Orzechów, Woświn).

Statistical analysis of population groups distinguished on the basis of geographical distribution considered the following parameters:
1. Expected heterozygosity:
1.1. Mean value of coefficient of expected heterozygosity per locus per population, $\overline{H}$;
1.2. Mean value of coefficient of expected heterozygosity per locus per population, H_S;
1.3. Coefficient of expected heterozygosity per locus for species, H_T;
1.4. Mean value of coefficient of expected heterozygosity per locus for species, $\overline{H}_T$.
2. Inter-population differences:
2.1. Coefficients of genetic similarity (I_N) and genetic distance (D_N) (NEI 1978);
2.2. Coefficient D_{ST} for a given locus and as a mean of all loci;
2.3. Coefficient G_{ST} for a given locus and as a mean of all loci;
2.4. Heterozygote excess coefficient, D.

D values in particular loci or population groups were compared with Student t-test.

The significance of differences in the distribution of alleles between population groups and between colonies within populations was tested with Chi-square test in nominal scale.

RESULTS

3.1. ELECTROPHORETIC PHENOTYPES OF THE ANALYSED ENZYMES AND THEIR GENETIC INTERPRETATION

Figure 2 shows electrophoretic phenotypes of the seven studied enzymes. Author's own numbering of alleles was used, because of the absence of data on band position and principles of allele numbering in literature describing electrophoretic phenotypes of *D. polymorpha* (HEBERT et al. 1989, MAY & MARSDEN 1992, SPIDLE et al. 1994). Bands and alleles were numbered according to the commonly accepted principles, though the numbers did not always correspond to mobility.

Each enzyme had a few electrophoretic phenotypes which were numbered according to the sequence adopted (phenotypic analysis, Fig. 2). The phenotypes were one-, two-, three-, four-, or five-banded and were exclusive within individuals.

In the genetic interpretation it was assumed that exclusive one-banded phenotypes of different electrophoretic mobility were single-locus homozygotes. Phenotypes of 2, 3, 4 and 5 bands were interpreted as heterozygotes for the respective locus.

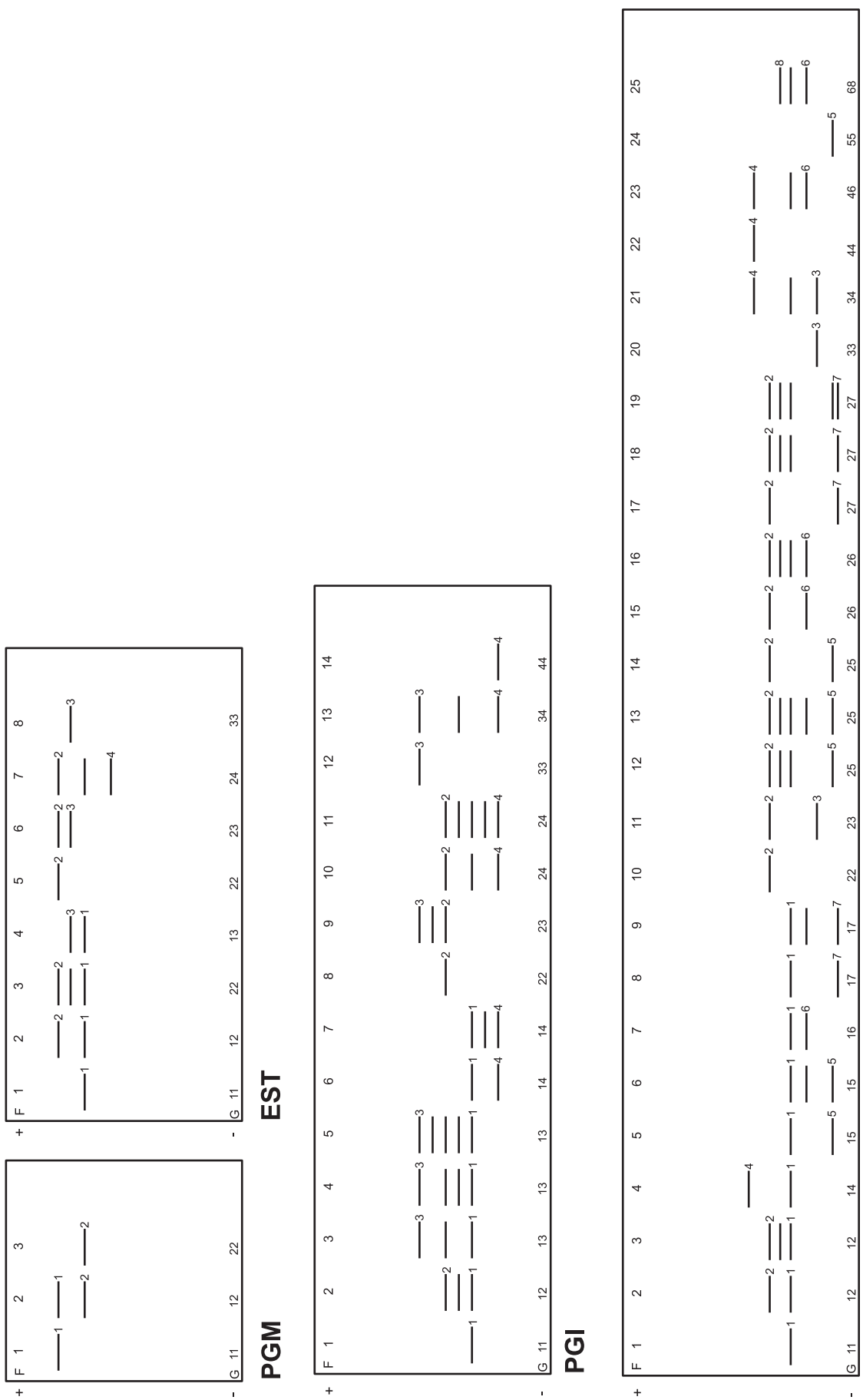

Fig. 2. Electrophoretic phenotypes of analysed enzymes in *D. polymorpha* and their genetic interpretation (F – phenotype number, G – genotype number)

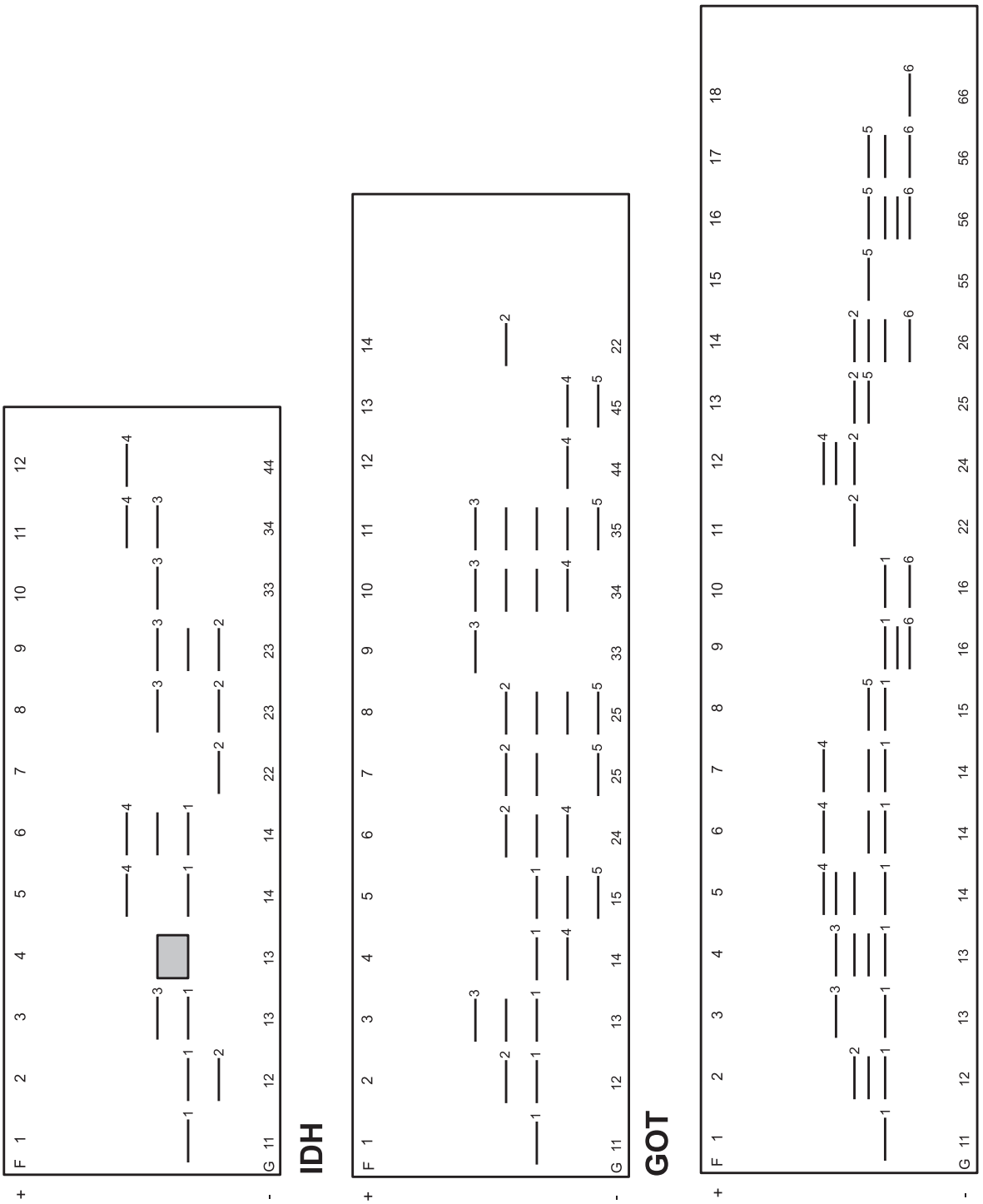

Fig. 2. continued

Seven enzymatic loci were identified, one for each enzyme.

The detailed genetic interpretation of the obtained electrophoretic phenotypes of the seven analysed enzymes is presented below (Fig. 2).

Aspartate aminotransferase (GOT): 14 phenotypes of one to five bands. It was assumed that the phenotypes were encoded by a single locus, *Got1*, of five alleles *Got1*-1 – *Got1*-5.

Esterase (EST): 8 phenotypes, encoded by locus *Est1*, with alleles *Est1*-1 – *Est1*-4.

Phosphoglucoisomerase (PGI): 14 phenotypes of one to five bands, treated as encoded by a single locus with four alleles *Pgi1*-1 – *Pgi1*-4.

NADP-dependent malate dehydrogenase (ME): 25 phenotypes of one to five bands. They were interpreted as encoded by eight alleles *Me1*-1 – *Me1*-8.

NAD-dependent malate dehydrogenase (MDH): 18 phenotypes of one to four bands, encoded by a single locus with six alleles: *Mdh1*-1 – *Mdh1*-6.

Isocitrate dehydrogenase (IDH): 12 phenotypes of one, two or three bands, encoded by one locus *Idh1* with four alleles.

Phosphoglucomutase (PGM): 3 phenotypes encoded by one locus *Pgm1* with two alleles *Pgm1*-1 and *Pgm1*-2.

3.2. PHENOTYPE ANALYSIS

3.2.1. Number of phenotypes

The mean number of phenotypes per enzyme, calculated based on analysis of 32 populations of *D. polymorpha*, was 13.4. The enzymes displayed the following numbers of phenotypes: ME – 25, MDH – 18, GOT and PGI – 14, IDH – 12, EST – 8, PGM – 3 (Tables 2, 3).

Table 2 shows numbers of phenotypes for particular enzymes in the 32 analysed populations. The mean number of phenotypes per population was the highest for enzymes ME – 10.8, PGI – 7.2 and GOT – 7.1. In particular populations the highest numbers of phenotypes were found for enzymes: ME, from 3 to 15, PGI – 4–11, GOT – 3–12. The lowest mean number of phenotypes per population was observed for enzyme PGM – 1.41.

With respect to all enzymes except MDH, on an average half of the phenotypes found within the species were present in each population. Only in the case of PGM in the population from lake Miedwie (no. 2) the maximum number of three phenotypes was present.

3.2.2. Phenotype frequency in populations

Table 3 presents the frequency of phenotypes in the populations of *D. polymorpha*. For all the enzymes except GOT, one of the phenotypes had a higher frequency than the remaining ones and was present in all the populations.

Phenotypes of frequencies exceeding 0.10, their number being 20, were present on an average in 93% populations. Twenty nine phenotypes were within the range of 0.01–0.10 and on an average each of them appeared in 61% populations. Phenotypes of frequencies below 0.01 were observed for all the enzymes, their total number being 45, and they were on an average present in 12% populations. The least numerous were rare phenotypes of PGM – 1 phenotype, the most numerous were those of ME and MDH – 13 in each case. The distribution of rare phenotypes among the populations varied. Over half of such phenotypes appeared in one or three populations. The remaining 20 phenotypes of this group were present in 4–10 populations.

The results suggest a widespread character of both high- and low-frequency phenotypes in the populations.

3.2.3. Genetic similarity and genetic distance between the populations

Values of genetic similarity I_H between the analysed populations of *D. polymorpha*, calculated according to HEDRICK (1975), were within the range of 0.452 to 0.958, the mean being 0.778 (Fig. 3). Three populations: Czarnogłowy (no. 7), Sitno (no. 14) and Ślesińskie (no. 32) had the lowest I_H values, which were 0.452, 0.469 and 0.516, respectively (Fig. 4). The highest number of populations were within the range 0.85–0.90 and 0.75–0.80 (Fig. 3). Figure 4 shows genetic similarity between the analysed populations.

Genetic distances between the 32 populations calculated according to HEDRICK (1975) were within the range of 0.042–0.548, the mean being 0.222.

I_H values varied somewhat between the enzymes (Fig. 5). MDH showed the highest genetic similarity, ranging from 0.548 to 1.000, the range for ME was 0.332–0.982, for PGI – 0.190–0.996, for IDH – 0.164–1.000. The lowest I_H values were those for GOT and EST, where the values varied widely and amounted to 0.017–0.992 and 0.035–1.000, respectively. All the lowest values of the coefficient for EST were observed in the population Ślesińskie (0.035–0.243). EST had high values of mean and median of genetic similarity, amounting to 0.798 and 0.917, respectively, while for GOT the corresponding values were 0.492 and 0.483. For the remaining enzymes the values of mean and median of genetic similarity were within 0.775–0.927 and 0.803–0.961, respectively.

3.3. GENETIC STRUCTURE OF POPULATIONS OF *D. POLYMORPHA*

3.3.1. Percentage of polymorphic loci

Based on electrophoretic analysis of 7 enzymes in 3,870 individuals of *D. polymorpha* from 32 populations, 7 enzymatic loci were identified. All the loci

were variable in that there were at least two alleles in a locus (see Table 8). The percentage of polymorphic loci at a polymorphism criterion 0.99 and 0.95 was 100 (Table 4).

The percentage of polymorphic loci per population, considering the above-mentioned polymorphism criteria, was 89.28 and 78.04, respectively. The difference was mainly due to locus *Pgm1* which, depending on the criterion adopted, was monomorphic in 62.5–87.5% populations. At the polymorphism criterion of 0.99 the percentage of polymorphic loci in the populations assumed values ranging from 85.7 (6 polymorphic loci) to 100, the mean being 89.3 (Table 5, Fig. 6). In 75% analysed populations variation was observed in 6 loci, the monomorphic locus being always *Pgm1*. The remaining populations showed variation in all the loci (Fig. 6). The lowest number of polymorphic loci (5) was

Table 2. Number of phenotypes in 7 enzymes in 32 populations of *D. polymorpha*

Population number	Population name	GOT	EST	PGI	ME	MDH	IDH	PGM
1	Dąbie	3	3	7	8	6	5	2
2	Miedwie	9	6	6	15	7	9	3
3	Gardzko	9	4	6	10	4	4	1
4	Orzechów	3	2	4	3	2	2	1
5	Chłop	3	4	6	12	5	7	1
6	Marwicko	6	5	6	9	4	8	2
7	Czarnogłowy	3	7	6	9	3	5	1
8	Woświn	5	6	6	11	8	9	2
9	Ińsko	7	3	11	13	6	8	1
10	Lubianka	6	4	8	10	5	7	2
11	Duże	5	5	7	7	5	6	1
12	Raduń	9	5	9	10	5	5	1
13	Adamowo	6	5	10	14	4	6	1
14	Sitno	4	4	5	10	3	3	1
15	Ostrowiec	6	5	10	10	3	4	2
16	Płociowe	8	4	11	9	2	6	1
17	Marta	4	6	5	10	3	4	1
18	Krzywe	3	1	9	11	4	4	1
19	Chycina	8	6	7	11	3	4	1
20	Jaroszewskie	6	3	10	8	5	8	1
21	Łeby-Redy	7	2	8	8	5	5	2
22	Vistula Bay	8	6	10	11	6	5	1
23	Śniardwy	7	5	10	11	3	4	1
24	Mikołajskie	8	3	5	9	2	3	1
25	Wersminia	8	6	4	10	3	4	1
26	Inulec	9	5	4	13	4	3	2
27	Majcz	9	7	8	14	4	3	1
28	Necko	9	6	7	14	2	4	1
29	Gosławskie	12	6	5	15	10	7	2
30	Pątnowskie	9	7	8	14	8	9	2
31	Mikorzyńskie	11	7	6	14	10	7	2
32	Ślesińskie	10	5	7	13	8	4	2
	$\bar{x}$:	**7.06**	**4.78**	**7.23**	**10.81**	**4.84**	**5.38**	**1.41**
	Range	3–12	2–7	4–11	3–15	2–10	2–9	1–3
N phenotypes/species		14	8	14	25	18	12	3

Table 3. Mean frequencies of phenotypes of 7 enzymes from 32 populations of *D. polymorpha* from Poland, and their percentage in the populations

Enzyme/phenotype	Frequency in species	% in population
GOT		
1	0.2719	94
2	0.1236	84
3	0.1090	72
4	0.1773	84
5	0.1184	81
6	0.0191	13
7	0.0711	81
8	0.0271	50
9	0.0018	6
10	0.0359	53
11	0.0396	53
12	0.0017	9
13	0.0032	19
14	0.0004	3
EST		
1	0.1455	81
2	0.7026	100
3	0.0620	63
4	0.0209	53
5	0.0353	81
6	0.0239	69
7	0.0003	3
8	0.0095	28
ME		
1	0.3820	100
2	0.0967	88
3	0.0795	63
4	0.0039	31
5	0.1207	94
6	0.0039	25
7	0.0781	100
8	0.0392	66
9	0.0115	31
10	0.0225	69
11	0.0006	6
12	0.0801	97
13	0.0044	22
14	0.0011	6
15	0.0104	59
16	0.0315	72
17	0.0066	31
18	0.0181	72
19	0.0016	13
20	0.0007	3
21	0.0042	28
22	0.0004	6
23	0.0003	3
24	0.0006	6
25	0.0013	6

Enzyme/Phenotype	Frequency in species	% in population
PGI		
1	0.1466	97
2	0.3130	100
3	0.0911	84
4	0.0010	6
5	0.0003	3
6	0.0149	41
7	0.0107	31
8	0.2340	100
9	0.1350	100
10	0.0206	44
11	0.0001	3
12	0.0291	69
13	0.0036	25
14	0.0027	19
MDH		
1	0.6137	100
2	0.2880	100
3	0.0057	22
4	0.0013	9
5	0.0017	16
6	0.0006	6
7	0.0003	3
8	0.0252	41
9	0.0018	16
10	0.0025	6
11	0.0426	91
12	0.0003	3
13	0.0146	50
14	0.0002	3
15	0.0003	3
16	0.0003	3
17	0.0003	3
18	0.0005	9
IDH		
1	0.3549	100
2	0.0020	16
3	0.1329	72
4	0.3259	97
5	0.0079	28
6	0.0050	19
7	0.0041	19
8	0.0011	13
9	0.0003	3
10	0.1206	94
11	0.0411	59
12	0.0042	22
PGM		
1	0.9843	100
2	0.0026	19
3	0.0131	22

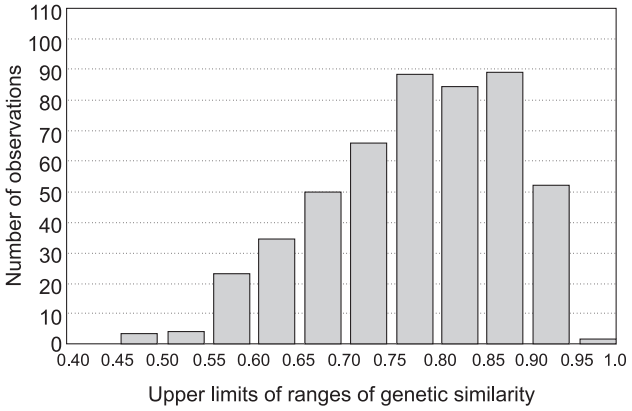

Fig. 3. Distribution of genetic similarities according to HEDRICK (1975) for 32 populations of *D. polymorpha*

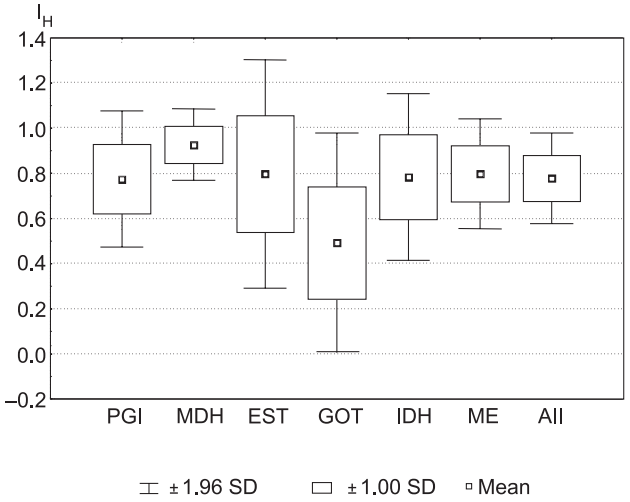

Fig. 5. Values of mean genetic similarity according to HEDRICK (1975) for 6 enzymes in 32 populations of *D. polymorpha*

found in the population from Orzechów (no. 4) at polymorphism criterion 0.95 which most probably resulted from the small sample size.

3.3.2. Number of alleles per locus

The number of alleles per locus and per polymorphic locus for the species was 4.7 (Table 4). Polymorphic loci showed the following numbers of alleles: *Me1* – 8, *Mdh1* – 6, *Got1* – 5, *Est1*, *Pgi1* and *Idh1* – 4, *Pgm1* – 2 (Table 6).

The mean number of alleles per locus and per polymorphic locus per population was 3.5 and 3.7, respectively (Table 5). The analysed populations differed in

Table 4. Parameters of genetic variation of *D. polymorpha* from Poland: N – number of individuals, PL% – percentage of polymorphic loci, A_1 – number of alleles in a locus, G_1 – number of genotypes in a locus, $\overline{H}_T$ – mean expected heterozygosity per locus for species, $\overline{H}_S$ – mean expected heterozygosity per locus per population

Coefficient	N	PL%	A_1	G_1	$\overline{H}_T$	$\overline{H}_S$
Value	3.870	100	4.71	10.29	0.473	0.447

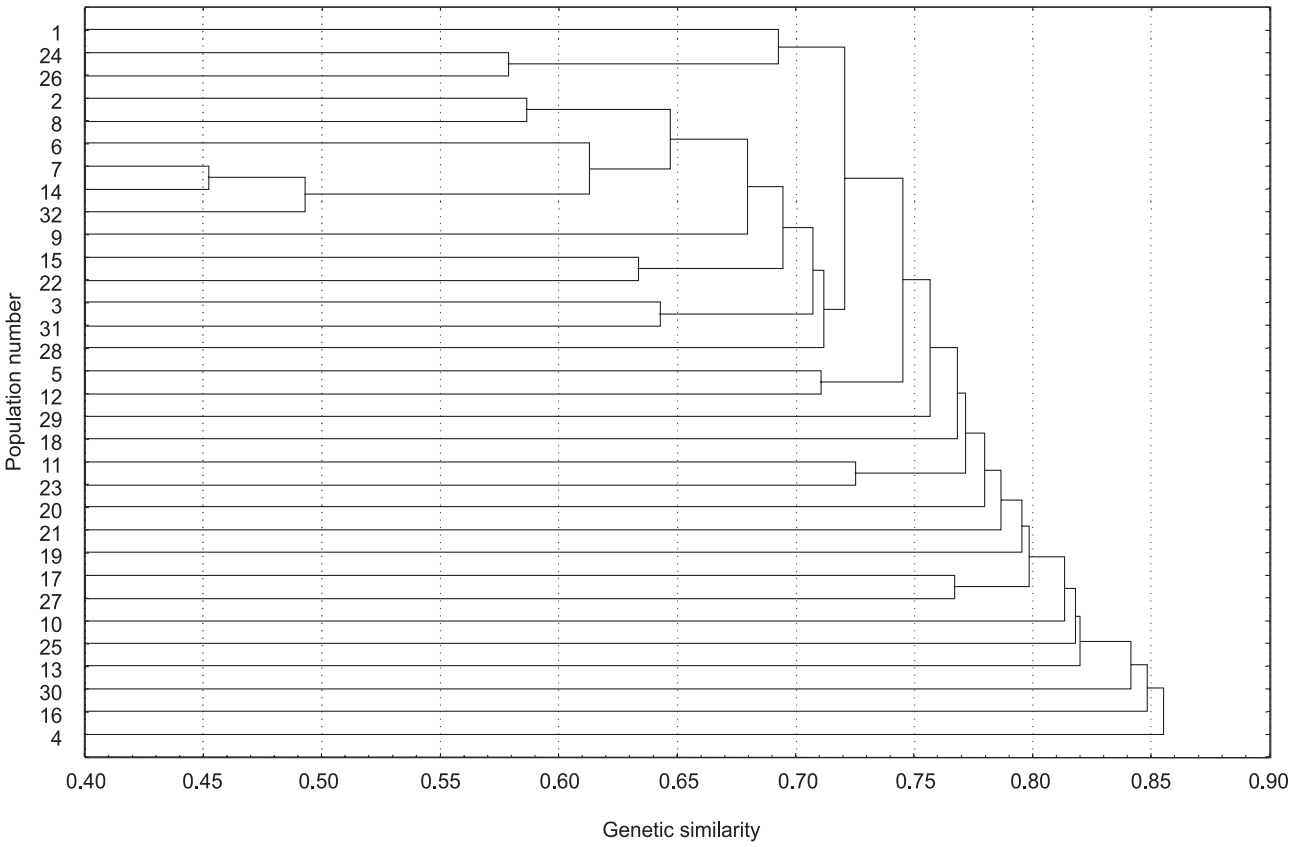

Fig. 4. UPGMA dendrogram based on coefficients of genetic similarity according to HEDRICK (1975) for 32 populations of *D. polymorpha*

Table 5. Polymorphism parameters for 32 populations of *D. polymorpha* from Poland: N – number of individuals, A_1 – mean number of alleles in locus, A_2 – mean number of alleles in polymorphic locus, P – percentage of polymorphic loci, H_S – mean expected heterozygosity per locus in population, SD – standard deviation, asterisk – polymorphism criterion 0.99, double asterisk – according to NEI (1978)

Population number	Population name	N	A_1	SD	A_2	P*	H_S**	SD
1	Dąbie	190	3.86	0.51	3.86	100.00	0.410	0.061
2	Miedwie	300	4.14	0.63	4.14	100.00	0.477	0.082
3	Gardzko	40	3.14	0.46	3.50	85.71	0.482	0.094
4	Orzechów	20	2.29	0.29	2.50	85.71	0.338	0.085
5	Chłop	110	3.29	0.47	3.67	85.71	0.460	0.081
6	Marwicko	110	3.57	0.43	3.57	85.71	0.402	0.083
7	Czarnogłowy	110	3.14	0.55	3.50	85.71	0.487	0.090
8	Woświn	200	4.00	0.62	4.00	85.71	0.449	0.079
9	Ińsko	310	3.86	0.74	3.33	85.71	0.398	0.086
10	Lubianka	150	3.86	0.51	3.86	85.71	0.426	0.081
11	Duże	110	3.71	0.57	4.17	85.71	0.461	0.086
12	Raduń	100	3.57	0.61	4.00	85.71	0.465	0.094
13	Adamowo	220	3.57	0.61	4.00	85.71	0.458	0.089
14	Sitno	40	3.00	0.53	3.33	85.71	0.444	0.085
15	Ostrowiec	120	3.29	0.42	3.29	100.00	0.431	0.087
16	Płociowe	100	3.29	0.57	3.29	85.71	0.362	0.086
17	Marta	100	3.00	0.62	3.33	85.71	0.412	0.080
18	Krzywe	160	3.14	0.59	3.50	85.71	0.431	0.083
19	Chycina	110	3.14	0.59	3.50	85.71	0.452	0.088
20	Jaroszewskie	140	3.29	0.57	3.67	85.71	0.459	0.082
21	Łeby-Redy	90	3.57	0.57	3.57	85.71	0.483	0.091
22	Vistula Bay	80	3.57	0.53	4.00	85.71	0.470	0.088
23	Śniardwy	100	3.14	0.59	3.50	85.71	0.442	0.084
24	Mikołajskie	40	3.00	0.58	3.33	85.71	0.429	0.102
25	Wersminia	80	3.43	0.75	3.83	85.71	0.425	0.085
26	Inulec	100	3.57	0.69	3.57	100.00	0.454	0.082
27	Majcz	140	3.43	0.53	3.83	85.71	0.455	0.090
28	Necko	100	3.29	0.57	3.67	85.71	0.427	0.087
29	Gosławskie	100	3.71	0.47	3.71	100.00	0.531	0.078
30	Pątnowskie	100	4.57	0.61	4.57	100.00	0.500	0.081
31	Mikorzyńskie	100	4.43	0.65	4.43	100.00	0.527	0.079
32	Ślesińskie	100	4.00	0.65	4.00	100.00	0.453	0.084
	$\bar{x}$:	**121**	**3.495**	**0.565**	**3.69**	**89.28**	**0.447**	**0.084**

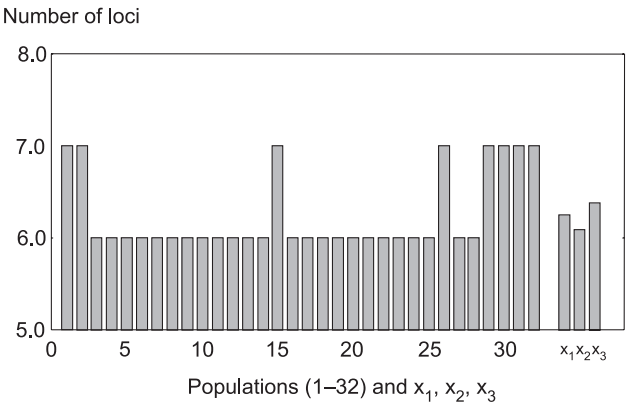

Fig. 6. Number of polymorphic loci in populations of *D. polymorpha* (x_1, x_2, x_3 – mean values at polymorphism criteria 0.99, 0.95 and with no criterion)

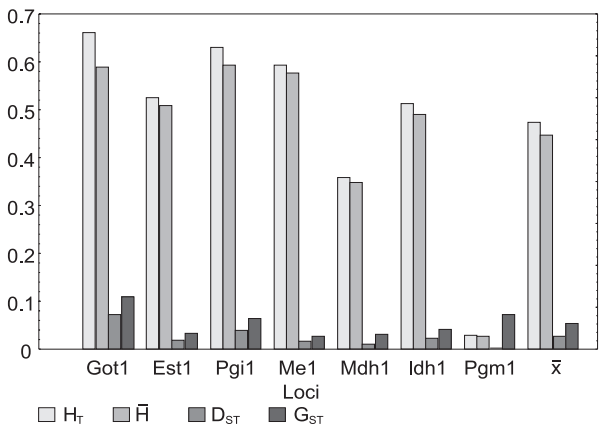

Fig. 7. Comparison of coefficients H_T, $\overline{H}$, D_{ST} and G_{ST} in 7 loci of *D. polymorpha*

Table 6. Number of alleles in 7 loci in 32 populations of *D. polymorpha*

Population number	Population name	*Got1*	*Est1*	*Pgi1*	*Me1*	*Mdh1*	*Idh1*	*Pgm1*
1	Dąbie	3	3	4	6	5	4	2
2	Miedwie	5	3	3	7	5	4	2
3	Gardzko	5	3	3	4	3	3	1
4	Orzechów	3	2	3	3	2	2	1
5	Chłop	3	3	3	5	4	4	1
6	Marwicko	5	3	3	5	3	3	2
7	Czarnogłowy	5	3	3	5	2	3	1
8	Woświn	4	3	3	7	5	4	2
9	Ińsko	4	2	4	7	5	4	1
10	Lubianka	5	3	4	6	4	3	2
11	Duże	4	3	4	6	4	4	1
12	Raduń	5	3	4	6	3	3	1
13	Adamowo	5	3	4	6	3	3	1
14	Sitno	4	3	4	5	2	2	1
15	Ostrowiec	4	3	4	5	2	3	2
16	Płociowe	5	3	4	5	2	3	1
17	Marta	4	3	3	6	2	2	1
18	Krzywe	3	2	4	6	3	3	1
19	Chycina	5	3	4	5	2	2	1
20	Jaroszewskie	5	2	4	5	3	3	1
21	Łeby-Redy	5	2	4	6	3	3	2
22	Vistula Bay	5	3	4	5	4	3	1
23	Śniardwy	5	3	4	5	2	2	1
24	Mikołajskie	5	3	3	5	2	2	1
25	Wersminia	5	3	3	7	2	3	1
26	Inulec	5	3	3	7	3	2	2
27	Majcz	5	3	4	5	3	3	1
28	Necko	5	3	4	5	3	2	1
29	Gosławskie	5	3	3	5	5	3	2
30	Pątnowskie	5	4	4	7	6	4	2
31	Mikorzyńskie	5	3	4	7	6	4	2
32	Ślesińskie	5	3	4	6	6	2	2
	$\bar{x}$:	**4.56**	**2.88**	**3.63**	**5.63**	**3.41**	**2.97**	**1.38**
	Range	3–5	2–4	3–4	3–7	2–6	2–4	1–2
N alleles/species		5	4	4	8	6	4	2

the mean number of alleles per locus per population. It ranged from 2.29 in the population from Orzechów (no. 4) to 4.57 in the population from Pątnowskie (no. 30). Six populations had 3.57 alleles per locus, five populations had 3.14 and another five 3.29 (Table 5). The number of alleles per polymorphic locus was distributed in an analogous way.

Table 6 presents the number of alleles in particular loci in the 32 analysed populations. The mean number of alleles per population was the highest for loci *Me1* – 5.6, *Got1* – 4.6 and *Pgi1* – 3.6. The number of alleles varied between populations: in locus *Me1* from 3 to 7, in locus *Got1* from 3 to 5 and in locus *Pgi1* from 3 to 4, the maximum number of alleles being 8, 5 and 4, respectively. The lowest mean number of alleles per population was observed in loci *Pgm1* – 1.4 and *Est1* – 2.9.

Some populations displayed maximum numbers of alleles in all loci except *Me1*. Twenty two populations had five alleles in locus *Got1*, and 20 populations had four alleles in locus *Pgi1*. The maximum number of alleles in loci *Pgm1* and *Idh1* was found in 12 and 8 populations, respectively. In three populations (numbers 30–32) there were maximum numbers of alleles in locus *Mdh1*. Populations Pątnowskie (no. 30) and Orzechów (no. 4) were characterised by the maximum and minimum number of alleles in all the loci, respectively. In the remaining populations the distribution of the number of alleles in particular loci varied.

3.3.3. Frequency of alleles in populations

The mean allele frequencies in *D. polymorpha* from Poland and their distribution in the 32 populations

Table 7. Allele frequencies in 7 loci of *D. polymorpha* from Poland and their occurrence in the populatons

Loci/alleles	Frequency in species	% in population
Got1		
1	0.536	100
2	0.121	91
3	0.094	81
4	0.119	91
5	0.130	100
Est1		
1	0.538	100
2	0.429	100
3	0.032	88
4	0.001	3
Pgi1		
1	0.361	100
2	0.467	100
3	0.144	100
4	0.028	63
Me1		
1	0.597	100
2	0.188	100
3	0.003	34
4	0.005	47
5	0.105	97
6	0.062	100
7	0.039	78
8	0.001	6
Mdh1		
1	0.777	100
2	0.194	100
3	0.004	25
4	0.002	22
5	0.020	63
6	0.003	31
Idh1		
1	0.592	100
2	0.006	41
3	0.371	100
4	0.031	59
Pgm1		
1	0.986	100
2	0.014	38

are contained in Table 7. In all the polymorphic loci except *Pgi1* one allele had a frequency over 0.53. Rare alleles, of frequencies below 0.01, were found in 4 loci, their total number being 8. The number of rare alleles varied from 0 to 3 between the loci.

Table 8 contains frequencies of alleles for particular loci in the 32 populations. The populations differed with respect to the presence of alleles and their frequency. The presence of alleles in the populations was correlated with the frequency of their occurrence in the species (Table 7). With increasing frequency in the species, the number of populations in which the allele appeared, increased. An exception was allele 6 in locus *Me1*, of a frequency of 6% but which was found in all the populations. Alleles found in all the populations showed a wide range of frequency. Out of 15 alleles in this group, six had frequencies over 0.53, three alleles over 0.36, and five alleles had a range of frequency of 0.06–0.20 (Table 7).

Alleles of frequencies from 0.05 to 0.12 were found on an average in 93% populations each, and two of them were present in every population. Alleles of frequencies from 0.01 to 0.05 were detected on an average in 65% populations each. One of the six alleles of this group, *Est1*-3, was present in as much as 88% populations. Another, *Pgm1*-2, was found only in 38% populations. Alleles of frequencies of 0.002 to 0.01 were detected on an average in 33% populations each. Among the six alleles of this group, the most often encountered was allele *Me1*-4 (47%), the least common being *Mdh1*-4 (22%). Alleles *Est1*-4 and *Me1*-8 which appeared in 1 and 2 populations, respectively, had a frequency of 0.001 (Table 7). The results suggest a wide distribution of alleles of low frequency in the populations.

3.3.4. Expected heterozygosity

The mean expected heterozygosity H_T in *D. polymorpha* was 0.473. The mean value of expected heterozygosity H_S per population was 0.447 and it ranged from 0.338 to 0.531 (Tables 4, 5, 9).

The values of expected heterozygosity H_T and $\overline{H}$ in the analysed loci are presented in Table 9. The mean value of H_T per locus was 0.473, and the mean value of $\overline{H}$ – 0.447. In all the loci except *Got1*, the values of H_T and $\overline{H}$ were similar. This resulted from the lack of differences between the frequencies of alleles in populations with respect to loci *Est1*, *Pgi1*, *Me1*, *Mdh1*, *Idh1* and *Pgm1*. Locus *Got1* proved to be the most variable, with $H_T = 0.661$, followed by loci *Pgi1* (0.631) and *Me1* (0.592). Locus *Pgm1* had the lowest values of H_T (0.028) and $\overline{H}$ (0.026). The values for the remaining loci were within the range of 0.348–0.525 (Fig. 7).

3.3.5. Number of genotypes per locus

The number of genotypes per locus estimated for the species was 10.3 (Table 4). The following numbers

Table 8. Allele frequencies in 7 enzymatic loci in 32 populations of *D. polymorpha* from Poland

	Population number										
Locus	1	2	3	4	5	6	7	8	9	10	11
Got1											
(N)	190	300	40	20	110	110	110	200	310	150	110
1	0.9320	0.4950	0.3750	0.5500	0.6050	0.8230	0.4450	0.7750	0.8660	0.6100	0.7910
2	0.0210	0.1930	0.1750	0.0000	0.0000	0.0590	0.0820	0.1100	0.0550	0.2000	0.0360
3	0.0470	0.0970	0.0870	0.0000	0.0000	0.0090	0.2050	0.0000	0.0000	0.0030	0.0000
4	0.0000	0.1150	0.2130	0.0750	0.3230	0.0820	0.1450	0.0630	0.0690	0.0970	0.1550
5	0.0000	0.1000	0.1500	0.3750	0.0730	0.0270	0.1230	0.0520	0.0100	0.0900	0.0180
Est1											
(N)	190	300	40	20	110	110	110	200	310	150	110
1	0.4760	0.5350	0.5000	0.4500	0.4820	0.6730	0.5090	0.5250	0.5470	0.5270	0.5410
2	0.5180	0.4530	0.4750	0.5500	0.5090	0.2550	0.4590	0.4470	0.4530	0.4700	0.2450
3	0.0050	0.0120	0.0250	0.0000	0.0090	0.0730	0.0320	0.0270	0.0000	0.0030	0.2140
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pgi1											
(N)	190	300	40	20	110	110	110	200	310	150	110
1	0.3680	0.3480	0.4000	0.1750	0.3450	0.3410	0.4730	0.2250	0.3870	0.2930	0.6770
2	0.4870	0.5150	0.3500	0.7000	0.5770	0.2680	0.2500	0.5950	0.3450	0.5570	0.2410
3	0.0760	0.1370	0.2500	0.1250	0.0770	0.3910	0.2770	0.1800	0.1710	0.1370	0.0680
4	0.0680	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0970	0.0130	0.0140
Me1											
(N)	190	300	40	20	110	110	110	200	310	150	110
1	0.7290	0.5800	0.5870	0.8000	0.4450	0.6640	0.5590	0.5850	0.6890	0.6170	0.4050
2	0.1340	0.1420	0.2130	0.1750	0.3140	0.1590	0.2450	0.2620	0.1600	0.1330	0.3860
3	0.0080	0.0070	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0350	0.0030	0.0050
4	0.0240	0.0100	0.0000	0.0000	0.0000	0.0050	0.0050	0.0070	0.0130	0.0070	0.0000
5	0.0450	0.1420	0.0630	0.0000	0.1500	0.1140	0.1000	0.0930	0.0600	0.1830	0.1090
6	0.0610	0.0650	0.1380	0.0250	0.0450	0.0590	0.0910	0.0400	0.0270	0.0570	0.0860
7	0.0000	0.0550	0.0000	0.0000	0.0450	0.0000	0.0000	0.0070	0.0160	0.0000	0.0090
8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mdh1											
(N)	190	300	40	20	110	110	110	200	310	150	110
1	0.7050	0.8750	0.7750	0.7000	0.6550	0.8360	0.7320	0.6550	0.7950	0.8470	0.5450
2	0.2450	0.1170	0.2130	0.3000	0.3230	0.1590	0.2680	0.2800	0.1900	0.1430	0.4050
3	0.0260	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0070	0.0000
4	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0020	0.0000	0.0000
5	0.0180	0.0030	0.0130	0.0000	0.0140	0.0050	0.0000	0.0550	0.0100	0.0000	0.0450
6	0.0000	0.0030	0.0000	0.0000	0.0090	0.0000	0.0000	0.0050	0.0030	0.0030	0.0050
Idh1											
(N)	190	300	40	20	110	110	110	200	310	150	110
1	0.6740	0.4880	0.6630	0.9750	0.6270	0.5180	0.5590	0.4900	0.5760	0.6470	0.4360
2	0.0260	0.0820	0.0130	0.0000	0.0050	0.0090	0.0000	0.0050	0.0030	0.0000	0.0050
3	0.2920	0.4080	0.3250	0.0250	0.3410	0.4180	0.4180	0.3850	0.3770	0.3170	0.4730
4	0.0080	0.0220	0.0000	0.0000	0.0270	0.0550	0.0230	0.1200	0.0440	0.0370	0.0860
Pgm1											
(N)	190	300	40	20	110	110	110	200	310	150	110
1	0.8420	0.9370	1.0000	1.0000	1.0000	0.9950	1.0000	0.9980	1.0000	0.9970	1.0000
2	0.1580	0.0630	0.0000	0.0000	0.0000	0.0050	0.0000	0.0020	0.0000	0.0030	0.0000

Table 8. continued

	Population number										
Locus	12	13	14	15	16	17	18	19	20	21	22
Got1											
(N)	100	220	40	120	100	100	160	110	140	90	80
1	0.5150	0.5270	0.4500	0.6750	0.7700	0.4650	0.7840	0.3770	0.6570	0.2220	0.4190
2	0.1100	0.3840	0.2250	0.0630	0.1200	0.1650	0.0000	0.0270	0.2180	0.1170	0.1810
3	0.0400	0.0730	0.2750	0.2460	0.0150	0.3150	0.0000	0.4230	0.0110	0.2670	0.0190
4	0.1750	0.0050	0.0000	0.0000	0.0550	0.0000	0.1840	0.0410	0.0860	0.0670	0.1440
5	0.1600	0.0110	0.0500	0.0170	0.0400	0.0550	0.0310	0.1320	0.0290	0.3280	0.2370
Est1											
(N)	100	220	40	120	100	100	160	110	140	90	80
1	0.6150	0.6180	0.4750	0.5540	0.8000	0.5000	0.5000	0.5270	0.5000	0.5170	0.4500
2	0.3600	0.3640	0.5120	0.4000	0.1600	0.4900	0.5000	0.4500	0.5000	0.4830	0.5120
3	0.0250	0.0180	0.0130	0.0460	0.0400	0.0100	0.0000	0.0230	0.0000	0.0000	0.0380
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pgi1											
(N)	100	220	40	120	100	100	160	110	140	90	80
1	0.4050	0.2820	0.2130	0.3670	0.2900	0.0600	0.1690	0.1640	0.4110	0.5170	0.2560
2	0.4000	0.4680	0.6130	0.4170	0.4500	0.8100	0.5160	0.4090	0.3250	0.3440	0.5750
3	0.1450	0.2050	0.1250	0.1460	0.0850	0.1300	0.2660	0.4230	0.2320	0.1280	0.0940
4	0.0500	0.0450	0.0500	0.0710	0.1750	0.0000	0.0500	0.0050	0.0320	0.0110	0.0750
Me1											
(N)	100	220	40	120	100	100	160	110	140	90	80
1	0.5250	0.5140	0.6250	0.5710	0.7500	0.6150	0.5750	0.5360	0.5680	0.5330	0.6940
2	0.1250	0.1570	0.1620	0.1250	0.1500	0.2300	0.2030	0.2860	0.2930	0.1780	0.1940
3	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0030	0.0000	0.0040	0.0060	0.0000
5	0.1450	0.0950	0.1000	0.0710	0.0450	0.0150	0.1060	0.1410	0.1070	0.2280	0.0630
6	0.1550	0.1930	0.0250	0.0080	0.0400	0.1300	0.0870	0.0230	0.0290	0.0440	0.0250
7	0.0450	0.0250	0.0870	0.2250	0.0150	0.0050	0.0250	0.0140	0.0000	0.0110	0.0250
8	0.0000	0.0160	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000
Mdh1											
(N)	100	220	40	120	100	100	160	110	140	90	80
1	0.8350	0.8140	0.7880	0.8830	0.9100	0.7500	0.7660	0.7820	0.7000	0.7610	0.7690
2	0.1400	0.1700	0.2130	0.1170	0.0900	0.2500	0.1970	0.2180	0.2820	0.1670	0.1620
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0060
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.0250	0.0160	0.0000	0.0000	0.0000	0.0000	0.0380	0.0000	0.0180	0.0720	0.0630
6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Idh1											
(N)	100	220	40	120	100	100	160	110	140	90	80
1	0.6150	0.5520	0.6880	0.4380	0.2850	0.6550	0.5590	0.7270	0.6180	0.6000	0.4750
2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000
3	0.3700	0.4250	0.3130	0.5580	0.5950	0.3450	0.3720	0.2730	0.3320	0.3940	0.4380
4	0.0150	0.0230	0.0000	0.0040	0.1200	0.0000	0.0690	0.0000	0.0500	0.0000	0.0870
Pgm1											
(N)	100	220	40	120	100	100	160	110	140	90	80
1	1.0000	1.0000	1.0000	0.9880	1.0000	1.0000	1.0000	1.0000	1.0000	0.9940	1.0000
2	0.0000	0.0000	0.0000	0.0130	0.0000	0.0000	0.0000	0.0000	0.0000	0.0060	0.0000

Table 8. continued

	Population number									
Locus	23	24	25	26	27	28	29	30	31	32
Got1										
(N)	100	40	80	100	140	100	100	100	100	100
1	0.6000	0.2370	0.4880	0.5100	0.4290	0.4700	0.2100	0.3850	0.3350	0.3600
2	0.1050	0.2000	0.0750	0.1000	0.1140	0.1250	0.1000	0.2500	0.2100	0.0450
3	0.0250	0.1000	0.0500	0.0350	0.0790	0.1200	0.2250	0.0200	0.0800	0.1450
4	0.1400	0.1000	0.2560	0.1800	0.1360	0.0850	0.1750	0.2300	0.2450	0.1850
5	0.1300	0.3630	0.1310	0.1750	0.2430	0.2000	0.2900	0.1150	0.1300	0.2650
Est1										
(N)	100	40	80	100	140	100	100	100	100	100
1	0.4950	0.4630	0.5440	0.6150	0.7070	0.7600	0.4450	0.4500	0.4600	0.4600
2	0.4950	0.5000	0.4250	0.3700	0.2070	0.2150	0.5000	0.4950	0.4800	0.5000
3	0.0100	0.0380	0.0310	0.0150	0.0860	0.0250	0.0550	0.0500	0.0600	0.0400
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050	0.0000	0.0000
Pgi1										
(N)	100	40	80	100	140	100	100	100	100	100
1	0.4050	0.5750	0.5000	0.5300	0.4680	0.4250	0.3600	0.3400	0.3800	0.3900
2	0.4500	0.3500	0.4880	0.4500	0.4460	0.5100	0.5450	0.4900	0.5050	0.5050
3	0.0850	0.0750	0.0130	0.0200	0.0460	0.0550	0.0950	0.1650	0.1050	0.1000
4	0.0600	0.0000	0.0000	0.0000	0.0390	0.0100	0.0000	0.0050	0.0100	0.0050
Me1										
(N)	100	40	80	100	140	100	100	100	100	100
1	0.6100	0.6630	0.6810	0.5550	0.5640	0.6200	0.5600	0.5700	0.4300	0.6950
2	0.1900	0.1120	0.1440	0.2450	0.1750	0.1650	0.1550	0.1250	0.1450	0.1350
3	0.0000	0.0000	0.0060	0.0100	0.0000	0.0000	0.0000	0.0050	0.0100	0.0000
4	0.0000	0.0000	0.0060	0.0150	0.0000	0.0000	0.0000	0.0050	0.0250	0.0150
5	0.1350	0.1250	0.0940	0.0750	0.1610	0.1000	0.0950	0.1450	0.2100	0.0700
6	0.0250	0.0630	0.0380	0.0400	0.0680	0.0500	0.0500	0.1050	0.0750	0.0150
7	0.0400	0.0380	0.0310	0.0600	0.0320	0.0650	0.1400	0.0450	0.1050	0.0700
8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mdh1										
(N)	100	40	80	100	140	100	100	100	100	100
1	0.8150	0.9380	0.8560	0.7700	0.8070	0.8150	0.6900	0.7700	0.7250	0.8000
2	0.1850	0.0630	0.1440	0.2150	0.1890	0.1700	0.1550	0.1250	0.1750	0.1400
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0200	0.0100	0.0150	0.0300
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0150	0.0100	0.0150	0.0050
5	0.0000	0.0000	0.0000	0.0150	0.0040	0.0000	0.1200	0.0750	0.0450	0.0050
6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0150	0.0000	0.0100	0.0250	0.0200
Idh1										
(N)	100	40	80	100	140	100	100	100	100	100
1	0.6000	0.5630	0.5810	0.5550	0.5710	0.6350	0.5550	0.6300	0.6900	0.6900
2	0.0000	0.0000	0.0130	0.0000	0.0040	0.0000	0.0000	0.0100	0.0100	0.0000
3	0.4000	0.4380	0.4060	0.4450	0.4250	0.3650	0.3150	0.3050	0.2750	0.3100
4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1300	0.0550	0.0250	0.0000
Pgm1										
(N)	100	40	80	100	140	100	100	100	100	100
1	1.0000	1.0000	1.0000	0.9900	1.0000	1.0000	0.9400	0.9600	0.9200	0.9800
2	0.0000	0.0000	0.0000	0.0100	0.0000	0.0000	0.0600	0.0400	0.0800	0.0200

Table 9. Values of expected heterozygosity coefficients H_T and $\overline{H}$, and genetic diversity D_{ST} and G_{ST} in the analysed loci of *D. polymorpha*

Loci	H_T	$\overline{H}$	D_{ST} (%)	G_{ST} (%)
Got1	0.661	0.589	7.16	10.85
Est1	0.525	0.508	1.78	3.38
Pgi1	0.631	0.592	3.98	6.31
Me1	0.592	0.576	1.56	2.63
Mdh1	0.359	0.348	1.07	2.99
Idh1	0.512	0.490	2.17	4.23
Pgm1	0.028	0.026	0.21	7.28
$\bar{x}$:	**0.473**	**0.447**	**2.56**	**5.42**

of genotypes were found in the analysed loci: *Me1* – 17, *Got1* and *Mdh1* – 13, *Pgi1* – 10, *Idh1* – 9, *Est1* – 7, *Pgm1* – 3 (Tables 10, 11).

Table 10 presents the numbers of genotypes in particular loci and populations of *D. polymorpha*. The mean number of genotypes per locus per population was 5.3, the extreme values being 2.43 and 7.29 (populations no. 4 and 30). The highest mean values of the number of genotypes per locus per population were observed in loci *Me1* – 8.7, *Pgi1* – 6.8 and *Got1* – 6.6. The number of genotypes in locus *Me1* varied between populations from 3 to 11, in locus *Pgi1* from 4 to 10 and in locus *Got1* from 3 to 10, the maximum numbers of genotypes being 17, 10 and 13, respectively. In two populations, 2 and 16, in loci *Pgm1* and *Pgi1* there were all genotypes found in the species. In the remaining populations in all the loci the number of genotypes was always lower compared to the maximum number for the species.

There was a strong linear correlation (r=0.9 at $p<0.001$) between the number of genotypes per locus and the number of alleles per locus (Fig. 8). With increasing number of alleles in a population the number of genotypes observed increases.

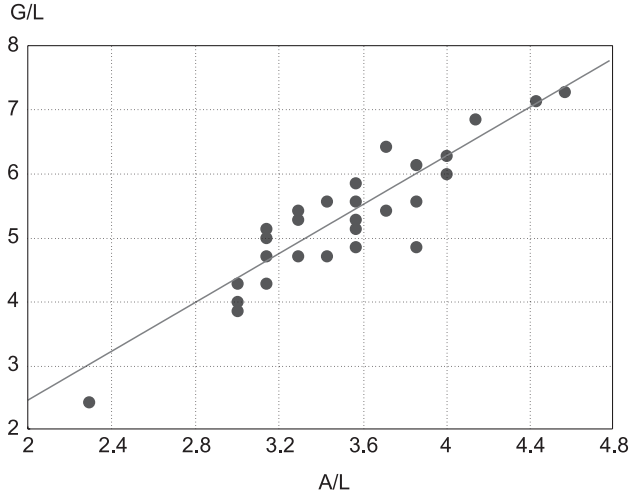

Fig. 8. Linear correlation between the number of genotypes in locus per population (G/L) and the number of alleles in locus per population (A/L)

3.3.6. Genotype frequencies in populations

The mean frequencies of genotypes and their distribution in the 32 populations of *D. polymorpha* are presented in Table 11. In loci *Est1*, *Mdh1* and *Pgm1* one of the genotypes was considerably more frequent than the others. In the remaining loci the number of genotypes was 3–5, and their frequencies ranged from 0.11 to 0.38. In all the loci rare genotypes were found to occur, of frequency below 0.01. The number of rare genotypes was 29, and for particular loci it ranged from 1 (*Pgm1*) to 8 (*Me1* and *Mdh1*). The frequencies of genotypes in the species were correlated with their frequencies in the populations. With increasing frequency of a genotype in the species, the number of populations where the genotype was present increased. Nine high-frequency (0.18–0.98) genotypes were present in all the populations. Only in locus *Me1* genotype 1–6, in spite of its 0.08 frequency, was also found in all the populations. Out of 20 genotypes of a frequency of 0.001–0.003, 7 were found in single populations, 6 in two and 2 in three populations. The remaining genotypes of this group were found in 5 or 7 populations (Table 11).

The hypothesis of panmictic character of populations of *D. polymorpha* was tested based on genotype frequency, using Chi square test (Table 12). Analysis of loci with respect to Hardy-Weinberg equilibrium showed that over half of them were not in equilibrium. On an average each population had 2.94 loci in Hardy-Weinberg equilibrium out of 6.4 loci tested per population. The extreme numbers of loci in equilibrium were 0 and 5 and were found in populations Dąbie and Łeba-Reda (Table 12).

Locus *Got1* was in Hardy-Weinberg equilibrium only in the population from Ińsko, *Est1* in populations Płociowe, Majcz and Necko (Table 12). The absence of equilibrium resulted from the high excess of heterozygotes in 94% populations in locus *Est1* and in 91% populations in *Got1* (Table 13). Locus *Pgi1* was the most often in equilibrium (84.4% populations); it

Table 10. Number of genotypes in 7 loci in 32 populations of *D. polymorpha*

Population number	Population name	*Got1*	*Est1*	*Pgi1*	*Me1*	*Mdh1*	*Idh1*	*Pgm1*	x̄:
1	Dąbie	3	3	7	8	6	5	2	4.86
2	Miedwie	9	5	6	10	7	8	3	6.86
3	Gardzko	8	3	6	7	4	4	1	4.71
4	Orzechów	3	2	4	3	2	2	1	2.43
5	Chłop	3	3	6	9	5	6	1	4.71
6	Marwicko	6	5	6	8	4	6	2	5.29
7	Czarnogłowy	8	6	6	8	3	4	1	5.14
8	Woświn	5	5	6	11	8	7	2	6.29
9	Ińsko	7	3	9	11	6	6	1	6.14
10	Lubianka	6	4	8	9	5	5	2	5.57
11	Duże	5	5	7	9	6	5	1	5.43
12	Raduń	8	4	8	9	5	4	1	5.57
13	Adamowo	6	4	9	10	4	5	1	5.57
14	Sitno	4	4	5	8	3	3	1	4.00
15	Ostrowiec	6	5	9	8	3	4	2	5.29
16	Płociowe	7	4	10	8	2	6	1	5.43
17	Marta	4	5	5	9	3	3	1	4.29
18	Krzywe	3	1	9	9	4	3	1	4.29
19	Chycina	7	5	7	9	3	3	1	5.00
20	Jaroszewskie	6	3	9	8	5	6	1	5.43
21	Łeby-Redy	6	2	7	8	5	4	2	4.86
22	Vistula Bay	7	5	9	9	6	4	1	5.86
23	Śniardwy	7	4	9	9	3	3	1	5.14
24	Mikołajskie	7	2	5	7	2	3	1	3.86
25	Wersminia	7	5	4	9	3	4	1	4.71
26	Inulec	8	4	4	11	4	3	2	5.14
27	Majcz	8	6	7	9	4	4	1	5.57
28	Necko	8	5	7	9	5	3	1	5.43
29	Gosławskie	10	5	5	9	8	6	2	6.43
30	Pątnowskie	9	6	7	9	7	8	2	7.29
31	Mikorzyńskie	10	6	6	11	9	6	2	7.14
32	Ślesińskie	10	4	7	9	7	3	2	6.00
	x̄:	**6.59**	**4.16**	**6.84**	**8.72**	**4.72**	**4.59**	**1.41**	**5.30**
	Range	3–10	1–6	4–10	3–11	2–9	2–8	1–3	
N genotypes/species		13	7	10	17	13	9	3	

was followed by loci *Mdh1* and *Idh1* (75.0% and 56.3% populations, respectively). Loci *Me1* and *Pgm1* were not in equilibrium in over half of the analysed populations (Table 13).

Table 14 presents the values of heterozygote excess coefficient (D) in the analysed loci in the 32 populations. A correlation was found between the D value and the Hardy-Weinberg equilibrium for particular loci. The highest and the lowest (negative) D values were observed in the loci which were not in equilibrium. These included *Est1*, *Got1* and *Pgm1* of the following mean D values: 0.59, 0.20 and –0.21. In the remaining loci the number of homo- and heterozygotes was in equilibrium ($D<0.05$), and the lack of equilibrium in some of the populations resulted from rare genotypes whose distributions departed from the expected.

3.3.7. Individual genotypic variation

Ninety two unique genotypes were found in the populations which constituted ca. 75.8% all genotypes. The percentage of unique genotypes varied between populations, from 36% to 98% (Table 15). The per-

Table 11. Mean genotype frequencies in 7 loci in *D. polymorpha* and their occurrence in the populations

Loci/Genotypes	Frequency in species	% population
Got1		
1–1	0.2719	94
1–2	0.1236	84
1–3	0.1090	72
1–4	0.1773	84
1–5	0.1184	81
2–2	0.0004	3
2–4	0.0191	13
2–5	0.0982	88
3–3	0.0018	6
3–4	0.0359	53
3–5	0.0396	53
4–4	0.0017	9
4–5	0.0032	19
Est1		
1–1	0.1455	81
1–2	0.7646	100
1–3	0.0209	53
2–2	0.0353	81
2–3	0.0239	69
2–4	0.0003	3
3–3	0.0095	28
Me1		
1–1	0.3820	100
1–2	0.1762	100
1–4	0.0039	31
1–5	0.1246	97
1–6	0.0781	100
1–7	0.0507	66
2–2	0.0225	69
2–3	0.0006	6
2–5	0.0856	97
2–6	0.0419	81
2–7	0.0263	75
3–3	0.0007	3
3–4	0.0042	28
4–4	0.0004	6
4–6	0.0003	3
5–5	0.0006	6
6–8	0.0013	6
Pgi1		
1–1	0.1466	97
1–2	0.3130	100
1–3	0.0924	84
1–4	0.0256	47
2–2	0.2340	100
2–3	0.1350	100
2–4	0.0207	44
3–3	0.0291	69
3–4	0.0036	25
4–4	0.0027	19
Mdh1		
1–1	0.6137	100
1–2	0.2880	100
1–3	0.0070	25
1–4	0.0026	22
1–5	0.0252	41
1–6	0.0043	22
2–2	0.0426	91
2–4	0.0003	3
2–5	0.0146	50
2–6	0.0002	3
5–5	0.0003	3
5–6	0.0006	6
6–6	0.0005	9
Idh1		
1–1	0.3549	100
1–2	0.0020	16
1–3	0.4588	100
1–4	0.0129	30
2–2	0.0041	19
2–3	0.0014	19
3–3	0.1206	94
3–4	0.0411	59
4–4	0.0042	22
Pgm1		
1–1	0.9843	100
1–2	0.0026	19
2–2	0.0131	22

centage of unique genotypes in the population depended on the number of analysed individuals: the more numerous specimens were analysed, the lower the percentage of unique genotypes (Fig. 9). The correlation was statistically significant ($r=-0.4$, $p<0.005$), though some populations behaved differently. For example, in the population from lake Marta (no. 17), with 100 individuals analysed, the percentage of unique genotypes was 36%, and in the population from lake Miedwie (no. 2), where 300 individuals were analysed, the percentage was high and amounted to almost 73%. No correlation was found between the num-

Table 12. Values of Chi square for 7 loci with respect to Hardy-Weinberg equilibrium (asterisk denotes p<0.05, degrees of freedom given in brackets)

	Chi^2						
Population	*Got1*	*Est1*	*Pgi1*	*Me1*	*Mdh1*	*Idh1*	*Pgm1*
Dąbie	169.0* (3)	159.8 * (3)	75.2* (6)	115.3* (15)	29.5* (10)	205.8* (6)	183.5 * (1)
Miedwie	158.7* (10)	138.0* (3)	1.2 (3)	350.0* (21)	75.4* (10)	348.1* (6)	168.2* (1)
Gardzko	30.7* (10)	28.6* (3)	1.3 (3)	0.17 (3)	2.3 (1)	0.0 (1)	–
Orzechów	9.5* (3)	11.0* (1)	0.5 (3)	0.2 (3)	2.3 (1)	0.0 (1)	–
Chłop	42.9* (3)	97.9* (3)	0.5 (3)	15.5 (10)	3.7 (6)	6.6 (6)	–
Marwicko	31.9* (10)	15.6* (3)	5.2 (3)	7.2 (10)	0.9 (3)	72.3* (6)	0.0 (1)
Czarnogłowy	79.2* (10)	87.3* (3)	2.8 (3)	25.6* (10)	0.1 (1)	8.2* (3)	–
Woświn	102.6* (6)	109.0* (3)	3.8 (3)	186.7* (21)	11.9 (10)	108.0* (6)	0.0 (1)
Ińsko	10.2 (6)	31.0* (1)	47.8* (6)	308.7* (21)	97.9* (10)	150.5* (6)	–
Lubianka	115.0* (10)	107.3* (3)	7.0 (6)	60.1* (15)	36.7* (6)	25.2* (3)	0.0 (1)
Duże	82.3* (6)	84.0* (3)	11.9 (6)	19.5 (15)	4.7 (6)	59.1* (6)	–
Raduń	35.4* (10)	30.4* (3)	11.5 (6)	24.0 (15)	16.5* (3)	3.3 (3)	–
Adamowo	152.2* (10)	65.7* (3)	3.9* (6)	94.0* (15)	1.4 (3)	33.8* (3)	–
Sitno	33.3* (6)	19.5* (3)	17.0* (6)	2.1 (10)	0.0 (1)	0.0 (1)	–
Ostrowiec	25.8* (6)	18.2* (3)	3.0 (6)	20.9* (10)	0.0 (1)	1.7 (3)	0.0 (1)
Płociowe	19.4* (10)	0.7 (3)	3.1 (6)	3.8 (10)	0.2 (1)	23.9* (3)	–
Marta	122.1* (6)	69.5* (3)	0.1 (3)	33.9* (15)	0.5 (1)	14.2* (1)	–
Krzywe	9.5* (3)	157.0* (1)	8.4 (6)	25.8* (15)	2.1 (3)	69.2* (3)	–
Chycina	66.2* (10)	71.8* (3)	2.1 (6)	13.5 (10)	1.0 (1)	0.7 (1)	–
Jaroszewskie	27.1* (10)	87.2* (1)	2.9 (6)	20.2* (10)	8.8* (3)	6.6 (3)	–
Łeby-Redy	69.9* (10)	75.9* (1)	1.8 (6)	23.2 (15)	4.6 (3)	0.0 (3)	0.0 (1)
Vistula Bay	76.4* (10)	60.7* (3)	5.3 (6)	2.7 (10)	0.7 (6)	17.3* (3)	–
Śniardwy	25.0* (10)	51.2* (3)	0.8 (6)	18.4 (10)	0.4 (1)	0.1 (1)	–
Mikołajskie	39.2* (10)	35.4* (3)	5.5 (3)	0.7 (10)	0.0 (1)	0.4 (1)	–
Wersminia	90.1* (10)	35.6* (3)	1.5 (3)	56.1* (21)	0.7 (1)	19.8* (3)	–
Inulec	71.9* (10)	28.0* (3)	5.5 (3)	103.9* (21)	11.2* (3)	1.3 (1)	25.1* (1)
Majcz	89.9* (10)	2.9 (3)	13.8* (6)	20.5* (10)	0.6 (3)	2.1 (3)	–
Necko	54.5* (10)	8.1 (3)	1.9 (6)	17.8 (10)	10.5* (3)	0.7 (1)	–
Gosławskie	46.3* (10)	46.1* (3)	4.0 (3)	23.0* (10)	2.1 (10)	0.0 (3)	84.0* (1)
Pątnowskie	48.6* (10)	61.3* (6)	2.9 (6)	67.3* (21)	2.8 (15)	10.8 (6)	76.6* (1)
Mikorzyńskie	39.6* (10)	76.0* (3)	9.1 (6)	84.5* (21)	1.3 (15)	1.2 (6)	87.8* (1)
Ślesińskie	50.2* (10)	87.3* (3)	1.1 (6)	1.0 (15)	0.4 (15)	0.0 (1)	56.3* (1)

ber of alleles in the population and the percentage of unique genotypes, and there was a small correlation, on the border of significance (r=0.31, p=0.09) between the number of genotypes in the population and the percentage of unique genotypes (Fig. 10).

3.3.8. Genetic diversity within colonies of *D. polymorpha*

Three hundred eighty seven colonies were analysed for 7 enzymatic loci. All the colonies proved to be polymorphic.

The genetic diversity within the colonies is presented as three examples: Orzechów (no. 4), Woświn (no. 8) and Gosławskie (no. 29). The populations represent the lowest (population 4), moderate (8) and the highest (29) values of the expected heterozygosity per locus per population (H_S, see Table 5) and the highest (population 29) and medium (4, 8) percentage of unique genotypes (see Table 15).

Each of the 32 colonies, including 2 from the population Orzechów, 20 from Woświn and 10 from Gosławskie, was polymorphic with respect to 5 to 7 enzymes, at the polymorphism criterion 0.99. The fewest polymorphic loci were found in two colonies, one from each of the populations Orzechów and Woświn (Table 16).

Table 13. Analysis of enzymatic loci with respect to Hardy-Weinberg equilibrium

Loci	Hardy-Weinberg equilibrium		Homozygote excess		Heterozygote excess	
	number of populations	% populations	number of populations	% populations	number of populations	% populations
Got1	1	3.1	3	9.4	29	90.6
Est1	3	9.4	2	6.2	30	93.8
Pgi1	27	84.4	13	40.6	19	59.4
Me1	15	46.9	8	25.0	24	75.0
Mdh1	24	75.0	19	59.4	13	40.6
Idh1	18	56.3	14	43.8	18	56.2
Pgm1	5	41.7	7	58.3	5	41.7

Table 14. Values of heterozygote excess coefficient (D) for particular loci in the 32 populations of *D. polymorpha*

Population number	Population name	*Got1*	*Est1*	*Pgi1*	*Me1*	*Mdh1*	*Idh1*	*Pgm1*
1	Dąbie	–0.68	0.91	–0.30	–0.05	–0.20	–0.55	–1.00
2	Miedwie	0.24	0.63	–0.05	0.15	0.00	–0.38	–0.78
3	Gardzko	0.20	0.81	0.03	0.07	0.08	0.29	–
4	Orzechów	0.63	0.82	0.08	0.22	0.43	0.03	–
5	Chłop	0.51	0.93	–0.08	–0.01	–0.05	0.02	–
6	Marwicko	0.02	0.14	–0.10	0.02	0.02	0.53	0.01
7	Czarnogłowy	0.08	0.68	0.04	–0.02	0.04	–0.18	–
8	Woświn	–0.04	0.69	–0.09	–0.24	–0.18	0.39	0.00
9	Ińsko	0.03	0.32	–0.09	–0.11	–0.04	0.37	–
10	Lubianka	0.03	0.85	–0.07	0.03	–0.01	0.31	0.00
11	Duże	–0.17	–0.15	0.03	0.05	–0.02	0.51	–
12	Raduń	0.26	0.53	0.09	0.16	–0.26	0.07	–
13	Adamowo	0.61	0.52	0.00	0.03	–0.06	0.33	–
14	Sitno	0.50	0.71	–0.15	0.02	–0.03	–0.01	–
15	Ostrowiec	0.30	0.21	0.12	0.16	–0.03	–0.13	0.01
16	Płociowe	0.03	0.02	0.11	0.05	0.10	–0.13	–
17	Marta	0.47	0.82	0.02	–0.13	–0.09	0.39	–
18	Krzywe	0.23	1.00	0.03	0.01	0.02	0.37	–
19	Chycina	0.43	0.77	0.02	0.07	0.12	0.10	–
20	Jaroszewskie	0.27	0.80	0.01	0.09	0.16	–0.04	–
21	Łeby-Redy	0.33	0.94	0.12	0.11	–0.14	–0.01	0.01
22	Vistula Bay	0.17	0.76	–0.15	0.05	0.12	0.13	–
23	Śniardwy	0.18	0.71	0.03	0.02	0.09	0.04	–
24	Mikołajskie	0.26	0.87	–0.26	0.14	0.07	–0.14	–
25	Wersminia	0.17	0.63	0.12	–0.06	–0.14	0.03	–
26	Inulec	0.14	0.49	0.16	–0.02	–0.25	–0.13	–1.00
27	Majcz	0.22	0.00	0.20	0.06	–0.02	–0.13	–
28	Necko	0.08	–0.15	0.13	0.02	–0.05	0.10	–
29	Gosławskie	0.26	0.60	0.15	0.05	0.13	0.01	–1.00
30	Pątnowskie	0.16	0.58	–0.03	0.14	0.12	–0.03	–1.00
31	Mikorzyńskie	0.18	0.71	0.09	0.16	–0.02	0.12	–1.00
32	Ślesińskie	0.28	0.83	–0.09	0.04	0.00	–0.02	–1.00

The number of alleles per locus per colony was on an average 2.62 and ranged from 2.00 to 3.43. The number of alleles per polymorphic locus ranged from 2.33 to 3.71, the mean being 2.86 (Table 16). In case of locus *Got1* some colonies had the maximum number of alleles for the species. In the remaining polymorphic loci the number of alleles in a colony was 2–6 (Tables 17, 18).

Most often in a colony there were 10 different genotypes in 10 analysed specimens (Table 16). The lowest numbers of alleles per locus and per polymorphic locus, numbers of genotypes and percentage of polymorphic loci were found in two colonies of the population Orzechów.

Tables 17 and 18 show frequencies of alleles in 32 colonies in the three above-mentioned populations. In loci *Me1*, *Mdh1* and *Pgm1* one of the alleles had a decidedly higher frequency compared to the other alleles. In the remaining loci the highest frequencies were observed in case of two alleles of similar frequency values. Colonies within a population differed with respect to the occurrence of rare alleles and low-frequency alleles. Colonies of different populations differed also in high-frequency alleles (Tables 17, 18). For example, allele *Got1*-5 in the population from Woświn was present in 55% colonies with a frequency of 0.05–0.20, while in the population from Gosławskie it was present in all the colonies and its frequency ranged from 0.10 to 0.45. A similar phenomenon was observed in case of allele *Me1*-7.

Table 15. Percentage of unique genotypes (UG) in the 32 populations of *D. polymorpha*

Population number	Population name	N	% UG
1	Dąbie	190	46.3
2	Miedwie	300	72.7
3	Gardzko	40	85.0
4	Orzechów	20	60.0
5	Chłop	110	62.7
6	Marwicko	110	74.6
7	Czarnogłowy	110	82.7
8	Woświn	200	61.5
9	Ińsko	310	39.7
10	Lubianka	150	60.0
11	Duże	110	80.9
12	Raduń	100	88.0
13	Adamowo	220	58.6
14	Sitno	40	80.0
15	Ostrowiec	120	84.2
16	Płociowe	100	75.0
17	Marta	100	36.0
18	Krzywe	160	50.6
19	Chycina	110	72.7
20	Jaroszewskie	140	81.4
21	Łeby-Redy	90	75.3
22	Vistula Bay	80	80.0
23	Śniardwy	100	85.0
24	Mikołajskie	40	90.0
25	Wersminia	80	81.3
26	Inulec	100	85.0
27	Majcz	140	87.1
28	Necko	100	92.0
29	Gosławskie	100	98.0
30	Pątnowskie	100	92.0
31	Mikorzyńskie	100	98.0
32	Ślesińskie	100	80.0

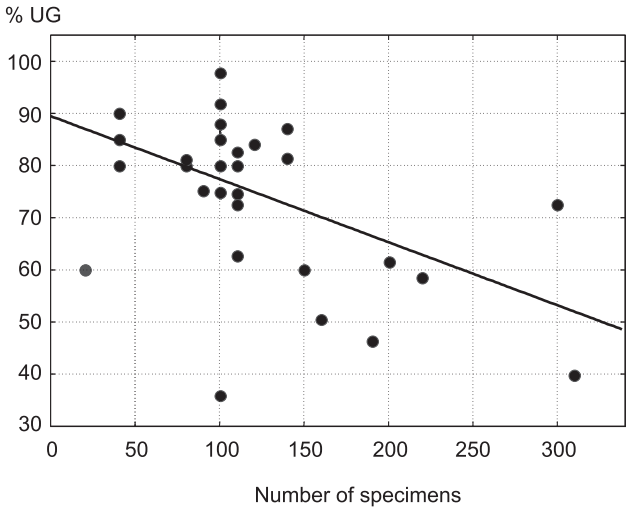

Fig. 9. Negative linear correlation between the percentage of unique genotypes (%UG) in the population and the number of analysed specimens

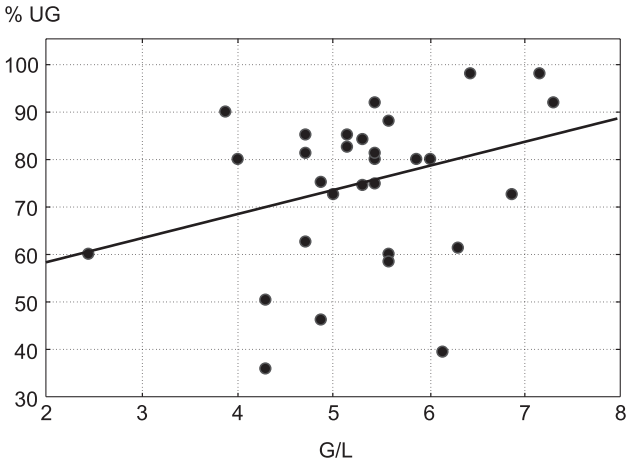

Fig. 10. Linear correlation between the percentage of unique genotypes in the population (%UG) and the number of genotypes in a locus per population (G/L)

Table 16. Parameters of polymorphism in the 32 colonies of *D. polymorpha* from the populations Woświn, Gosławskie and Orzechów (A_1 – mean number of alleles per locus, A_2 – mean number of alleles per polymorphic locus, PL% – percentage of polymorphic loci, G_1 – number of different genotypes, x – mean values per colony)

Lake/colony number	A_1	A_2	PL%	G_1	Lake/colony number	A_1	A_2	PL%	G_1
Woświn					Gosławskie				
1	2.43	2.67	85.7	10	1	2.86	3.17	85.7	10
2	2.86	3.17	85.7	10	2	3.00	3.33	85.7	10
3	2.57	2.83	85.7	9	3	3.00	3.00	100.0	10
4	3.14	3.50	85.7	10	4	3.43	3.83	85.7	10
5	2.86	3.17	85.7	10	5	3.00	3.00	100.0	10
6	2.57	2.83	85.7	10	6	3.00	3.00	100.0	10
7	2.86	3.17	85.7	10	7	3.71	3.71	100.0	10
8	2.86	3.17	85.7	10	8	3.14	3.14	100.0	10
9	3.00	3.00	100.0	10	9	3.43	3.43	100.0	10
10	2.57	2.83	85.7	10	10	2.86	3.17	85.7	10
11	2.28	2.50	85.7	10					
12	2.57	2.83	85.7	10	Orzechów				
13	2.43	3.00	71.4	10	1	2.00	2.40	71.4	9
14	2.43	2.67	85.7	10	2	2.14	2.33	85.7	8
15	2.57	2.83	85.7	10					
16	2.28	2.50	85.7	10					
17	2.57	2.83	85.7	10					
18	2.57	2.83	85.7	10					
19	2.57	2.83	85.7	10					
20	3.00	3.33	85.7	10					
x:	**2.62**	**2.86**	**86.2**	**9.5**					

The spread of alleles in the colonies increased with increasing frequency of alleles in the population (Fig. 11, Tables 17, 18). Alleles of frequencies of ca. 0.5 occurred in 100% colonies in each population. Alleles of frequencies of up to 0.06 were present in 5–60% colonies. The significance of differences between the distributions of alleles in the analysed colonies for each enzyme was tested with Chi-square test. In case of locus *Got1* colonies from the population Gosławskie showed significant statistical differences at the adopted significance level of 0.05. No statistically significant differences were found in the remaining loci.

The genetic similarity between the colonies in the populations of *D. polymorpha* calculated according to NEI (1978) assumed values from 0.881 to 0.994 in the population from Woświn, 0.847–0.986 in the population from Gosławskie and 0.982 in the population from Orzechów.

3.4. INTER-POPULATION VARIATION IN *D. POLYMORPHA*

3.4.1. Genetic similarity and genetic distance between populations

In the analysed populations of *D. polymorpha* the values of genetic similarity I_N calculated according to NEI (1978) were within 0.828–0.999, most populations being within 0.901–0.999. Figure 12 is a dendrogram illustrating genetic similarity between the studied populations.

The genetic distance D_N between the populations was within 0.001–0.189 (NEI 1978). For 7 populations the values did not exceed 0.060, for 8 populations they were over 0.100. Populations Duże and Orzechów showed the highest genetic distances, of 0.189 and 0.167, respectively.

The clusters in the dendrograms were the same with respect to the genetic similarity and distance.

A correlation was observed between the value of genetic similarity of the populations and their geographical distance. Besides, genetic similarities between the populations connected by water courses were higher compared to isolated populations (for description see Material and methods). Figure 13 presents values of genetic similarity between connected and isolated populations compared to closely located and geographically distant populations. For connected populations I coefficient assumed values from 0.965 to 0.997, for isolated closely located populations – 0.899–0.994, for geographically distant populations – 0.866–0.966 (Table 19).

Table 17. Frequencies of alleles in 20 colonies from the population Woświn

Loci/Allele		Woświn																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Got1	1	0.90	0.80	0.65	0.50	0.85	0.95	0.75	0.70	0.40	0.90	0.90	0.95	1.00	0.90	0.75	0.80	0.95	0.65	0.55	0.60
	2	–	0.10	0.05	0.25	0.10	–	0.15	0.15	0.30	–	0.10	0.05	–	–	0.20	0.15	0.05	0.20	0.25	0.15
	4	0.10	0.10	0.25	0.20	–	–	0.15	0.10	0.05	0.10	–	–	–	0.10	–	0.05	–	0.05	–	0.10
	5	–	–	0.05	0.05	0.05	0.05	0.05	0.05	0.25	–	–	–	–	–	0.05	–	–	0.10	0.20	0.15
Est1	1	0.50	0.50	0.50	0.75	0.75	0.45	0.55	0.60	0.45	0.50	0.50	0.65	0.55	0.55	0.45	0.45	0.45	0.45	0.45	0.45
	2	0.50	0.50	0.50	0.25	0.25	0.50	0.45	0.40	0.55	0.45	0.50	0.20	0.35	0.45	0.50	0.55	0.55	0.50	0.50	0.50
	3	–	–	–	–	–	0.05	–	–	–	0.05	–	0.15	0.10	–	0.05	–	–	0.05	0.05	0.05
Pgi1	1	0.35	0.20	0.35	0.20	0.15	0.35	0.35	0.05	0.25	0.15	0.25	0.10	0.20	0.25	0.20	0.25	0.30	0.15	0.20	0.15
	2	0.55	0.60	0.50	0.55	0.80	0.50	0.55	0.70	0.55	0.60	0.55	0.80	0.55	0.55	0.65	0.60	0.40	0.80	0.65	0.55
	3	0.10	0.20	0.15	0.25	0.05	0.15	0.10	0.25	0.20	0.25	0.20	0.10	0.25	0.20	0.15	0.15	0.30	0.05	0.15	0.30
Me1	1	0.70	0.55	0.60	0.35	0.45	0.70	0.60	0.45	0.55	0.85	0.75	0.60	0.80	0.40	0.35	0.70	0.60	0.55	0.60	0.55
	2	0.25	0.20	0.40	0.25	0.20	0.25	0.15	0.30	0.30	0.10	0.25	0.30	0.05	0.40	0.45	0.30	0.20	0.35	0.25	0.30
	3	–	–	–	0.05	0.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	0.05	–	0.05	0.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	0.05	0.20	–	0.20	0.25	0.05	0.20	0.15	0.15	0.05	–	0.05	0.10	–	0.20	–	0.05	0.10	–	0.10
	6	–	–	–	0.10	–	–	0.05	–	–	–	–	0.05	0.05	0.20	–	–	0.15	–	0.15	0.05
	7	–	–	–	–	–	–	–	0.10	0.05	–	–	–	–	–	–	–	–	–	–	–
Mdh1	1	0.60	0.55	0.65	0.60	0.55	0.65	0.70	0.65	0.70	0.70	0.80	0.75	0.90	0.30	0.50	0.90	0.65	0.60	0.75	0.55
	2	0.30	0.25	0.30	0.35	0.25	0.30	0.15	0.30	0.25	0.20	0.15	0.25	0.10	0.60	0.50	0.10	0.25	0.40	0.25	0.40
	4	–	0.05	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–
	5	0.10	0.15	0.05	0.05	0.20	0.05	0.15	0.05	–	0.10	0.05	–	–	0.10	–	–	–	–	–	0.05
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10	–	–	–
Idh1	1	0.62	0.55	0.60	0.55	0.40	0.45	0.50	0.45	0.35	0.45	0.50	0.45	0.40	0.30	0.60	0.50	0.45	0.70	0.50	0.50
	2	–	0.10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	0.35	0.35	0.35	0.35	0.45	0.50	0.45	0.45	0.50	0.30	0.40	0.50	0.40	0.35	0.30	0.45	0.40	0.30	0.45	0.35
	4	0.05	–	0.05	0.10	0.15	0.05	0.05	0.10	0.15	0.25	0.10	0.05	0.20	0.35	0.10	0.05	0.15	–	0.05	0.15
Pgm1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–	–	–	–	–

Table 18. Frequencies of alleles in 10 colonies from Gosławskie and 2 from Orzechów

Loci/Allele		Gosławskie										Orzechów	
		1	2	3	4	5	6	7	8	9	10	1	2
Got1	1	0.35	0.05	0.20	0.20	0.10	0.05	0.15	0.40	0.05	0.55	0.60	0.50
	2	0.10	0.15	–	0.05	0.20	0.20	0.10	–	–	0.20	–	–
	3	0.05	0.35	0.35	0.20	0.20	0.30	0.30	0.05	0.45	–	–	–
	4	0.15	0.15	0.15	0.25	0.15	0.20	0.15	0.10	0.40	0.05	0.15	–
	5	0.35	0.30	0.30	0.30	0.35	0.25	0.30	0.45	.10	0.20	0.25	0.50
Est1	1	0.45	0.40	0.50	0.55	0.50	0.45	0.40	0.45	0.35	0.40	0.45	0.45
	2	0.55	0.55	0.45	0.30	0.50	0.55	0.45	0.55	0.55	0.55	0.55	0.55
	3	–	0.05	0.05	0.15	–	–	0.15	–	0.10	0.05	–	–
Pgi1	1	0.35	0.45	0.30	0.55	0.45	0.20	0.35	0.35	0.30	0.30	0.25	0.10
	2	0.55	0.40	0.65	0.40	0.50	0.55	0.55	0.55	0.60	0.70	0.70	0.70
	3	0.10	0.15	0.05	0.05	0.05	0.25	0.10	0.10	0.10	–	0.05	0.20
Me1	1	0.50	0.50	0.70	0.40	0.65	0.30	0.70	0.50	0.75	0.60	0.80	0.80
	2	0.15	0.30	–	0.25	0.15	0.25	0.05	0.10	0.05	0.25	0.20	0.15
	5	0.25	–	0.10	0.20	–	0.10	0.10	0.15	0.05	–	–	–
	6	–	0.15	0.05	0.10	–	–	0.05	0.10	0.05	–	–	0.05
	7	0.10	0.05	0.15	0.05	0.20	0.35	0.10	0.15	0.10	0.15	–	–
Mdh1	1	0.85	0.65	0.90	0.55	0.65	0.85	0.70	0.85	0.55	0.35	0.65	0.75
	2	0.15	0.35	–	0.25	0.10	0.10	0.05	0.05	0.10	0.40	0.35	0.25
	3	–	–	–	0.05	–	–	0.05	0.05	0.05	–	–	–
	4	–	–	–	–	–	–	0.05	0.05	–	0.05	–	–
	5	–	–	0.10	0.15	0.25	0.05	0.15	–	0.30	0.20	–	–
Idh1	1	0.55	0.50	0.70	0.45	0.60	0.85	0.40	0.55	0.30	0.65	1.00	0.95
	3	0.35	0.30	0.20	0.40	0.25	0.15	0.35	0.45	0.45	0.25	–	0.05
	4	0.10	0.20	0.10	0.15	0.15	–	0.25	–	0.25	0.10	–	–
Pgm1	1	1.00	1.00	0.90	1.00	0.90	0.90	0.90	0.90	0.90	1.00	1.00	1.00
	2	–	–	0.10	–	0.10	0.10	0.10	0.10	0.10	–	–	–

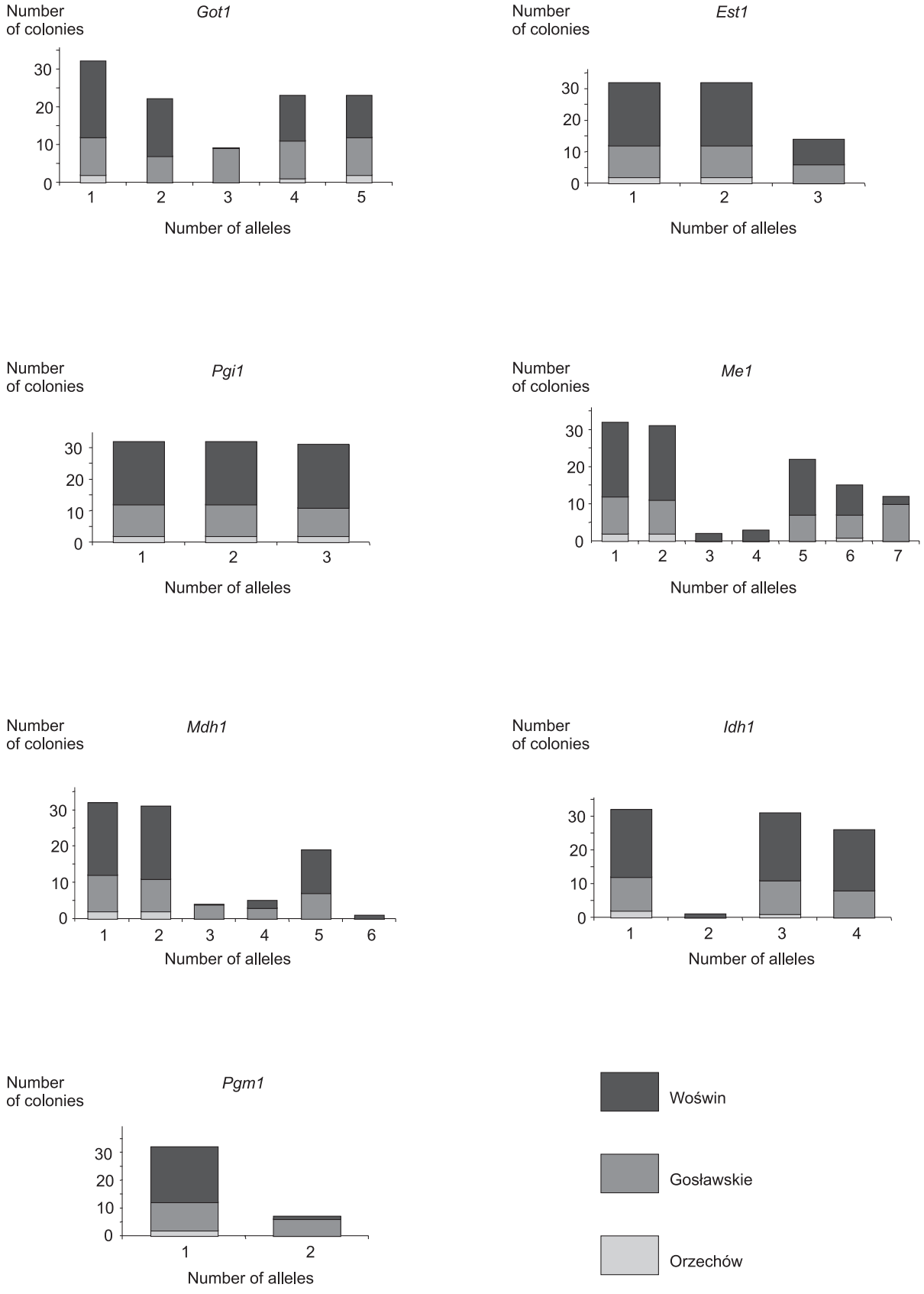

Fig. 11. Occurrence of alleles in colonies of *D. polymorpha*. Data from 20 colonies from the population Woświn, 10 from the population Gosławskie and 2 from the population Orzechów

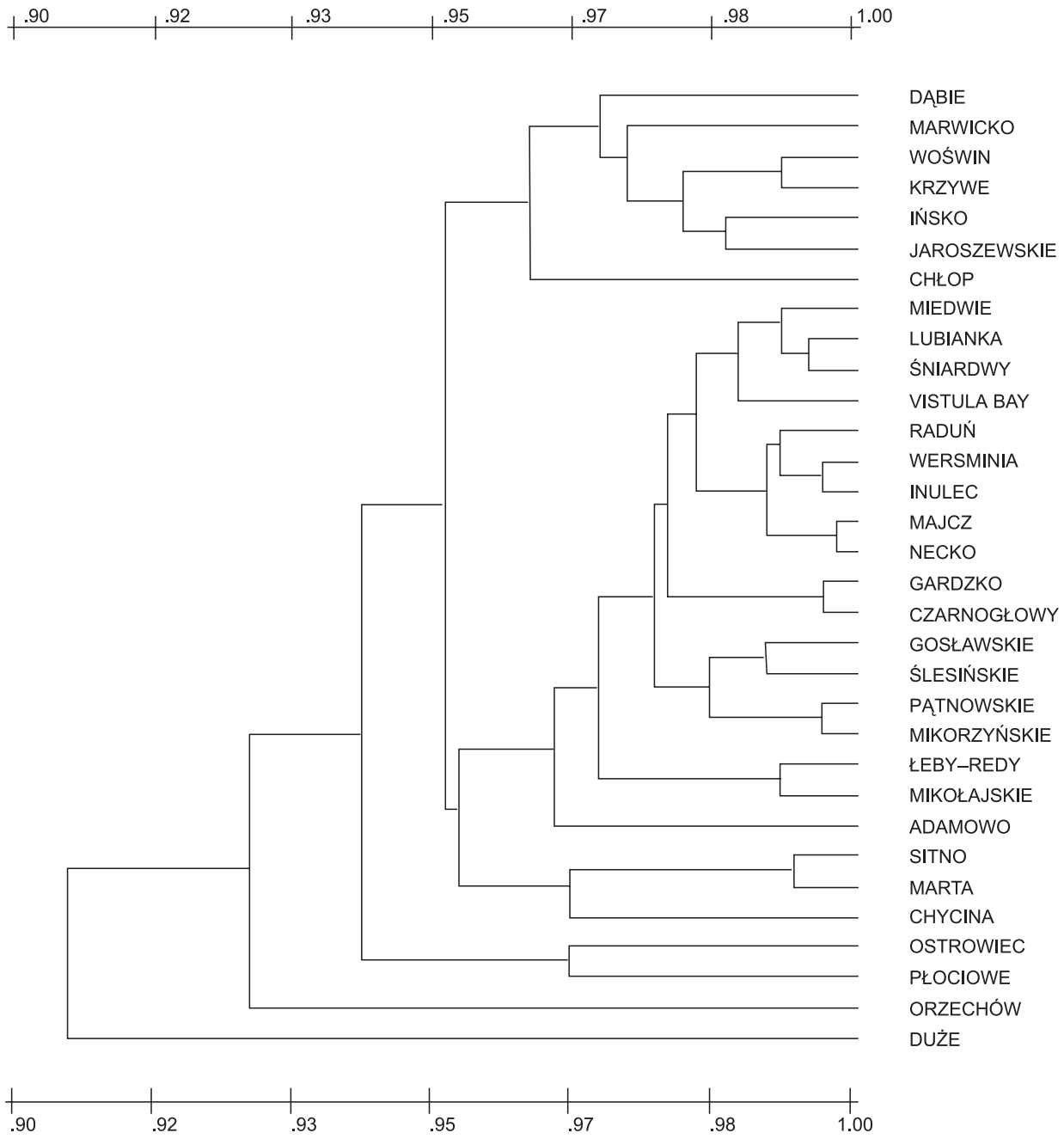

Fig. 12. UPGMA dendrogram based on genetic similarity coefficient (NEI 1978) for the 32 populations of *D. polymorpha*

Fig. 13. Values of genetic similarity (NEI 1978) for connected, isolated, closely located and distant populations of *D. polymorpha*

Table 19. Values of genetic similarity (I) between connected and isolated populations of *D. polymorpha*

Population number	Population name	I
Connected populations		
8–9	Woświn-Ińsko	0.980
13–14	Adamowo-Sitno	0.976
13–15	Adamowo-Ostrowiec	0.965
14–15	Sitno-Ostrowiec	0.965
23–24	Śniardwy-Mikołajskie	0.970
26–27	Inulec-Majcz	0.993
29–30	Gosławskie-Pątnowskie	0.980
29–31	Gosławskie-Mikorzyńskie	0.982
29–32	Gosławskie-Ślesin	0.990
30–31	Pątnowskie-Mikorzyńskie	0.997
30–32	Pątnowskie-Ślesin	0.987
31–32	Mikorzyńskie-Ślesin	0.984
Isolated populations – close		
4–5	Orzechów-Chłop	0.942
4–6	Orzechów-Marwicko	0.889
4–2	Orzechów-Miedwie	0.924
4–3	Orzechów-Gardzko	0.947
7–1	Czarnogłowy-Dąbie	0.935
7–8	Czarnogłowy-Woświn	0.972
17–14	Marta-Sitno	0.994
17–18	Marta-Krzywe	0.952
17–15	Marta-Ostrowiec	0.937
17–12	Marta-Raduń	0.941
11–10	Duże-Lubianka	0.900
11–12	Duże-Raduń	0.930
Isolated populations – remote		
4–22	Orzechów-Vistula Bay	0.946
4–28	Orzechów-Necko	0.931
4–29	Orzechów-Gosławskie	0.939
7–22	Czarnogłowy-Vistula Bay	0.962
7–28	Czarnogłowy-Necko	0.966
7–29	Czarnogłowy-Gosławskie	0.960
16–22	Płociowe-Vistula Bay	0.941
16–28	Płociowe-Necko	0.956
16–29	Płociowe-Gosławskie	0.885
17–22	Marta-Vistula Bay	0.964
17–28	Marta-Necko	0.945
17–29	Marta-Gosławskie	0.952
11–22	Duże-Vistula Bay	0.891
11–28	Duże-Necko	0.918
11–29	Duże-Gosławskie	0.866

3.4.2. Coefficients of genetic diversity

Values of D_{ST} and G_{ST} coefficients for the analysed loci, considering the correction for the sample size and the number of populations, are presented in Table 9 and Fig. 7. The highest intra-population variation compared to the variation in the species expressed by D_{ST} coefficient was observed in loci *Got1*, *Pgi1* and *Idh1* for which these values were 7.16%, 3.98% and 2.17%, respectively. In locus *Pgm1* D_{ST} value was the lowest (0.21%). The mean D_{ST} value per locus was 2.56%. The highest proportion of inter-population variation compared to the total variation within the species, as expressed by G_{ST} coefficient, was found in loci *Got1* (10.85%), *Pgm1* (7.28%) and *Pgi1* (6.31%), the lowest – in locus *Me1* (2.63%). The mean G_{ST} value per locus was 5.42%.

3.4.3. Genetic differentiation between distributional groups of *D. polymorpha*

Genetic similarity between populations within the geographical regions of Pomerania, Mazurian Lakeland and Konin was high and ranged from 0.9749 to 0.9863. Genetic distances between these groups ranged from 0.0138 to 0.0254 (Table 20). Within the regions the highest mean similarity was that between populations from the Konin group (0.9868), the lowest – between populations from the Pomerania group (0.9495, Table 21). The mean genetic distance was the highest in Pomeranian populations, the lowest within the Konin group (Table 21).

The frequencies of alleles in the groups from Pomerania, Mazurian Lakeland and Konin are presented in Table 22. The frequencies were similar between the groups except loci *Got1* and *Est1* where they were much variable. For example, allele *Got1*-1 in the group of Pomerania had a frequency of 0.646, in the group of Konin – 0.323, while its frequency in the species was 0.536. The groups differed in the occurrence of low-frequency alleles. Alleles *Est1*-4 and *Me1*-8 were characteristic of the groups Konin and

Table 20. Genetic similarities (above) and distances (below) between the three groups of populations of *D. polymorpha*

	Pomerania	Mazurian Lakeland	Konin
Pomerania	–	0.9863	0.9749
Mazurian Lakeland	0.0138	–	0.9835
Konin	0.0254	0.0166	–

Table 21. Genetic similarities and distances for the three groups of populations of *D. polymorpha*

Group	Mean genetic similarity	Range	Mean genetic distance	Range
Pomerania	0.9495	0.8277–0.9969	0.0523	0.0031–0.1891
Mazurian Lakeland	0.9836	0.9630–0.9988	0.0166	0.0012–0.0377
Konin	0.9868	0.9801–0.9972	0.0133	0.0028–0.0201

Table 22. Allele frequencies for the three groups of populations of *D. polymorpha*

Loci/alleles	Pomerania N 2830	Mazurian Lakeland N 560	Konin N 400
Got1			
1	0.646	0.476	0.323
2	0.121	0.112	0.151
3	0.082	0.066	0.117
4	0.087	0.150	0.209
5	0.064	0.196	0.200
Est1			
1	0.545	0.621	0.454
2	0.431	0.341	0.494
3	0.024	0.038	0.051
4	0.000	0.000	0.001
Pgi1			
1	0.334	0.473	0.368
2	0.451	0.458	0.511
3	0.178	0.047	0.116
4	0.037	0.022	0.005
Me1			
1	0.592	0.604	0.564
2	0.195	0.179	0.140
3	0.006	0.003	0.004
4	0.006	0.004	0.011
5	0.103	0.118	0.130
6	0.069	0.048	0.061
7	0.028	0.044	0.090
8	0.001	0.000	0.000
Mdh1			
1	0.774	0.820	0.746
2	0.204	0.173	0.149
3	0.002	0.000	0.019
4	0.001	0.000	0.011
5	0.017	0.004	0.061
6	0.002	0.003	0.014
Idh1			
1	0.564	0.585	0.641
2	0.012	0.003	0.005
3	0.385	0.412	0.301
4	0.039	0.000	0.053
Pgm1			
1	0.982	0.998	0.950
2	0.018	0.002	0.050

Pomerania, where their frequencies corresponded to the frequencies within the species and amounted to 0.001.

Distribution of alleles in the groups was compared with Chi-square test. The resulting values of Chi-square statistics indicate significant differences in all the loci between the studied groups at significance level 0.05.

Table 23 presents values of coefficients $\overline{H}$, H_T, D_{ST} and G_{ST} for 7 loci in the three groups of populations of *D. polymorpha*. Values of $\overline{H}$ and H_T were the highest in the Konin group, for 6 and 5 loci, respectively. The lowest values of these coefficients in the Mazurian Lakeland group were found for 5–6 loci.

D_{ST} was the highest between populations from the Pomerania group and the lowest between Mazurian and Konin populations. G_{ST} was the highest in the Pomerania group, except locus *Est1*, and the lowest in groups of Mazurian Lakeland and Konin (Table 23).

H_S values for the three groups of populations were varied and ranged from 0.438 to 0.503 (Table 24). Likewise, the mean H_T varied from 0.448 in the Mazurian Lakeland region to 0.510 in the Konin region. Differentiation of populations within groups, expressed as mean D_{ST} value, was the lowest in the region of Konin (D_{ST}=0.007) and the highest in the region of Pomerania (D_{ST}=0.029). Similarly, the proportion of inter-population variation in the variation of the group, expressed as mean value of G_{ST}, was the lowest in populations of the Konin group (0.014), and the highest in the populations from Pomerania (0.063) (Table 24).

Table 24. Values of H_s coefficient and mean values of coefficients H_T, D_{ST} and G_{ST} for the three groups of populations of *D. polymorpha*

Group	H_S	H_T	D_{ST}	G_{ST}
Pomerania	0.4376	0.4668	0.0292	0.0626
Mazurian Lakeland	0.4385	0.4478	0.0093	0.0208
Konin	0.5030	0.5102	0.0072	0.0141

Table 23. Values of coefficients $\overline{H}$, H_T, D_{ST} and G_{ST} for 7 loci in the three groups of populations of *D. polymorpha*

Loci	Pomerania				Mazurian Lakeland				Konin			
	$\overline{H}$	H_T	D_{ST}	G_{ST}	$\overline{H}$	H_T	D_{ST}	G_{ST}	$\overline{H}$	H_T	D_{ST}	G_{ST}
Got1	0.523	0.598	0.076	0.126	0.688	0.713	0.025	0.035	0.755	0.784	0.028	0.036
Est1	0.505	0.521	0.016	0.030	0.482	0.511	0.029	0.057	0.550	0.548	0.000	0.000
Pgi1	0.600	0.650	0.049	0.076	0.558	0.563	0.005	0.009	0.591	0.591	0.000	0.000
Me1	0.573	0.592	0.019	0.032	0.573	0.575	0.003	0.004	0.623	0.639	0.016	0.025
Mdh1	0.354	0.366	0.012	0.034	0.276	0.280	0.005	0.016	0.415	0.418	0.003	0.008
Idh1	0.486	0.516	0.030	0.058	0.490	0.488	0.000	0.000	0.492	0.497	0.005	0.011
Pgm1	0.021	0.024	0.002	0.103	0.003	0.003	0.000	0.000	0.095	0.095	0.001	0.008

DISCUSSION

The electrophoretic studies on the variation of 7 loci in *D. polymorpha* from Poland showed a strong protein polymorphism in the species. The level of genetic variation in the zebra mussel is higher than in the populations from the Great Lakes of North America which the species started to invade in 1985 (HEBERT et al. 1989, ROSE & ECKROAT 1991, GARTON & STOECKMANN 1992, BOILEAU & HEBERT 1993, SPIDLE et al. 1994).

Parameters of genetic variation of *D. polymorpha* from Poland were: percentage of polymorphic loci 100, number of alleles per locus 4.7, mean expected heterozygosity per locus per population 0.447.

In 13 American populations, analysed for 12 loci, the variation parameters were lower: percentage of polymorphic loci 92.8, number of alleles per locus 3.4, mean expected heterozygosity per locus per population 0.35 (HEBERT et al. 1989, GARTON & HAAG 1991, MAY & MARSDEN 1992, BOILEAU & HEBERT 1993, SPIDLE et al. 1994). A relatively wide variation of the zebra mussel populations on the American continent indicates that the populations were founded by a considerable number of individuals, and thus underwent no bottleneck effect (HEBERT et al. 1989, GARTON & HAAG 1991).

Like populations of *D. polymorpha*, most other introduced mollusc species in the Great Lakes of North America, especially those with planktonic larvae, show a level of variation similar to that found in their founder populations (WARD 1990). An absence of allozymic variation was observed only in an American population of *Corbicula fluminea*, an introduced species in North America (SMITH et al. 1979).

Compared to *D. polymorpha*, a higher level of polymorphism was found in several species of Black Sea molluscs which colonised the Mediterranean Sea through the Sues Canal. They include snails *Cerithium scabridum* and *C. caeruleum* in which each of 20 loci turned out to be polymorphic, the mean number of alleles per locus being 3.9–4.9 and the heterozygosity (H_o) was 0.61–0.66 (RITTE & PASHTAN 1982), and a bivalve *Brachidontes variabilis* characterised by the mean number of alleles per locus 5.5 (17 loci examined) and the heterozygosity of 0.62–0.63 (SAFRIEL & RITTE 1986). All these three species, like *D. polymorpha*, are invasive organisms of a wide distribution. Their life cycle includes external fertilisation and a stage of planktonic larva which enables them to be transported with sea currents and in consequence be dispersed widely which implies an intense gene flow (STAŃCZYKOWSKA 1977, RITTE & PASHTAN 1982, SAFRIEL & RITTE 1986, BORCHERDING 1991).

A high variation, similar to that found in the zebra mussel, was observed in such marine bivalves as *Macoma baltica*, *M. incongrua* and *Mulinia lateralis*. In these species 75–100% loci were polymorphic, the mean number of alleles per locus being 4.1–6.6, and the mean expected heterozygosity 0.365–0.456 (WENNE 1992, 1993). In marine bivalves *Crassostrea virginica* (BUROKER 1983), *Macoma irus* (WENNE 1993), *Patinopecten yessoensis* (KIJIMA et al. 1984) and snails *Littorina saxatilis* and *L. arcana* (KINGHT et al. 1987) genetic variation was twice lower than in the Polish populations of *D. polymorpha*.

Western European populations of *D. polymorpha* and the population from Lithuania also turned out to be highly polymorphic: the percentage of polymorphic loci was 98.5%, the number of alleles per locus 2.5 and the mean expected heterozygosity per population 0.402 (ZAPKUVIENNE 1992, BOILEAU & HEBERT 1993, SPIDLE et al. 1994).

In Poland all the analysed populations of *D. polymorpha* showed a high level of genetic variation, a high genetic similarity and a low inter-population differentiation. The mean expected heterozygosity per locus per population (H_S) assumed values ranging from 0.338 to 0.531 and was on an average 0.447. Its lowest value, 0.338, and the lowest number of alleles per locus, 2.29, was observed in a small isolated population Orzechów, which seems to be associated with the low number of examined specimens (20) rather than with isolated character of the population.

Dispersal of larvae of *D. polymorpha* in the water, the possibility of transport of adult individuals along inland routes, and consequent high potential gene flow, may account for the relatively poor genetic differentiation between the Polish populations of the zebra mussel.

Indices of genetic similarity (I) and genetic distance (D) (NEI 1978) are often used to compare populations based on allele frequencies. I values for the analysed populations of *D. polymorpha* ranged from 0.828 to 0.997, D was within 0.001–0.189. Three populations: Duże, Orzechów and Płociowe, had the lowest values of genetic similarity, of 0.828, 0.880 and 0.847, respectively. Most of the remaining populations were within the range of 0.901–0.999; the values correspond to conspecific populations (AYALA 1982). Figure 12 is a graphical interpretation of genetic similarity between the 32 analysed populations.

A small genetic distance was observed also between American and W European populations of *D. polymorpha*. For six populations from the Great Lakes and two Dutch populations D assumed values smaller than 0.02 (MAY & MARSDEN 1992, SPIDLE et al. 1994). However, two continental groups of the zebra mussel were 0.068 away, and though the distance is much higher, it is still within the genotypic and geographical variation of the species (SPIDLE et al. 1994).

The population groups corresponding to the geographical regions in Poland: Pomerania, Mazurian Lakeland and Konin, were also genetically highly uniform. The genetic similarity between the regions was very high (0.975–0.986), at a low genetic distance (0.014–0.025) (Table 20). The widest range of genetic similarity values was observed among Pomeranian populations (Table 21) which results most probably from their wide scatter in Pomerania and the small number of populations connected by natural or artificial water courses (Table 19). The highest genetic similarity was that between the Konin populations which are located close to each other and connected by canals. The data suggest unequivocally that a high genetic uniformity of populations of *D. polymorpha* results from biological dispersal abilities of the species and depends on the geographical distance between the populations and the presence/absence of water connections between them (Table 19, Fig. 13).

The high level of homogeneity of the species is correlated with the small genetic differentiation of the populations as expressed by G_{ST} coefficient.

Analysis of G_{ST} coefficient in various marine mollusc species indicates a relation between its value and the presence/absence of a planktonic veliger in their life cycle (WARD 1990). Low G_{ST} values, within 0.011–0.065 were found in five snail species of the genus *Littorina* which have veliger larvae. Their presence favours gene flow which is regarded as the main factor decreasing genetic differences between populations. A fairly high G_{ST} (0.076 and 0.085) found in *Littorina plena* and *L. scutulata* is rather surprising, as they both have pelagic larvae (MASTRO et al. 1982). In spite of this, the mean genetic differentiation of molluscs with veliger larvae is by 1/3 lower compared to species with no such larva (WARD 1990).

In *D. polymorpha* G_{ST} mean value per locus was 0.054 and ranged from 0.026 in locus *Me1* to 0.109 in locus *Got1*, and for 6 loci it did not exceed 0.073 (Table 9, Fig. 7).

The G_{ST} value was the lowest in the Konin region (0.014), slightly higher in Mazurian Lakeland (0.021) and Pomerania (0.063) (Table 24). With respect to the mean genetic similarity (I) the regions followed the same order, the values being 0.987, 0.984 and 0.950, respectively (Table 21). The results indicate that the larger the area of the region, the smaller the rate of gene flow, and as a result the higher inter-population differentiation and the smaller the genetic similarity. In the regions the G_{ST} values varied widely between loci (Table 23). In loci *Got1* and *Pgm1* in Pomerania G_{ST} assumed its highest values and was 0.126 and 0.103, respectively. In the regions of Mazurian Lakeland and Konin loci *Est1* (0.057) and *Got1* (0.036) showed the highest inter-population differentiation. Loci *Idh1* and *Pgm1* in the Mazurian populations and loci *Est1* and *Pgi1* in the Konin populations proved to be uniform as testified to by the G_{ST} value 0 (Table 23).

Genetic variation of *D. polymorpha* from small water bodies of Western Pomerania did not essentially depart from the variation of populations from large water bodies. Populations from small forest or midfield lakes, such as Czarnogłowy, Duże, Płociowe and Marta, showed a wide genetic variation and did not differ in this respect from other populations from very large lakes. H_S values for these four populations ranged from 0.362 to 0.487 (mean 0.431), and the number of alleles per locus was 3.0–3.7 (mean 3.3) (Table 5).

The high H_S value in the population from lake Czarnogłowy (0.487), an excavation reservoir formed in the 50s of the 20th c., indicates an invasion which was not accompanied by a decrease in the population gene pool. On the contrary, the invasion was effected by genetically differentiated individuals or it was multiple, with a participation of founding populations from various water bodies. It should be conjectured that the remaining isolated populations were founded in a similar way, as evidenced by their similar variation parameters. The population from lake Sitno is also noteworthy; there the zebra mussel does not form colonies but few individuals live singly on the bottom. In spite of the low abundance, the population showed a high genetic variation (H_s=0.444). This testifies to a mass invasion of the lake by *D. polymorpha* in the past and a constant immigration of new individuals from the abundant population of lake Ostrowiec, connected by the Płociczna River.

The zebra mussel in small and isolated populations showed no decreased genetic variation compared to other populations of the species. Decreased genetic variation in small, isolated or newly established populations as a result of genetic drift was observed in many animal species (NEI 1987, LEBERG 1992). The lack of this phenomenon in *D. polymorpha* may be accounted for by its mass way of invading new water bodies and large dispersal abilities resulting from the biology of the species.

In the analysed populations of *D. polymorpha* all the loci except *Pgm1* were polymorphic; *Pgm1* turned out to be monomorphic in 20 populations (63%). The highest number of alleles per locus was found in *Me1* – 8, *Mdh1* – 6 and *Got 1* – 5, the lowest number being 2 alleles in *Pgm1*. Four alleles were found in each of the remaining loci. The mean number of alleles per locus per population was also the highest in locus *Me1* – 5.6 and then in *Got1* – 4.6, the lowest in *Pgm1* – 1.4 (Table 6).

The highest variation as expressed by the expected heterozygosity per locus for the species (H_T) was noted for loci *Got1* (0.661), then for *Pgi1* (0.631), *Me1* (0.592), *Est1* (0.525), *Idh1* (0.512), *Mdh1* (0.359) and *Pgm1* (0.028). The analysed loci were similar with respect to expected heterozygosity per locus per popula-

tion ($\overline{H}$) which was slightly lower than H_T. Exceptions were loci *Got1* and *Pgi1*, characterised by the highest values of H_T and $\overline{H}$, respectively (Table 9).

Locus *Pgi1* showed a high variation also in American populations of *D. polymorpha* from lakes St. Clair (H=0.620 and 0.759, HEBERT et al. 1989, BOILEAU & HEBERT 1993), Erie (H=0.650, GARTON & HAAG 1991) and Oneida (H=0.654, BOILEAU & HEBERT 1993) where 4 and 5, and 6 and 5 alleles were identified, respectively. A high variation in locus *Pgi* was also observed by BOILEAU & HEBERT (1993) in W European populations of the zebra mussel (H=0.634) where they distinguished 4 alleles.

The highest number of alleles – 7 – in locus *Pgi1*, including 4 high-frequency alleles and 3 unique alleles, was found in populations from the Great Lakes of North America (GARTON et al. 1991, GARTON & STOECKMANN 1992).

Some American populations of *D. polymorpha* showed a higher variation in loci *Est* and *Pgm*, compared to the Polish populations. Heterozygosity in locus *Est* in lake St. Clair was 0.70 (HEBERT et al. 1989), in lake Erie 0.580 (GARTON & HAAG 1991), where 4 and 5 alleles were found, respectively. Different results on the variation in locus *Pgm* were obtained by MAY & MARSDEN (1992) and SPIDLE et al. (1994). In lake Oneida studied by MAY & MARSDEN (1992) the locus was monomorphic, while SPIDLE et al. (1994) found in that lake a variation of H=0.173 and identified 5 alleles. In the remaining 5 American lakes from 2 to 5 alleles were found in locus *Pgm*, and its heterozygosity ranged from 0.120 to 0.235 (MAY & MARSDEN 1992). In two Dutch populations two alleles were found in locus *Pgm*, and the heterozygosity was 0.097 and 0.100 (SPIDLE et al. 1994). In Polish populations which were polymorphic with respect to locus *Pgm1*, one of two alleles had a high frequency (Table 8). In these populations the expected heterozygosity ranged from 0.020 to 0.267.

For loci *Idh*, *Mdh*, *Me* and *Got* the variation in American populations was lower (HEBERT et al. 1989, GARTON & HAAG 1991, MAY & MARSDEN 1992, BOILEAU & HEBERT 1993, SPIDLE et al. 1994) compared to the Polish populations. In the population from lake St. Clair the expected heterozygosity for locus *Idh* was: according to HEBERT et al. (1989) 0.400, according to BOILEAU & HEBERT (1993) 0.408, and in lake Oneida 0.337 (BOILEAU & HEBERT 1993). In the American populations the above-cited authors identified from 2 to 3 alleles in locus *Idh*. In European populations of the zebra mussel 4 alleles were identified in this locus, H value ranging from 0.260 (BOILEAU & HEBERT 1993) to 0.412 (ZAPKUVIENNE 1992). Some authors (HEBERT et al. 1989, GARTON & HAAG 1991, MAY & MARSDEN 1992, SPIDLE et al. 1994) found that NAD-dependent malate dehydrogenase was encoded by 2 loci, *Mdh1* and *Mdh2*, one of them being more variable. In loci *Me1* and *Mdh* lower H values were noted in the population from lake Erie (*Me1* – 0.12, *Mdh1* – 0.17, *Mdh2* – 0.29, GARTON & HAAG 1991) compared to the European populations (ZAPKUVIENNE 1992, BOILEAU & HEBERT 1993).

A higher genetic variation of the Polish populations of *D. polymorpha* compared to the American ones results most probably from historical differences in the rate of expansion. A relatively high variation of American populations of ca. 10 years age suggests great adaptive abilities of the species and its invasive character. The obtained electrophoretic results indicate that the zebra mussel expands massively, using all its genetic potential.

Analyses of allelic and genotypic differentiation of *D. polymorpha* in colonies and populations attempted in this study are of pioneer character. A high polymorphism was found within colonies (86% polymorphic loci), the presence of 9.5 different genotypes in a colony and a high genetic similarity (Table 16). Values of genetic similarity between colonies in populations ranged from 0.847 to 0.994.

In almost 3/4 colonies there were 6 polymorphic loci, at a monomorphic locus *Pgm1*, 6 colonies had 7 polymorphic loci and only 2 colonies – 5 polymorphic loci (Table 16). The colonies analysed in this paper differed mainly in the frequency of rare alleles, low frequency alleles and, to a lesser degree, some alleles of higher frequency (Tables 17, 18). From each colony 10 individuals were analysed, and 8–10 different genotypes were found. The high genetic differentiation within colonies may be accounted for by a high population polymorphism, the presence of external fertilisation and the free-swimming veliger larva.

Subpopulations from lakes Dąbie and Ińsko have a genetic structure similar to that of the colonies which justifies treating *D. polymorpha* from these lakes as one population of panmictic reproduction (SOROKA et al. 1997, PIESIK et al. 1998).

Based on analysis of many electrophoretic phenotypes, multilocus genotypes were identified, their number varying between the populations. There were unique genotypes (UG) which appeared once only. The value of UG coefficient in populations varied from 36 to 98%, the mean being 75.8% per population (Table 15). The percentage of unique genotypes in populations depended on the number of analysed specimens (negative correlation, Fig. 9) and on the mean number of genotypes per locus per population (positive correlation, Fig. 10).

In *D. polymorpha* the mean number of genotypes per locus was 10.3 (Table 4). The highest number was found in locus *Me1* – 17, then in loci *Got1*, *Mdh1* – 13 in each, *Pgi1* – 10, the lowest number was recorded for locus *Pgm1* – 3 (Table 10). The genotypes that were present in all the populations varied considerably in their frequency which ranged from 0.08 to 0.94 (Table 11). The results of phenotypic interpretation of electrophoretic pictures were similar (Table

3). It can be supposed that heterozygotes of different number of bands with respect to a given enzyme (genotype) may result from the presence of exogenous proteins in the tissue of the zebra mussel. *D. polymorpha* is an intermediate host of larval stages of various trematode species, among others the genera *Bucephalus* and *Cataptroides* (POKORA, personal communication).

In this study, it was observed that some genotypes appeared more frequently, and other more rarely than it would follow from the Hardy-Weinberg equilibrium. Some genotypic combinations in loci *Me1*, *Mdh1*, and *Est1* did not appear at all. In loci *Est1*, *Got1*, *Me1*, *Pgi1* and *Idh1* an excess of heterozygotes was observed, while in loci *Pgm1* and *Mdh1* there was an excess of homozygotes (Tables 13, 14). Loci *Pgi1* and *Mdh1* were the most frequently in the Hardy-Weinberg equilibrium (84.4% and 75.0% populations, respectively) (Tables 12, 13). Because of the possibility of various genotype combinations (high fertility, external cross-fertilisation) the absence of some homo- and heterozygotes in some loci is surprising. The reason for their absence may be their low adaptive value.

There are no unequivocal literature data on the Hardy-Weinberg equilibrium in *D. polymorpha*. Many authors either did not consider the equilibrium (MAY & MARSDEN 1992, SPIDLE et al. 1994) or did not report unambiguous results. HEBERT et al. (1989) in each of the 17 analysed polymorphic loci from lake St. Clair found the distribution of genotypes to be similar to that expected from the Hardy-Weinberg equilibrium. This was confirmed by GARTON & HAAG (1991) for 4 out of the 5 analysed loci (except *Me*) in the population from lake Erie. Similar results pertaining to the Hardy-Weinberg equilibrium of 5 loci, except *Lap1*, were obtained by ZAPKUVIENNE (1992) for an E European population from lake Dringis. BOILEAU & HEBERT (1993) reported on the lack of Hardy-Weinberg equilibrium in 54% analysed loci (including *Mdh*) from lake St. Clair and in 45% loci from lake Erie (including *Idh*). Different results were described by ROSE & ECKROAT (1991) who found the lack of the Hardy-Weinberg equilibrium and an excess of heterozygotes in all the 13 analysed loci except *Est* in 5 populations of *D. polymorpha*, among others from lakes Erie and St. Clair. The lack of consistent results pertaining to the Hardy-Weinberg equilibrium may result from an erroneous interpretation of electrophoregrams which would complicate the whole problem. It cannot be excluded that the presence/absence of the Hardy-Weinberg equilibrium depends also on the geographical location of the population, environment conditions and selection, and that it can involve various loci to various extent.

In over 25 species of marine bivalves a lack of Hardy-Weinberg equilibrium was observed, resulting from an excess of homozygotes (ZOUROS et al. 1980, SINGH & GREEN 1984, ZOUROS & FOLTZ 1984). There are many hypotheses explaining the phenomenon, the most important being: presence of zero alleles (FOLTZ 1986), Wahlund's spatial effect (TRACY et al. 1975, KOEHN et al. 1976), partial division of a local population into reproductive groups i.e. dependence of the breeding season on the kind of genotypes (ZOUROS & FOLTZ 1984) and selection (TRACY et al. 1975). The explanation of the problem is additionally complicated by reports on marine snails of the genus *Littorina* which are in the Hardy-Weinberg equilibrium (WARD 1990). However, rare cases of the lack of equilibrium have different reasons, such as deficit of heterozygotes in locus *Est* in *Littorina saxatilis* and *L. obtusata* (NEWKIRK & DOYLE 1979), excess of heterozygotes in locus *Pgd* in *L. littorea* (FEVOLDEN & GARNER 1987), or they pertain to many loci in single populations, e.g. in *L. angulifera* (GAINES et al. 1974).

Marine molluscs are also subject to studies on correlation between heterozygosity and such adaptive parameters as growth rate, body size, shell length, metabolic rate and resistance to environment factors. Positive correlation between the growth rate and individual heterozygosity was found in many species, including *Crassostrea virginica* (SINGH & ZOUROS 1978, ZOUROS et al. 1980), *Macoma baltica* (GREEN et al. 1983), *Mulinia lateralis* (GARTON et al. 1984, KOEHN et al. 1988) and *Mytilus edulis* (KOEHN & GAFFNEY 1984, ZOUROS et al. 1988). There are also a few reports on the absence of such a correlation in the above-mentioned species (GOSLING 1989, GAFFNEY 1990, WENNE 1993) and in other marine molluscs (BEAUMONT 1982, FOLTZ & ZOUROS 1984). The problem is the more complex that the authors report on a positive correlation between the growth rate and the heterozygosity which is not confirmed by other students. An example is an analysis of the population of *Mytilus edulis* from Long Island, New York by KOEHN & GAFFNEY (1984), DIEHL et al. (1985) and GAFFNEY (1990). KOEHN & GAFFNEY (1984) observed a positive correlation between the hetrozygosity and the body size (the most distinct for loci *Lap* and *Odh*) and a decrease tendency in the heterozygote deficit in larger size classes. Contradictory results were obtained by DIEHL et al. (1985) who analysed the same population two months later. No correlations were found in the same population by GAFFNEY (1990) who analysed it twice with a half year interval. There are suggestions that environment changes may have a significant effect on the relation between heterozygosity and the growth rate in marine bivalves (GENTILI & BEAUMONT 1988, SCOTT & KOEHN 1990).

Similarly complicated is the dependence between the heterozygosity and adaptation in *D. polymorpha*. GARTON & HAAG (1991) found a positive correlation between heterozygosity and shell length of the zebra mussel and the lack of correlation between heterozygosity and oxygen consumption per unit body mass. BOILEAU & HEBERT (1993) observed no correlation

between heterozygosity and shell length or settling of juvenile *D. polymorpha*. Differences in shell length and body mass between individuals of *D. polymorpha* from various depths were observed by SOROKA et al. (1997). A higher body mass and longer shells in individuals living at a depth of 20 m found no reflection in isozyme data. The differences resulted probably from changes in environment conditions. At the depth of 20 m the conditions are more stable and food availability is higher; it can be thus conjectured that the observed morphological variation is of a fluctuating character.

Morphological differences between shallow- and deep-water forms of *Macoma baltica* in the Gulf of Gdańsk were not reflected in electrophoretic differences between them (WENNE 1993).

Based on genetic similarity (I) or distance (D), taxa can be classified or genetic changes during speciation described. Local populations of a species are genetically very similar and their I ranges from 0.90 or 0.95 to 1.00, species in statu nascendi (semispecies and subspecies) ca. 0.79, sibling species 0.50–0.60, and taxonomic species from 0.30 to 0.87 (AYALA 1982).

In the genus *Dreissena* the degree of genetic similarity at the inter-population and specific level is similar to that found in most other species. The form "quagga" found by MAY & MARSDEN (1992) in the Great Lakes of North America differed considerably from *D. polymorpha* in the occurrence and frequency of alleles. Because of the low value of genetic similarity, I=0.30, it was regarded as a distinct species, and then identified as *D. bugensis* (SPIDLE et al. 1994). Populations of *D. polymorpha* and *D. bugensis* showed minimum values of genetic distance (MAY & MARSDEN 1992, SPIDLE et al. 1994). Both species occur sympatrically in the Great Lakes, the abundance of *D. bugensis* increasing with depth and decreasing temperature (MILLS et al. 1993). The absence of hybrids between the two taxa confirms their genetic separateness (SPIDLE et al. 1995).

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