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Genetic Relationship Among Three Forms of Cabomba[1]

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ABSTRACT

The genetic relationship between the fanworts *Cabomba caroliniana* var. *caroliniana, C.c.* var. *multipartita,* and *C. pulcherrima* from eight sites in Florida was evaluated using gel electrophoresis. This technique permits the detection and quantification of gene differences between populations. Allele frequencies at 12 genetic loci were found to be extremely similar in each taxon indicating very high genetic identities. The lack of genetic differentiation suggests that morphological differences may best be ascribed to environmental factors.

Key words: genetic differentiation, electrophoresis, isozymes, fanwort, genetic identity, phenotypic plasticity.

INTRODUCTION

Fanworts (*Cabomba* sp.) are submersed plants which grow in ponds, lakes, and quiet streams of the southeastern United States. The distribution extends westward into Texas and northward to Illinois and Michigan with naturalized populations ranging from Virginia to southern New England (Fassett 1953). Three taxa of *Cabomba* are commonly reported in the literature for the United States: *C. caroliniana* var. *caroliniana* Gray (fanwort), *C. caroliniana* var. *multipartita* (green fanwort), and *C. pulcherrima* (Harper) Fass. (purple fanwort). It is believed that variety *multipartita* is a cultivated form of *C. c. caroliniana,* owing its existence to the aquarium industry (Tarver *et al.* 1978). *C. pulcherrima* is a purple colored segregate of *C. caroliniana* (Fassett 1953). There is some question, however, whether these taxa are actually genetically distinct populations or merely environmental variants.

The primary purpose of this study was to determine the genetic relationship among these three taxa of *Cabomba.* Genetic differentiation can be detected electrophoretically as differences in allele frequencies and in the most extreme case as fixation of alternate alleles. Genetically differentiated populations often differ physiologically and may therefore have pronounced effects on biological and chemical control programs.

MATERIALS AND METHODS

Allozyme data are based upon samples from eight geographically separate study sites in Florida. *Cabomba caroliniana* var. *caroliniana* was collected from the Suwannee River, Levy Co., population (CP). *C. c. multipartita* was sampled from Orange Lake, Alachua Co., population (MO), the Suwannee River (MS), and an experimental population (MF) provided by the USDA-ARS, Ft. Lauderdale, Florida, Broward Co. *C. pulcherrima* was collected from Brim Lake, Columbia Co., population (PB), Lake Miccosukee, Jefferson Co., population (PM), and Lake Carr, Leon Co., population (PC). Healthy individuals were randomly collected during the spring and summer of 1982 and maintained under growth lights in the laboratory. Prior to electrophoresis, leaf tips were macerated by a Plexiglass rod in a drop of 0.1 M HEPES, 0.025 M β-mercaptoethanol, pH 7.0 grinding solution. The crude extract was absorbed onto paper wicks, and inserted into a 12.4% starch gel. Each individual was assayed for six different protein/enzyme systems. Acid phosphatase (Acph), esterase (Est), anodal peroxidase (Apx), and malate dehydrogenase (Mdh) were separated as anodally migrating bands on a 0.0152 M Tris, 0.0037 M citrate, pH 7.9 gel. The bridge buffer was a 0.030 M, pH 8.6 boric acid solution. Gels were run at 300 v for 12 minutes, at which time the wicks were removed and the run continued until the borate front migrated 8.0 cm from the origin (2-2.5 h). Glutamate dehydrogenase (Gdh), and General Protein (GP) were separated as anodally migrating bands on a 0.0115 M Tris, 0.057 M citrate, pH 8.0 gel. The bridge buffer was a 1:29 dilution of the gel buffer. Gels were run at 300 v for 12 minutes, at which time the paper wicks were removed and the run continued for 3 hours. After electrophoresis, and prior to isozyme staining, the gels were sliced horizontally into thirds. Isozyme staining procedures are similar to those described previously (Wain 1982). All gels were stained until banding sites reached optimum clarity, and then were fixed in 45% ethanol and wrapped in plastic wrap. Those gels exhibiting unusual banding patterns were photographed.

The six gene systems were chosen solely on the basis of availability of staining reagents and degree of resolution and consistency of banding. A total of 12 zones of electrophoretic activity (presumed loci) were analyzed for this study.

Genetic differentiation among populations of *Cabomba* was calculated using the statistic of genetic identity described by Nei (1972, 1978). Genetic identity may range from zero to one. A value of zero indicates that two populations are completely dissimilar, a value of one indicates that two populations share the same alleles in essentially the same frequencies. Individuals of all three taxa were run on the same gel in order to make scoring of the bands more accurate. Certain individuals of each form were electrophoresed several times during the course of the study, their isozyme banding patterns did not change with age.

RESULTS AND DISCUSSION

Table 1 lists the sample sizes and allele frequencies of the 12 genetic loci analyzed in *Cabomba.* Numerical suf-

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TABLE 1. ALLELE FREQUENCIES AND SAMPLE SIZES, N, FOR LOCI SURVEYED IN POPULATIONS OF *Cabomba*.

System	allele	populations CP N=42	CS N=40	MS N=45	MF N=45	MO N=43	PB N=42	PM N=45	PC N=42
Acph-1	3.0	.048	.062	.089	.071	.070	.071	.089	.048
	3.2	.952	.938	.911	.929	.930	.929	.911	.952
Acph-2	5.0	.976	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	5.2	.024							
Apx-1	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Apx-2	2.9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Apx-3	3.3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Apx-4	3.7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Apx-5	6.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh	5.2	.048	.100	.060	.022	.023		.022	.024
	5.9	.952	.900	.940	.978	.977	1.00	.978	.976
Est	5.0						.012	.044	
	5.2	1.00	1.00	1.00	1.00	1.00	.988	.956	1.00
Gp-1	1.5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Gp-2	2.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Gdh	0.4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

fixes following a protein name denote the existence of multiple gene loci. Electromorphs (alleles) are referenced by the distance in centimeters migrated from the gel origin. Acph-1, Mdh, and Est are diallelic systems, yet allele frequencies are very similar in each taxon. For example, the frequency of Acph-1 allele 3.2 ranges only from a low of 0.911 in populations MS and PM to a high of 0.952 in populations CP and PC. The remaining nine genic loci (Acph-2, Apx-1, Apx-2, Apx-3, Apx-4, Apx-5, Gp-1, Gp-2, and Gdh) are monomorhpic for the same allele in each population. These similarities in allele frequencies are reflected by high values of genetic identity. Mdh is the only locus which exhibits an average genetic identity below 1.0 (Table 2), yet the values are still quite high. The average genetic identity for each pair-wise comparison of taxa is presented in Table 3. Our sample of genetic markers indicates that these taxa of *Cabomba* are genetically indistinguishable.

Phenotypically plastic morphological traits are well documented in aquatic plants (Sculthorpe 1967, Hutchinson 1975), and we suggest that morphological differences found among these three taxa of *Cabomba* can best be explained in terms of environmentally induced variation. Godfrey and Wooten (1981) report that *Cabomba* with high levels of purple pigment are commonly found in very warm waters, whereas individuals with little or no purple pigment inhabit cooler waters. They could find no characters other than color to distinguish *C. pulcherrima* from *C. caroliniana*. Likewise, little or no genetic differentiation has been reported among growth forms of the waterhyacinth, *Eichhornia crassipes* Mart. (Wain and 1980), or life history forms of the eelgrass, *Zostera marina L.* (Gagnon *et al.* 1980).

TABLE 2. GENETIC IDENTITY, I, AMONG POPULATIONS OF *Cabomba* FOR MDH.

	1	2	3
C. c. caroliniana	0.986	0.992	0.990
C. c. multipartita		0.999	1.000
C. pulcherrima			1.000

TABLE 3. AVERAGE GENETIC IDENTITY, I, AMONG TAXA OF *Cabomba*.

	1	2	3
C. c. caroliniana	0.999	0.999	0.999
C. c. multipartita		1.000	1.000
C. pulcherrima			1.000

Morphological characters such as color are often used to separate varieties, subspecies, and even species. When differences in such characters do have a genetic basis, their expression may be controlled by only one or a few genes (Grant 1971). In such cases, genetic differences among the color morphs are trivial and do not represent a significant form of differentiation. The process of species formation, however, is generally accompanied by substantial genetic differentiation (Ayala 1977, Gottlieb 1977), and often reflects basic ecophysiological differentiation among the population systems (Lewontin 1974).

The lack of genetic differentiation between *Cabomba caroliniana* and *C. pulcherrima* strongly suggests that these taxonomic entities are actually part of a common gene pool and thus will react similarly to applied programs of biological and chemical control.

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