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GENETIC VARIATION IN POLISH POPULATIONS OF *CALLITRICHE COPHOCARPA*

KATARZYNA BUCZKOWSKA[1*], KRZYSZTOF SZOSZKIEWICZ[2] AND ALINA BĄCZKIEWICZ[1]

[1]*Department of Genetics, Adam Mickiewicz University, ul. Umultowska 89, 61–614 Poznań, Poland,*
[2]*Department of Ecology and Environmental Protection, Agricultural University, ul. Piątkowska 94, 61–691 Poznań, Poland*

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Callitriche cophocarpa Sendtn. is typically a submerged macrophyte, widespread throughout Poland. It is a monoecious plant with unisexual flowers which are produced only in the floating rosettes. We studied the allozyme variation of 9 populations of *C. cophocarpa* from Polish rivers in order to establish its genetic structure, mode of reproduction, clonal structure, and gene flow between populations. Genetic variation of the examined populations was low. Only 7 of the 12 loci studied were polymorphic. One of the 24 detected multilocus genotypes was widespread, found in 8 populations, and the remaining genotypes were rare. The mean percentage of polymorphic loci within populations was 19.44 and the mean number of alleles per locus was 1.231, with mean values of 0.0219 observed and 0.0421 expected heterozygosity. Fixation indices calculated for each population showed a significant deficit of heterozygotes. The study showed high differentiation between populations, with almost 50% of the total variation in *C. cophocarpa* located between populations. Allozyme studies showed a high contribution of clonal reproduction. The populations of *C. cophocarpa* are isolated from each another, and the existing physical barriers decrease the probability of gene flow between populations and cross-fertilization. Gene flow within rivers is more probable.

Key words: *Callitriche*, water starwort, macrophytes, allozyme, genetic structure, inbreeding, clonal growth.

INTRODUCTION

The genus *Callitriche* (water starworts) comprises 40–50 species, many of them widely distributed all over the world. They are annuals or perennials, depending on their environment. Most of them can grow in shallow to deep water or on wet ground, so their vegetative parts are highly variable morphologically, as in the case of leaf shape, which can vary depending on light level and water depth (Stace, 1997).

The biology of water starworts has become an important subject since these species have been found to be good indicators of water quality degradation. Currently, systems based on macrophytes are under intensive development and are being introduced into the regular monitoring according to the requirements of the EU Water Framework Directive. Most European monitoring methods based on aquatic plants, such as France's Macrophyte Biological Index for Rivers (Haury et al., 2006), Germany's Reference Index (Schaumburg et al., 2004) or Great Britain's Mean Trophic Rank (Holmes at al., 1999) involve *Callitriche* species. Non-genetic methods of identifying *Callitriche* species are of low reliability; to fully utilize the potential of this group of plants, the existing data on their taxonomy, genetic variation, distribution and ecology need to be revised. Since *C. cophocarpa* Sendtn. is one of to the most frequent water starworts in Europe (Schotsman, 1972), studies on this plant have particularly wide application.

Callitriche cophocarpa is one of six species of the genus *Callitriche* occurring in Poland (Podbielkowski and Tomaszewicz, 1982; Zając and Zając, 2001). It is widespread throughout Poland and is the most common representative of the genus here. It is a perennial, typically growing in flowing or stagnant water (Zając and Zając, 2001), mostly submerged, with a terminal leaf rosette on the water surface (amphibious species); occasionally it occurs as a terrestrial plant growing in mud (Stace, 1997).

*e-mail: androsac@amu.edu.pl

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TABLE 1. Localities of 9 studied populations of *C. cophocarpa* and number of samples collected in each population. Population numbers correspond to those given in Figure 1

No.	Population (river)	Location of sampling point	No. of samples
		Western Poland	
1	Grabia	Ldzań village S of Łódź	8
2	Mała Wełna	Kiszkowo town W of Gniezno	1
3	Płytnica	Płytnica town N of Piła	7
4	Dojca	Site N of Wolsztyn	5
5	Flinta	Ryczywół town S of Piła	7
6	Parsęta	Stare Dębno village S of Koszalin	10
		Eastern Poland	
7	Mławka	Szreńsk village W of Mława	5
8	Drwęca	Samborowo town S of Ostróda	5
9	Łutownia	Site N from Białowieża village	2

C. cophocarpa is a monoecious plant with unisexual flowers which are produced only in the floating rosettes, not when the plants grow in totally submerged form. According to Rich and Jermy (1998), *C. cophocarpa* exhibits aerial pollination. Cooper et al. (2000) suggested that the pollen morphology and ultrastructure may indicate the possibility of epihydrophily in this species. Aside from sexual reproduction, *C. cophocarpa* can also reproduce vegetatively (Podbielkowski and Tomaszewicz, 1982).

Genetic studies of vascular aquatic plants indicate low genetic variability and weak population differentiation below the species level (Les, 1988; Barrett et al., 1993; Santamaría, 2002). Santamaría (2002) contended that the low intrapopulation variation and high differentiation of populations of aquatic plants are most likely related to widespread clonal multiplication. Crawford et al. (1997) suggested a correlation between the level of genetic variation and the range of species distribution: broadly distributed species tend to have higher genetic variation than species with a restricted distribution. Little is known about genetic variation in *Callitriche* species because the genetic structure of water starwort populations has not been well studied. Recently, isozyme analysis was used for identification of *Callitriche* species in Great Britain; it also revealed some intraspecific variation in *C. stagnalis*, *C. obtusangula*, *C. brutia* and *C. hamulata* (Demars and Gornal, 2003). *C. cophocarpa* has not been studied in this respect. Isozyme data can provide additional information about the biology of this species, for example its mode of reproduction, clonal structure, mating system and gene flow between populations. Its genetic variability can be compared with that of with other aquatic species. Knowledge of genetic variability in *C. cophocarpa* – its isozyme patterns, the number of alleles per locus, allele frequencies, etc. – is important because this species has been suggested to be one of the parents of *C. platycarpa* (Savidge, 1960). Our study estimates the level of genetic variation within and between populations of *C. cophocarpa*, based on material collected in Poland, where it is very abundant (Podbielkowski and Tomaszewicz, 1982).

MATERIALS AND METHODS

PLANT MATERIAL

Nine populations of *C. cophocarpa* from nine rivers in different regions of Poland were examined (Tab. 1, Fig. 1). Samples from the upper course of each river were taken at intervals of no less than 100 m over a distance of 1–2 km. Depending on population size, between 1 and 10 samples were collected from each river, and from each sample 10–15 plants were randomly taken for analysis. In total, 554 plants from 50 samples were examined. Fruits are essential for precise identification of *Callitriche* spp. (Stace, 1997). Since the collected plants were mainly sterile, species were identified on the basis of genetic markers such as chromosome number, DNA sequences (according to Philbrick and Les, 2000) and isozyme patterns (Bączkiewicz et al., 2007).

ELECTROPHORETIC ANALYSIS

Isozyme separation was conducted according to the procedure described by Wendel and Weden (1989). Crude cell extract was prepared by homogenization of shoot apices with 2–3 leaves in 40 μl extraction buffer (Wendel and Weden, 1989). Isozymes were separated on 10% starch gel in 2 buffer systems: ADH (alcohol dehydrogenase, E.C. 1.1.1.1), GDH (glutamate dehydrogenase, E.C. 1.4.1.2), PGI (glucose phosphate dehydrogenase, E.C. 5.3.1.9.) and GOT (glutamate oxaloacetate transaminase, E.C. 2.6.1.1.) in tris-citrate (pH 8.2); lithium-borate (pH 8.3), MDH (malate dehydrogenase, E.C. 1.1.1.37), PGD (phosphoglucose dehydrogenase, E.C. 1.1.1.44), SDH (shikimate dehydrogenase, E.C. 1.1.1.25), and ME (malic enzyme, E.C. 1.1.1.40) were separated in morpholine citrate (pH 6.1) in a 1:14 dilution of electrode buffer (Wendel and Weeden, 1989). Lithium-borate gels were separated at constant voltage and morpholine-citrate gels were separated at constant current. After separation the isozymes were detected on the gel slabs by standard staining methods (Wendel and Weeden, 1989).

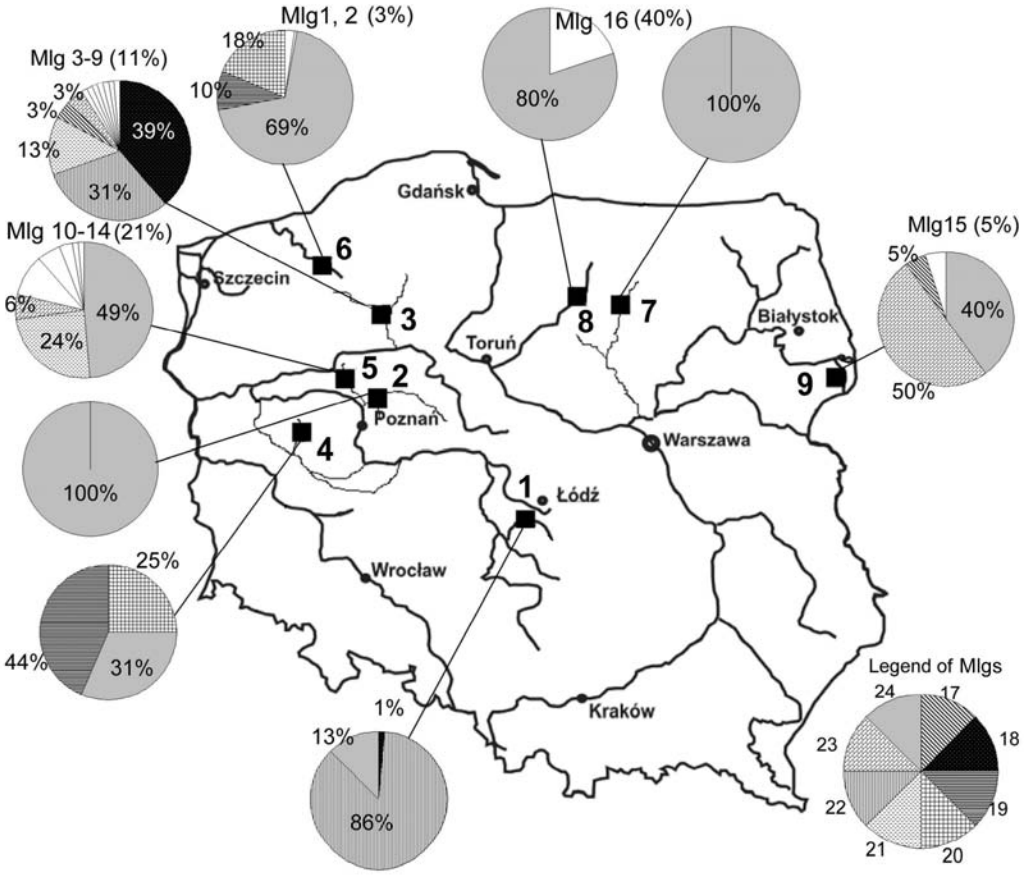

Fig. 1. Map of Poland showing locations of the studied populations of *C. cophocarpa*, with pie charts of multilocus genotypes. 1 – Grabia; 2 – Mała Wełna; 3 – Płytnica; 4 – Dojca; 5 – Flinta; 6 – Parsęta; 7 – Mławka; 8 – Drwęca; 9 – Łutownia; correlated with the frequencies of genotypes. Unshaded portions of pie charts with Mlg numbers indicate unique Mlgs in particular populations. Mlg numbers correspond to those given in Table 4.

DATA ANALYSIS

Allele frequencies, the percentage of polymorphic loci (P), the mean number of alleles per locus (A) and per polymorphic locus (A_p), and observed and expected heterozygosity (H_O, H_E) were estimated for each population and for the whole species using POPGENE-1.32 (Yeh et al., 2000). FSTAT version 2.9.3.2 (Goudet, 2001) was used to compute Wright's (1965) F statistics: inbreeding within individuals in populations (F_{IS}), inbreeding due to population subdivision as an indicator of the degree of differentiation between populations (F_{ST}), and overall level of inbreeding (F_{IT}). F statistics were calculated with Weir and Cockerham's (1984) multilocus estimators (f, F, θ) to measure deviation from Hardy-Weinberg equilibrium (HWE) at each polymorphic locus. A locus was considered polymorphic if more than one allele was observed. The statistical significance of F_{IS}, F_{ST}, F_{IT} was determined from 1500 permutations of alleles between individuals within samples, genotypes between samples and alleles between samples, respectively. Means and standard errors over loci were calculated by jackknifing over polymorphic loci; bootstrap confidence intervals (95% confidence intervals – CI) were constructed around jackknifed means of F statistics (Weir, 1996). Gene flow (N_m) was estimated with Wright's (1951) formula: $N_m = 0.25(1-F_{ST})/F_{ST}$. Genetic distance between pairs of populations were computed according to Nei (1972).

Three measures of clonal diversity were also estimated: number of genotypes per population (G), proportion of distinguishable genotypes (G/N) and multilocus genotype diversity (D_G) as a modification of the Simpson index (Piélou, 1969; Ellstrand and Roose, 1987). D_G values range from 0 to 1, and higher values correspond to greater clonal diversity. G/N values range from 0 to 1, and higher values correspond to greater clonal diversity. In both cases the potential level of diversity increases with the number of loci assayed. The formula of Aspinwall and Christian (1992) was used to calculate the probability that ramets with the same multilocus genotype belong to the same genet.

TABLE 2. Allele frequencies for 7 polymorphic loci in 9 populations of *C. cophocarpa* from western and eastern Poland. N – sample size

Locus	Allele	Western Poland						Eastern Poland		
		1 Grabia	2 Mała Wełna	3 Płytnica	4 Dojca	5 Flinta	6 Parsęta	7 Mławka	8 Drwęca	9 Łutownia
Mdh-1	1	—	—	0.013	—	0.157	—	—	—	—
	2	1.000	1.000	0.987	1.000	0.843	1.000	1.000	1.000	1.000
Mdh-2	1	—	—	0.045	—	—	0.030	—	—	—
	2	1.000	1.000	0.955	1.000	1.000	0.970	1.000	1.000	1.000
Adh	1	0.006	—	0.260	—	0.029	—	—	—	0.300
	2	0.988	1.000	0.480	1.000	0.942	1.000	1.000	1.000	0.400
	3	0.006	—	0.260	—	0.029	—	—	—	0.300
Gdh	1	—	—	—	—	—	—	—	0.200	0.050
	2	1.000	1.000	0.994	0.875	1.000	0.907	1.000	0.800	0.950
	3	—	—	0.006	0.125	—	0.093	—	—	—
Got-1	1	1.000	1.000	1.000	0.313	1.000	0.711	1.000	1.000	1.000
	2	—	—	—	0.687	—	0.289	—	—	—
Sdh	1	—	—	0.039	—	0.071	—	—	—	—
	2	1.000	1.000	0.961	1.000	0.929	1.000	1.000	1.000	1.000
Me	1	0.125	1.000	0.136	1.000	0.829	1.000	1.000	1.000	0.975
	2	0.875	—	0.864	—	0.171	—	—	—	0.025
N		80	10	75	64	70	135	50	50	20

RESULTS

In the 8 studied enzyme systems, 12 isozyme loci were detected. Only 7 of the 12 examined loci were polymorphic (*Mdh-1, Mdh-2, Adh, Gdh, Got-1, Sdh, Me*), with 2 or 3 alleles. The remaining ones (*Pgi-1, Pgi-2, Pgd-1, Pgd-2* and *Got-2*) were monomorphic in all populations studied and displayed a homozygous pattern (Tab. 2). Two of the studied populations (from the Mławka River and Mała Wełna River) were monomorphic in all loci. The remaining populations were polymorphic in 1–6 loci. The most variable population was the one from the Płytnica River (Tabs. 2, 3). Using 7 polymorphic loci, 24 different multilocus genotypes were detected in the studied populations. One genotype, representing almost 50% of all plants, was widespread and found in 8 populations; 6 genotypes occurred in 2 populations, one occurred in 3 populations, and 16 were found only once (Tab. 4). The commonest genotype was homozygous in all loci. Genotypes 1–14 and 18–22 occurred only in the western part of Poland; genotypes 15 and 16 were present only in the east. All these genotypes were rare, found only in one or two populations (Fig. 1). Allele 1 was detected in genotypes exclusive to western Poland (loci *Mdh-1, Mdh-2, Sdh*) and in a genotype exclusive to eastern Poland (*Gdh*). Different rare multilocus genotypes were found in the neighboring Parsęta (nos. 1, 2) and Płytnica (3–9) Rivers. The two unique genotypes from the Parsęta River were homozygous for the rare allele 1 at *Mdh-2*. Three unique genotypes (3, 4, 5) from the Płytnica were heterozygous at this locus. The number of genotypes in the populations ranged from 1 to 12, with a mean of 4.3. The highest number of genotypes were detected in the population from the Płytnica, and 7 of them were exclusive to this population. In two populations (Mała Wełna and Mławka Rivers) only one multilocus genotype, the commonest one, was noted (Tabs. 4, 5). The average probability that ramets with the same multilocus genotype belong to the same genet was 0.551. The mean proportion of distinguishable genotypes (G/N) was 0.0882. The Simpson index (D_G) ranged from 0.00 to 0.752, with a mean of 0.4024 (Tab. 5).

A total of 21 alleles were found in all loci. One rare allele (frequency <0.05) was found at *Mdh-2* (allele 1), which occurred in only two populations, from the Płytnica and Parsęta Rivers (Tab. 2). The percentage of polymorphic loci (P) within the studied populations ranged from 0.0 to 50.0, with a mean of 19.44 (Tab. 3). The mean number of alleles per locus (A) ranged from 1.00 to 1.58, with a mean of 1.231. The mean number of alleles per polymorphic locus (A_p) ranged from 1.00 to 2.00, with a mean of 1.403. The mean values of observed and expected heterozy-

TABLE 3. Sample size (N), percentage of polymorphic loci (P), number of alleles per locus (A), number of alleles per polymorphic locus (A_p), observed (H_O) and expected (H_E) heterozygosity, and fixation index (F) for the studied populations of *Callitriche cophocarpa*. SD – standard deviation; NA – not applicable; *** – $p < 0.001$; ** – $p < 0.01$; * – $p < 0.05$

No.	Population	N	P	A	A_p	H_O±SD	H_E±SD	F
1	Grabia	80	16.7	1.25	1.43	0.001±0.003	0.020±0.063	0.949**
2	Mała Wełna	10	0.0	1.00	1.00	0.00	0.00	NA
3	Płytnica	75	50.0	1.58	2.00	0.073±0.158	0.089±0.186	0.193*
4	Dojca	64	16.7	1.17	1.29	0.021±0.072	0.055±0.135	0.619**
5	Flinta	70	33.3	1.42	1.71	0.033±0.098	0.066±0.109	0.500**
6	Parsęta	135	25.0	1.25	1.43	0.015±0.053	0.053±0.123	0.711**
7	Mławka	50	0.0	1.00	1.00	0.00	0.00	NA
8	Drwęca	50	8.3	1.08	1.20	0.00	0.027±0.093	1.000**
9	Łutownia	20	25.0	1.33	1.57	0.054±0.172	0.069±0.194	0.216*
Population mean		62	19.44	1.231	1.403	0.0219	0.0421	0.5983
Overall species mean			58.33	1.750	2.286	0.023±0.039	0.083±0.128	0.7386

Total N = 554

gosity were 0.0219 and 0.0421, respectively. All the parameters had the highest values in the population from the Płytnica River. Fixation indices (F) calculated for each population ranged from 0.193 to 1.000; all populations showed a significant deficit of heterozygotes (Tab. 3). All measures of genetic diversity (P, A, A_p, H_O, H_E) were higher at the species level than at the population level (Tab. 3).

No polymorphic loci were very heterozygous at local and total population levels. Levels of inbreeding within local populations and in the sample as a whole were significant, as indicated by jackknife estimation of F_{IS} and F_{IT} calculated over polymorphic loci: 0.538 and 0.761, respectively (Tab. 6).

Jackknife estimation of mean differentiation between populations based on polymorphic loci showed that almost 50% of the total variation found in *C. cophocarpa* is between populations (F_{ST} = 0.48). The mean value of gene flow (N_m) between populations was 0.268 (Tab. 6).

Nei's genetic distances between the populations of *C. cophocarpa* were low, ranging from 0.0000 (between the Mławka River and Mała Wełna River populations) to 0.1381 (between the Płytnica River and Dojca River populations); the mean genetic distance between populations was 0.0429.

DISCUSSION

Genetic variation of the Polish populations of *C. cophocarpa* we examined was low; among the 554 examined plants only 24 different multilocus genotypes were found. One genotype was common and occurred in every population but one. The most variable was the population from the Płytnica River.

TABLE 4. Frequency of multilocus genotypes (Mlgs) in the studied populations of *C. cophocarpa*

MLG	Frequency of MLG	No. of rivers	Name of river
1	0.005	1	Parsęta
2	0.002	1	Parsęta
3	0.002	1	Płytnica
4	0.002	1	Płytnica
5	0.002	1	Płytnica
6	0.002	1	Płytnica
7	0.002	1	Płytnica
8	0.003	1	Płytnica
9	0.003	1	Płytnica
10	0.012	1	Flinta
11	0.002	1	Flinta
12	0.007	1	Flinta
13	0.003	1	Flinta
14	0.002	1	Flinta
15	0.002	1	Łutownia
16	0.018	1	Drwęca
17	0.006	2	Płytnica, Łutownia
18	0.056	2	Płytnica, Grabia
19	0.074	2	Parsęta, Dojca
20	0.074	2	Parsęta, Dojca
21	0.048	2	Flinta, Płytnica
22	0.166	2	Grabia, Płytnica
23	0.025	3	Płytnica, Łutownia, Flinta
24	0.480	8	Parsęta, Łutownia, Grabia, Mławka, Drwęca, Mała Wełna, Dojca, Flinta

TABLE 5. Number of samples (N_1), number of examined plants (N_2), number of polymorphic loci (N_3), number of multilocus genotypes observed (G), average probability of two ramets in a sample with the same allozyme pattern belonging to the same genet ($\overline{P}$ 1), proportion of ramets distinguishable (G/N), and Simpson's diversity index (D_G) for the studied populations of *Callitriche cophocarpa*

No.	Population	N_1	N_2	N_3	G	$\overline{P}$	G/N	D_G
1	Grabia	8	80	2	3	0.26	0.038	0.243
2	Mała Wełna	1	10	0	1	–	0.100	0.00
3	Płytnica	7	75	6	12	0.76	0.160	0.752
4	Dojca	5	64	2	3	0.68	0.047	0.655
5	Flinta	7	70	4	8	0.57	0.114	0.542
6	Parsęta	10	135	3	5	0.59	0.037	0.485
7	Mławka	5	50	0	1	–	0.058	0.00
8	Drwęca	5	50	2	2	0.34	0.040	0.329
9	Łutownia	2	20	3	4	0.66	0.200	0.616
Population mean		5.6	61.6	2.4	4.3	0.551	0.0882	0.4024

TABLE. 6. Summary of genetic diversity for 7 polymorphic loci in *C. cophocarpa*. SE – standard error; CI – confidence interval; *** – $p < 0.001$; ** – $p < 0.01$

Locus	F_{IS}	F_{IT}	F_{ST}	N_m
Mdh-1	1.000***	1.000***	0.109**	2.044
Mdh-2	0.521***	0.529***	0.015**	16.417
Adh	0.212***	0.486***	0.348***	0.468
Gdh	0.252***	0.323***	0.095***	2.382
Got-1	1.000***	1.000***	0.442***	0.316
Sdh	1.000***	1.000***	0.037**	6.507
Me	0.229**	0.821***	0.768***	0.076
Mean	0.538	0.761	0.483	0.2676
± SE	0.226	0.111	0.175	
95% CI	0.270-0.869	0.497-0.928	0.172-0.665	

Unfortunately we cannot compare this level of genetic variation with other populations of *C. cophocarpa*, since no other studies of the genetic variability of this species have been published. Some intraspecific isozyme phenotypic variation was reported by Demars and Gornall (2003) for *C. hermaphroditica*, *C. stagnalis*, *C. obtusangula*, *C. brutia* and *C. hamulata* in populations from Great Britain, but their genetic parameters were not computed.

Our results confirm the general observation that aquatic species have lower genetic variation than terrestrial plants (Les, 1988; Laushman, 1993). Some data on aquatic plants show widespread monomorphism. In some cases, populations have a single allozyme multilocus phenotype (Vermeersch and Triest, 2006). The average expected heterozygosity at the population level for 338 dicotyledonous taxa was estimated at $H_E = 0.096$ (Hamrick and Godt, 1989); for *C. cophocarpa*, a monoecious aquatic plant, we found $H_E = 0.0421$. Similar expected heterozygosity has been reported for other monoecious species of the genera *Ceratophyllum* (Les, 1991), *Potamogeton* and *Myriophyllum* (Hofstra et al., 1995; Hollingsworth et al., 1996; Kaplan and Štěpánek, 2003). A level of genetic variation higher than in *C. cophocarpa* was found for the dioecious *Vallisneria americana* ($H_E = 0.085$; Laushman, 1993). High levels of genetic variation in monoecious species with restricted geographic ranges, such as *Sagittaria isoetiformis* ($H_E = 0.218$) and *S. teres* ($H_E = 0.101$) (Edwards and Sharitz, 2000), seem to be an exception among aquatic species, but this may be due to their entomophily.

Our results suggest two main reasons for the low genetic variation of *C. cophocarpa*: clonal reproduction and domination of inbreeding. Infrequent flowering and the ability to propagate vegetatively by shoot fragmentation mean that a high contribution of clonal reproduction in this species is probable, as confirmed by the low value of the mean proportion of distinguishable genotypes ($G/N = 0.0882$); that is much lower than the mean value ($G/N = 0.25$) reported by Diggle et al. (1998) for taxa characterized by the absence or rarity of sexual reproduction. A number of authors have reported high rates of vegetative propagation in other aquatic species (Hofstra et al., 1995; Hollingsworth et al., 1996; Kaplan and Štěpánek, 2003). The clonal structure of the studied populations contributes to self-pollination in the species. The deficiency of heterozygotes observed in the studied populations of *C. cophocarpa* ($F_{IS} = 0.538$) indicates a high level of inbreeding or nonrandom matting in subdividing populations, attributable to limited pollen and seed transport. The observed heterozygosity of the examined populations was lower than that expected from HWE.

Monoecy and epihydrophilous pollination can promote self-fertilization in *C. cophocarpa*. Silander (1985) suggested a relationship between clone size and selfing rate: large clone size may increase the likelihood of geitonogamous pollen transfer, leading to self-fertilization and inbreeding. However, the presence of rare heterozygotes indicates that cross-pollination and gene flow between *C. cophocarpa* populations sometimes takes place. Limited sexual reproduction has been observed in other highly clonal aquatic species (Barrett et al., 1993).

Isozyme studies revealed high differentiation between populations of *C. cophocarpa*, reaching almost 50% of total genetic diversity (F_{ST} = 0.483). This confirms its clonal structure and limited gene flow. The F_{ST} value in *C. cophocarpa* was similar to G_{ST} reported by Hamrick and Godt (1989) for annual selfing species, which have more than 50% of their variation between populations. High G_{ST} values are also expected in clonal species, in which a majority of genetic variation is often found between populations (Ellstrand and Roose, 1987). The studied populations of *C. cophocarpa* are isolated from each other, and the existing physical barriers decrease the probability of gene flow between populations; gene flow within rivers is more probable. The differing, rare multilocus genotypes present in the neighboring Parsęta and Płytnica Rivers confirmed this finding. A similar situation was reported in populations of *Mimulus caespitosus*, a clonal perennial species growing in mountain streams (Ritland, 1989). The differentiation of *C. cophocarpa*, which occurs in discrete water bodies (rivers, lakes, ponds), is similar to that found in other aquatic freshwater plants (Les, 1991; Laushman, 1993; Edwards and Sharitz, 2000). It is higher than in aquatic species occurring in continuous marine water systems, such as the sea-grasses *Thallasia testudinum* (G_{ST} = 0.139; Schlueter and Guttman, 1998) and *Zostera marina* (G_{ST} = 0.107; Laushman, 1993).

Our allozyme studies showed that, apart from selfing and a high contribution of clonal reproduction, limited sexual reproduction (infrequent flowering) and limited gene flow (N_m = 0.268) are probably the main reasons for the generally low genetic variation and for the high level of differentiation between populations of *C. cophocarpa*. The founder effect resulting from the specificity of the species habitat (discrete water bodies) has an important influence on high differentiation between populations.

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