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GENETIC DIVERSITY IN THE MONOECIOUS HYDROPHILE CERATOPHYLLUM (CERATOPHYLLACEAE)[1]

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This study surveys genetic variation in two clonal, monoecious, water-pollinated species that differ in their extent of sexuality and distributional range. Electrophoresis was used to quantify allozyme variability in 12 Wisconsin populations of the widespread *Ceratophyllum demersum* and the rare *C. echinatum*. Electrophoretic data indicate that populations of both species have low levels of sexual recombination, low levels of variation, and are structured genetically like inbreeding terrestrial plants. *Ceratophyllum* populations differ from "typical" clonal terrestrial plants by lower genetic diversity, lower proportions of multiclonal populations, and fewer genotypes per population. In two populations where sexual recombination is documented, heterozygosity is low with significant deficiencies. Monoecy in *Ceratophyllum* may be related to historical evolutionary factors, whereas vegetative reproduction has a greater influence on the genetic population structure of extant populations. The low genetic identity between *C. demersum* and *C. echinatum* supports their recognition as distinct species.

Breeding systems and the degree of vegetative reproduction can be important determinants of genetic population structure in plants capable of clonal growth (Murawski and Hamrick, 1990). Water-pollinated plants (hydrophiles) are interesting species to study in this respect because they possess unusually high incidences of dicliny and undergo extensive vegetative reproduction. Dioecy, which occurs in only 4% of flowering plants, and monoecy, found in roughly 5% of all angiosperms (Richards, 1986), together characterize 92% of hydrophile species (Les, 1988a). Consequently, several authors have predicted that hydrophiles are outcrossed and possess large amounts of genetic variability (Hartog, 1970; Pettitt, Ducker, and Knox, 1981). Dioecy (sex separation between individuals) is typically equated with outcrossing breeding systems (Richards, 1986), but less is understood of the genetic consequences of monoecy (sex separation on one individual). Although monoecious species are mostly self-compatible and capable of both selfing and outcrossing (Grant, 1975; Richards, 1986), they are predicted to show high intrapopulational genetic variation, low interpopulational differentiation, and reduced subdivision within populations (Loveless and Hamrick, 1984).

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Complicating the understanding of breeding systems in perennial hydrophiles is their low sexuality (plants are seldom observed in flower) and their efficient mechanisms for vegetative reproduction, which together promote extensive clonal growth (Les, 1988a). Clonal growth of hydrophytes is widely recognized (Sculthorpe, 1967; Hutchinson, 1975), yet a recent review of genetic diversity in clonal plants (Ellstrand and Roose, 1987) entirely excludes submersed aquatic plants. Most clonal terrestrial plant species possess intermediate levels of genetic diversity and exist as multiclonal populations. There are few empirical data, however, pertaining to the question of genetic structure and diversity in clonal submersed aquatic plants.

Overall, patterns of genetic diversity within and among aquatic angiosperm populations have been poorly studied and are not well understood. Recent reviews of more than 100 plant species surveyed for patterns of genetic variation (Hamrick, 1983; Loveless and Hamrick, 1984) include only one submersed aquatic plant. Genetic data summarized from other electrophoretic studies of aquatic plant populations indicate that many species are characterized by extensive fixation and possess miniscule amounts of detectable variability (Wain, Haller, and Martin, 1985). A difficulty with interpreting many of these reports, however, is that they frequently fail to provide quantitative genetic data, thereby precluding the computation of meaningful statistics (Les, 1988a).

Because water pollination is abiotic, the ge-

netic structure of hydrophile populations presumably would be similar to that of wind-pollinated species. Fundamental differences, however, occur in the pollen dispersal range of wind- and water-pollinated species, which can limit pollen gene flow in the latter (Les, 1988a). Therefore, genetic studies are necessary for clarification of similarities and differences existing between hydrophiles and anemophilous plants.

The genus *Ceratophyllum* L. (Ceratophyllaceae) is an ideal group for studying patterns of genetic variation in clonal water-pollinated plants. These are obligately submersed, perennial, self-compatible, monoecious, freshwater plants that represent the only hydrophilous genus of dicots (Les, 1988a, b). Taxonomically, the genus has been thoroughly studied (Les, 1985, 1986a, 1988b, c, 1989) and consists of six well-defined species.

All species of *Ceratophyllum* reproduce vegetatively by fragmentation. Because all *Ceratophyllum* are suspended, rootless species, fragments are highly successful at colonizing new sites and are the major dispersal propagules (Les, 1986a). The frequency of sexual reproduction and extent of distributional range contrast in two sympatric North American species, *C. demersum* and *C. echinatum.* Low levels of sexuality have long been recognized in *C. demersum* (e.g., Jones, 1931; Martin and Uhler, 1939). In preparation of a monograph, less than 15% of North American specimens examined represented reproductive material (Les, 1986b, c). Sexuality in *C. echinatum* is more commonplace with about 35% of the specimens examined representing reproductive material (Les, 1986b). Even these figures probably overestimate the sexuality of both species because specimens of this genus are much more likely to be collected when the "interesting" fruits are present.

A relationship exists between the degree of sexuality in these species and their pattern of morphological variability. The less sexual *C. demersum* is uniform morphologically within populations but not between populations (Les, 1986a, 1988a), whereas the more sexual *C. echinatum* is characterized by similar patterns of intra- and interpopulational morphological variability (Les, 1988a, b). Whether the same patterns exist with respect to allozyme variability, however, has not yet been ascertained. Only one electrophoretic study (a small survey of one population of *C. demersum*) has been reported previously for the genus (Les, 1986a). For terrestrial plants, no reliable correlation has been established between the extent of intra- or interpopulational allozyme variability and the mode of reproduction (Hamrick and Godt, 1990).

Ceratophyllum demersum is a common, cosmopolitan species, whereas, *C. echinatum* is restricted to North America and is becoming rare throughout much of its range (Les, 1986b, 1989). Although there have been many electrophoretic studies of variation among terrestrial species with different geographical distributions, such data for aquatic plants are nearly nonexistent. This is unfortunate because aquatic plants offer the best examples of species with cosmopolitan distributions. Because the extent of geographic range is highly correlated with allozyme diversity in terrestrial plants (Hamrick and Godt, 1990), electrophoretic data are appropriate for comparing levels of genetic variation between aquatic plants such as *C. demersum* and *C. echinatum* that reflect different extremes of geographic range.

The fossil record indicates relatively slight morphological evolution in the genus *Ceratophyllum,* which has apparently undergone a prolonged period of "stasis" (Herendeen, Les, and Dilcher, 1990). The superficial morphological similarity of *C. demersum* and *C. echinatum* has influenced some taxonomists to regard them as conspecific varieties (e.g., Eyles and Robertson, 1944). Although sufficient morphological evidence now exists for maintaining *C. demersum* and *C. echinatum* as distinct species (Les, 1985, 1988b), electrophoretic data offer an independent source of evidence pertinent to the question of their taxonomic distinctness. Reductions in electrophoretically determined genetic identities from conspecific populations to congeneric species have been well documented for terrestrial plants (Crawford, 1983) and should also characterize these aquatic species.

In this study, electrophoretic data are used to quantify and compare genetic variation in populations of *Ceratophyllum demersum* and *C. echinatum.* Study populations were selected within a small geographic region (state of Wisconsin, USA) to facilitate the detection of interpopulational and interspecific gene flow. Specifically, this study compares the population structure and pattern of genetic variability of these hydrophile species to data summarized for terrestrial plants.

MATERIALS AND METHODS

A sample of 341 plants representing 262 individuals from nine populations of *Ceratophyllum demersum* and 79 individuals from three populations of *C. echinatum* was surveyed (Table 1). Collections were made using

TABLE 1. *Sources of* Ceratophyllum *populations examined. Sexual condition at time of collection = f (flowering) or v (vegetative)*

Ceratophyllum demersum: WI: Ozaukee Co., Saukville Twp, T11N, R21E, NE¼ sect 29, *Mud Lake* (f); 1–2 m water in stagnant inlet to lake; 17 June 1988; *Les s.n.* (UWM). Price Co., Elk Twp, T37N, R1E, SE¼ sect 7, *Elk Lake* (f); 1–2 m water along shoreline at south and southeast edge of lake; 20 June 1988; *Les s.n.* (UWM). Vilas Co., Arbor Vitae Twp, T40N, R6E, N½ of SE¼ sect 26, *Little Muskie Lake* (f); 2 m water along SE shoreline of lake; 24 June 1988; *Les s.n.* (UWM). Vilas Co., Boulder Junction Twp, T41N, R7E, sect 19, *Trout Lake* (v); 1–2 m water along shoreline at south end of lake; 22 October 1988; *Remley s.n.* (UWM). Vilas Co., Presque Isle Twp, T43N, R7E, NW¼ sect 34, *Wildcat Lake* (v); 1–2 m water along shoreline at public access site, SE end of lake; 25 June 1988; *Les s.n.* (UWM). Washington Co., West Bend Twp, T11N, R19E, W½ sect 22, *Lucas Lake* (f); in 1–2 m water along S shore at W end of lake; 18 June 1988; *Les s.n.* (UWM). Waukesha Co., Mukwanago Twp, T5N, R18E, NE¼ sect 35, *Mukwanago River* (v); shallow water along banks of river at parking lot just before railroad bridge; 14 September 1988; *Remley s.n.* (UWM). Waukesha Co., Oconomowoc Twp., T8N, R18E, SW¼ of SE¼ sect 19, *Okauchee Lake* (f); 1 m water along W shore of inlet to lake at access site; 3 July 1988; *Les s.n.* (UWM). Winnebago Co., Algoma Twp, T18N, R16E, SW¼ sect 10, *Lake Butte Des Morts* (f); 1–2 m water along SW shore of bay along E side of highway 41 overpass; 1 July 1988; *Les s.n.* (UWM).

Ceratophyllum echinatum: WI: Ashland Co., Gordon Twp, T43N, R2W, SW¼ sect 14, *Knab Lake* (v); 1–2 m water, 8–10 m from shore directly east of public access site, 3.2 km SE of Morse; 21 June 1988; *Les s.n.* (UWM). Chippewa Co., T30N, R8W, SW¼ of SW¼ of sect 2, *Cornell Pond* (f); 0.5–1 m water along shore of small pond 12 km east of Bloomer, 1.2 km SE of Cornell Lake; 22 June 1988; *Les s.n.* (UWM). Vilas Co., T42N, R11E, NW¼ of NW¼ of sect 13, *Lac Vieux Desert* (f); in 1–1.5 m water on W side of road over creek at entrance to Simpson estate; 16 July 1990; *Les and Gerber s.n.* (UWM).

a rake or by snorkeling, and at widely spaced intervals to minimize resampling of clonally derived ramets. Populations observed to be in flower and/or fruit at the time of collection were recorded (Table 1). Plants were grown in greenhouse culture during the study period.

For electrophoresis, small, apical shoot fragments were washed thoroughly and placed in cool distilled water. These were ground in a chilled mortar and pestle using three drops of extracting buffer (Gottlieb, 1981b). Extracts were centrifuged for 3.5 minutes at 12,000 rpm. Supernatants were absorbed onto Whatman 3MM paper wicks and loaded into 12% starch gels. Aldolase (ALD, EC 4.1.2.13), aspartate aminotransferase (AAT, EC 2.6.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.2), phosphoglucoisomerase (PGI, EC 5.3.1.9), and triosephosphate isomerase (TPI, EC 5.3.1.1) were resolved using a pH 8.3 lithium-borate buffer system (Gottlieb, 1981b). Isocitrate dehydrogenase [$NADP^+$] (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), phosphoglucomutase (PGM, EC 2.7.5.1), and shikimate dehydrogenase (SKDH, EC 1.1.1.25) were resolved using a pH 6.5 buffer system (Cardy, Stuber, and Goodman, 1981). Staining procedures for all enzymes followed Soltis et al. (1983) using agarose overlays for ALD, IDH, PGI, TPI, 6-PGDH, PGM, and SKDH.

Genetic interpretations of banding patterns were inferred from known isozyme numbers in diploid plants and the active subunit structure of each enzyme (Gottleib, 1981a, 1982; Crawford, 1983). Isozymes were numbered sequentially beginning with the most anodally migrating; allozymes were labeled alphabetically, also beginning with the most anodal form. These numbers and letters correspond to inferred encoding alleles.

Allele frequencies were calculated for each population. The proportion of polymorphic loci (P, where the frequency of the most common allele is <0.95), mean number of alleles at polymorphic loci (K_p), mean number of alleles/locus (K), observed heterozygosity (H_o, scored by direct count), expected heterozygosity (H_{exp}), and percent of total observed alleles of a species present in a population (P_a) were calculated manually. Values of P_s and K_s were calculated for each species. A correlation between P and P_a was evaluated by calculation of the "Pearson" product-moment correlation coefficient and Bartlett chi-square test using the SYSTAT statistical software package (Wilkinson, 1988). Frequency data were used to calculate genetic identities (Nei, 1972) and gene diversity statistics. Gene diversity in the total population (H_T), gene diversity within subpopulations (H_S), gene diversity between subpopulations (D_{ST}), and proportion of total genetic diversity among populations (G_{ST}) (Nei, 1973) were calculated using version 2.1 of GENESTAT-PC (Lewis and Whitkus, 1989). An average linkage cluster analysis (UPGMA) of genetic identities was carried out using version 1.3 of the MVSP multivariate statistical package (Kovach, 1986). Deviation from expected heterozygote frequencies at polymorphic enzyme loci was assessed by calculation of Wright's fixation index (F) and a chi-square test of F (Li and Horvitz, 1953).

Numbers of discernible multilocus genotypes and their relative frequencies were calculated manually for each population directly from electrophoretic data. Calculations of genotypes/population, proportion distinguisha-

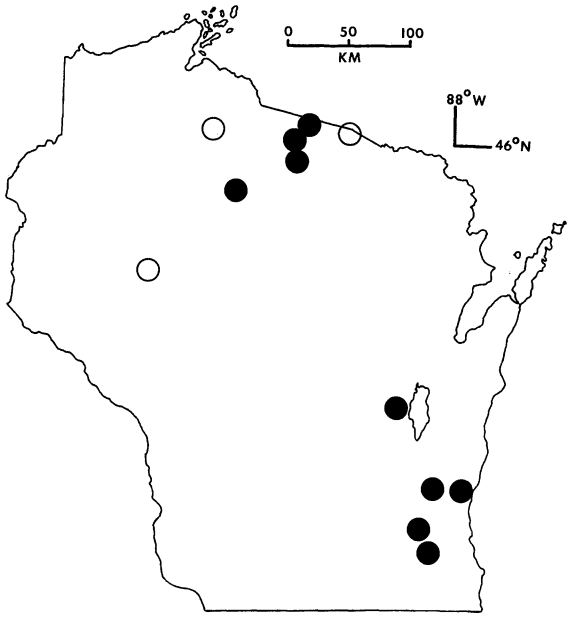

Fig. 1. Map of Wisconsin, USA showing location of study populations for *Ceratophyllum demersum* (closed circles) and *C. echinatum* (open circles).

ble, percent multiclonal populations, multilocus genotype diversity, populations/genotype, and percent of local and widespread genotypes were made for *C. demersum* following the methods of Ellstrand and Roose (1987). These calculations were omitted for *C. echinatum* which was uniclonal in all three populations.

RESULTS

Seven of the 12 populations surveyed electrophoretically (Fig. 1) were flowering at the time of collection (Table 1). Ten enzymes putatively coded by 17 genetic loci were resolved by electrophoresis. PGI-1 and TPI-3 were monomorphic. Both *Ceratophyllum demersum* and *C. echinatum* possessed from one to three alleles at a locus (Table 2), with only three populations fixed at all loci surveyed. Mean values of P, K_p, K, and H_o were higher for *C. demersum* than *C. echinatum* (Table 3). Species values for the proportion of polymorphic loci (P_s) and number of alleles per locus (A_s) were higher in *C. demersum* (0.53; 1.82) than in *C. echinatum* (0.14; 1.57). The percent of total species alleles observed in a given population (P_a) was slightly higher in *C. echinatum* (Table 3). In both species, the proportion of polymorphic loci (P) is significantly correlated with the percent total species alleles (P_a) in populations (*C. demersum*: $r = 0.974$, $P < 0.001$; *C. echinatum*: $r = 1.000$, $P < 0.004$).

For polymorphic loci, the mean total gene diversity (H_T) was higher for *C. echinatum* with relatively equal partitioning within (H_S) and between (D_{ST}) populations (Table 4). For *C. demersum,* more of the total diversity was partitioned among populations (Table 4). The relative proportion of among populational variation to the total variation (G_{ST}) was similar and very high for both species (Table 4).

Seven populations of *C. demersum* showed significant deviations from expected heterozygote frequencies at most polymorphic loci (Table 5). These deviations mostly represented heterozygote deficiencies except for Little Muskie Lake, Lucas Lake, Mud Lake (where populations showed "fixed" heterozygotes at all polymorphic loci), and one locus (PGM-2) in Okauchee Lake (Table 5). Two populations of *C. echinatum* also showed "fixed" heterozygosity at both polymorphic loci (Table 5).

Eight of the 12 populations surveyed consisted of only one electrophoretically discernible genotype (Table 6). In populations where two, three, six, and eight genotypes were observed, the frequencies of the most common genotype were 0.879, 0.842, 0.563, and 0.545, respectively (Table 6). Values of genotypes/population, the proportion of clones distinguishable, percent multiclonal populations, multilocus genotype diversity, and populations/genotype were all much lower for *C. demersum* than average values reported for terrestrial plants (Table 7). Sampled populations of *C. demersum* possessed a high percentage of local genotypes and lacked widespread genotypes (Table 7).

Genetic identity (Table 8) was high among *C. demersum* populations (mean = 0.8899), lower among *C. echinatum* populations (mean = 0.6926), and was substantially reduced between *C. demersum* and *C. echinatum* (mean = 0.1708). A cluster analysis of genetic identities among all populations studied (Fig. 2) groups northern Wisconsin populations (Wildcat Lake, Trout Lake, Elk Lake) with one southern Wisconsin population (Mukwanago River). In southern Wisconsin, populations from "protected" sites (Mud Lake, Lucas Lake) and populations from more disturbed sites (Little Muskie Lake, Okauchee Lake, Lake Butte Des Morts) also clustered. The two populations of *C. echinatum* closest geographically (Lac Vieux Desert, Knab Lake) did not cluster closely (Fig. 2).

DISCUSSION

Levels of sexuality in Ceratophyllum populations—During the collection of study ma-

TABLE 2. *Allele frequencies (— no activity) at polymorphic loci for 12* Ceratophyllum *populations. Sample size (N) indicated*

Locus/allele		Wildcat Lake (32)	Trout Lake (32)	Little Muskie (21)	Elk Lake (33)	Butte Des Morts (33)	Okau-chee Lake (32)	Lucas Lake (28)	Mud Lake (32)	Mukwa-nago River (19)	Cornell Pond (32)	Knab Lake (25)	Vieux Desert (22)
AAT-1	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	—	1.000	1.000	0.0
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—	0.0	0.0	1.000
ALD-1	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0	0.0
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000
	c	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	0.0	0.0
GDH-1	*a*	0.0	0.0	0.0	0.061	0.0	0.047	0.500	0.500	0.079	1.000	1.000	1.000
	b	1.000	1.000	1.000	0.879	1.000	0.906	0.0	0.0	0.842	0.0	0.0	0.0
	c	0.0	0.0	0.0	0.061	0.0	0.047	0.500	0.500	0.079	0.0	0.0	0.0
IDH-1	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.500	0.500
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.500	0.500	0.0	1.000	0.500	0.500
	c	1.000	1.000	1.000	1.000	1.000	1.000	0.500	0.500	1.000	0.0	0.0	0.0
MDH-1	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0	1.000
	b	0.0	0.0	0.0	0.0	0.0	0.031	0.0	0.0	0.0	0.0	0.0	0.0
	c	0.0	0.0	1.000	0.121	1.000	0.906	1.000	1.000	0.158	0.0	0.0	0.0
	d	1.000	1.000	0.0	0.879	0.0	0.063	0.0	0.0	0.842	0.0	1.000	0.0
MDH-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	0.0	0.0
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000	1.000
6-PGDH-1	*a*	0.0	0.0	0.500	0.121	0.909	0.0	0.0	0.0	0.158	—	—	—
	b	1.000	1.000	0.500	0.879	0.091	1.000	1.000	1.000	0.842	—	—	—
	c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.500
6-PGDH-2	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0	0.0
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	0.0	0.500
PGI-2	*a*	0.0	0.0	0.0	0.121	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	b	1.000	1.000	1.000	0.879	1.000	1.000	1.000	1.000	0.158	0.0	0.0	0.0
	c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0	1.000
	d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0
	e	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.842	0.0	0.0	0.0
PGI-3	*a*	1.000	1.000	1.000	1.000	0.758	1.000	1.000	1.000	0.842	—	—	—
	b	0.0	0.0	0.0	0.0	0.242	0.0	0.0	0.0	0.158	—	—	—
PGM-1	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0	1.000
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0
	c	1.000	1.000	1.000	0.879	1.000	1.000	0.500	0.0	1.000	0.0	0.0	0.0
	d	0.0	0.0	0.0	0.0	0.0	0.0	0.500	1.000	0.0	0.0	0.0	0.0
	e	0.0	0.0	0.0	0.121	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PGM-2	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000	1.000
	b	1.000	1.000	1.000	0.879	0.197	0.875	0.500	0.500	0.895	0.0	0.0	0.0
	c	0.0	0.0	0.0	0.121	0.803	0.125	0.500	0.500	0.105	0.0	0.0	0.0
SKDH-1	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	0.0	0.0
TPI-1	*a*	0.0	0.0	0.0	0.0	0.0	0.063	0.0	0.0	0.0	0.0	0.0	0.0
	b	1.000	1.000	0.500	1.000	1.000	0.937	0.500	0.500	1.000	0.0	0.0	0.0
	c	0.0	0.0	0.500	0.0	0.0	0.0	0.500	0.500	0.0	1.000	1.000	1.000
TPI-2	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	0.0	0.0

terial in 1988–1990, seven of the 12 populations (58%) were sexual (unusually low water levels in 1988 may have influenced the high observed sexuality). This observation is reported to emphasize that most populations studied were at least capable of sexual reproduction and were not obligately apomictic. Facultative sexuality in clonal plants is not unusual and occurs commonly in terrestrial species (Silander, 1985; Ellstrand and Roose, 1987).

The influence of sexual reproduction on genetic diversity in *Ceratophyllum* species is difficult to ascertain because significant correlations have not been established between common measures of genetic variation (e.g., *P, K*) and levels of sexuality in plants (Hamrick, Linhart, and Mitton, 1979). Minor correlations have been indicated between reproductive modes and gene diversity, namely values of H_T and H_S (Loveless and Hamrick, 1984). Values of both H_T and H_S were found to be lower

TABLE 3. *Proportion of polymorphic loci (P), mean number of alleles per polymorphic locus (K_p), mean number of alleles per locus (K), mean observed loci heterozygous per individual (H_o), expected heterozygosity (H_{exp}), and percent of total alleles per population (P_a) in 12* Ceratophyllum *populations*

Species/population	P	K_p	K	H_o	H_{exp}	P_a
Ceratophyllum demersum						
Elk Lake	0.35	2.17	1.41	0.007	0.076	77.4
Lake Butte Des Morts	0.18	2.00	1.18	0.005	0.050	64.5
Little Muskie Lake	0.12	2.00	1.12	0.118	0.059	61.3
Lucas Lake	0.29	2.00	1.29	0.294	0.147	71.0
Mud Lake (inlet)	0.24	2.00	1.24	0.235	0.118	67.7
Mukwanago River	0.35	2.17	1.41	0.009	0.090	76.7
Okauchee Lake	0.24	2.50	1.35	0.020	0.040	74.2
Trout Lake	0.00	—	1.00	0.000	0.000	54.8
Wildcat Lake	0.00	—	1.00	0.000	0.000	54.8
Mean	0.20	2.12	1.22	0.076	0.064	66.9
Ceratophyllum echinatum						
Cornell Pond	0.00	—	1.00	0.000	0.000	63.6
Knab Lake	0.07	2.00	1.07	0.071	0.036	68.2
Lac Vieux Desert	0.14	2.00	1.14	0.143	0.071	72.7
Mean	0.07	2.00	1.07	0.071	0.036	68.2

in an obligate apomict than in sexual species, with facultative apomicts showing the highest values (Loveless and Hamrick, 1984). More recent tabulations comparing only sexual vs. mixed sexual/asexual species have found no significant correlation with gene diversity statistics (Hamrick and Godt, 1990). Total diversity (H_T for both *C. demersum* (0.211) and *C. echinatum* (0.529) is higher than the value reported by Loveless and Hamrick (1984) for an obligate apomict (0.172). Within-population diversity (H_S) is less in *C. demersum* (0.085) and higher in *C. echinatum* (0.255) than an obligate apomict (0.159). This result probably does not reflect the relatively higher sexuality of *C. echinatum,* because all populations examined of this species were monoclonal.

TABLE 4. *Gene diversity statistics (after Nei and Chesser, 1983) for polymorphic loci and all loci in* Ceratophyllum demersum *and* C. echinatum

Locus	H_T	H_S	D_{ST}	G_{ST}
C. demersum				
GDH-1	0.4234	0.1893	0.2341	0.5529
IDH-1	0.1978	0.1131	0.0846	0.4279
MDH-1	0.4915	0.0739	0.4176	0.8497
6-PGDH-1	0.3050	0.1294	0.1756	0.5756
PGI-2	0.1937	0.0542	0.1396	0.7204
PGI-3	0.0851	0.0716	0.0135	0.1584
PGM-1	0.3000	0.0806	0.2194	0.7312
PGM-2	0.3645	0.2190	0.1455	0.3992
TPI-1	0.2897	0.1831	0.1067	0.3681
Mean	0.2112	0.0853	0.1260	0.4948
All loci	0.1559	0.0655	0.0904	0.5797
C. echinatum				
IDH-1	0.4466	0.3399	0.1067	0.2389
6-PGDH-2	0.6122	0.1700	0.4422	0.7224
Mean	0.5294	0.2550	0.2745	0.4807
All loci	0.2343	0.0364	0.1979	0.8446

The proportion of the total alleles observed for a species found within each population (P_a) has also been considered as an indicator of sexuality. Comparing reproductive races of *Panicum maximum,* Usberti and Jain (1978) observed P_a values of 98.5% for sexual populations and 65.7% for asexual populations. From a tabulation of 114 studies, Hamrick (1983) reported an average P_a value of 62% for sexually reproducing species, with a higher value (73.2%) for mixed mating systems. Again, the single value for an asexual species in that report (65%) provides for no meaningful evaluation. The mean P_a values for populations of *C. demersum* (66.9%) and *C. echinatum* (68.2%) are not particularly informative (Table 3), falling midway between those averages reported for sexual and mixed reproductive modes. *Ceratophyllum* populations with the lowest P_a (54.8%) are uniclonal; however, much higher values (71%–73%) also occur in other uniclonal populations (Tables 3, 7).

The significant positive correlation between P_a and P in populations of *C. demersum* and *C. echinatum* indicates that polymorphism in these populations is mainly a function of the proportion of total allelic variation of the species present. Although this relationship is not surprising, it is worth considering that most of the total allelic variation in sexual outcrossing species resides within populations (Gottlieb, 1981a; Crawford, 1983). In such instances, the correlation should not be as obvious because both the P and P_a values should be uniformly high among sexual outcrossing populations.

TABLE 5. *Deviations from expected heterozygote frequencies at polymorphic enzyme loci in nine* Ceratophyllum *populations. P_{1-3} are allelic frequencies; calculation of Wright's fixation index, F, and chi-square test of F follows methods of Li and Horvitz (1953)*

Species/ population	(N)	Locus (# alleles)	P_1	P_2	P_3	Frequency of heterozygotes: Observed	Frequency of heterozygotes: Expected	F	χ^2 of F
C. demersum									
Elk Lake	(33)	GDH-1 (3)	0.879	0.061	0.061	0.121	0.222	0.455	13.66**
		MDH-1 (2)	0.879	0.121	—	0.000	0.213	1.000	33.00***
		6PGDH-1 (2)	0.879	0.121	—	0.000	0.213	1.000	33.00***
		PGI-2 (2)	0.879	0.121	—	0.000	0.213	1.000	33.00***
		PGM-1 (2)	0.879	0.121	—	0.000	0.213	1.000	33.00***
		PGM-2 (2)	0.879	0.121	—	0.000	0.213	1.000	33.00***
Lake Butte Des Morts	(33)	6PGDH-1 (2)	0.909	0.091	—	0.000	0.165	1.000	33.00***
		PGI-3 (2)	0.758	0.242	—	0.000	0.367	1.000	33.00***
		PGM-2 (2)	0.803	0.197	—	0.091	0.316	0.713	16.78***
Little Muskie Lake	(21)	6PGDH-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	21.00***
		TPI-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	21.00***
Lucas Lake	(28)	GDH-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	28.00***
		IDH-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	28.00***
		PGM-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	28.00***
		PGM-2 (2)	0.500	0.500	—	1.000	0.500	−1.000	28.00***
		TPI-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	28.00***
Mud Lake	(32)	GDH-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	32.00***
		IDH-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	32.00***
		PGM-2 (2)	0.500	0.500	—	1.000	0.500	−1.000	32.00***
		TPI-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	32.00***
Mukwanago River	(19)	GDH-1 (3)	0.842	0.079	0.079	0.158	0.279	0.433	7.13
		MDH-1 (2)	0.842	0.158	—	0.000	0.266	1.000	19.00***
		6PGDH-1 (2)	0.842	0.158	—	0.000	0.266	1.000	19.00***
		PGI-2 (2)	0.842	0.158	—	0.000	0.266	1.000	19.00***
		PGI-3 (2)	0.842	0.158	—	0.000	0.266	1.000	19.00***
		PGM-2 (2)	0.895	0.105	—	0.000	0.188	1.000	19.00***
Okauchee Lake	(32)	GDH-1 (3)	0.906	0.047	0.047	0.094	0.169	0.462	13.66**
		MDH-1 (3)	0.906	0.063	0.031	0.000	0.175	1.000	64.00***
		PGM-2 (2)	0.875	0.125	—	0.250	0.219	−0.143	0.65
		TPI-1 (2)	0.937	0.063	—	0.000	0.118	1.000	32.00***
C. echinatum									
Knab Lake	(25)	IDH-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	25.00***
Lac Vieux Desert	(22)	IDH-1 (2)	0.500	0.500	—	1.000	0.500	−1.000	22.00***
		6PGDH-2 (2)	0.500	0.500	—	1.000	0.500	−1.000	22.00***

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$.

TABLE 6. *Number and frequencies of electrophoretically discernible multilocus genotypes in 12* Ceratophyllum *populations*

Population	Number of genotypes	Genotype frequencies
Ceratophyllum demersum		
Elk Lake	2	0.879; 0.121
Lake Butte Des Morts	8	0.545; 0.182; 0.091; 0.061; 0.030; 0.030; 0.030; 0.030
Little Muskie Lake	1	1.000
Lucas Lake	1	1.000
Mud Lake	1	1.000
Mukwanago River	3	0.842; 0.105; 0.053
Okauchee Lake	6	0.563; 0.250; 0.094; 0.031; 0.031; 0.031
Trout Lake	1	1.000
Wildcat Lake	1	1.000
Ceratophyllum echinatum		
Cornell Pond	1	1.000
Knab Lake	1	1.000
Lac Vieux Desert	1	1.000

TABLE 7. *Mean diversity within and among clonal populations of* Ceratophyllum demersum *(this study) compared with values from studies of terrestrial clonal plants (data from Ellstrand and Roose, 1987)*

	C. demersum	Clonal terrestrial species
Genotypes/population	2.7	16.1
Proportion distinguishable	0.08	0.17
Multiclonal populations (%)	44.4	70.0
Multilocus genotype diversity (D)	0.45	0.62
Populations/genotype	1.2	2.9
Local genotypes (%)	90.0	75.8
Widespread genotypes (%)	0.0	27.1

Furthermore, if the same relationship is explored among a large number of different species with widely differing P values (calculated from data in Hamrick, 1983), a significant negative correlation is observed ($N = 177$, $r = -0.437$, $P < 0.001$). This seemingly counterintuitive result can be explained by considering the partitioning of allelic variation at the intraspecific vs. interspecific level. Because the total polymorphism of any one species is a fixed value, individual populations should show a positive relationship between P and P_a. Among species, however, P differs widely. Species with large P values probably show lower P_a values because it is less likely that the total allelic diversity will be fully represented among all populations. Conversely, populations of species with low P are more likely to possess most of the species alleles. Consequently, the P_a value is a better predictor of interpopulational differentiation than it is of sexuality. This conclusion is consistent with the significant negative correlation between G_{ST} and P_a values (calculated from data in Hamrick, 1983; $N = 117$, $r = -0.648$, $P < 0.001$). In that review, only one of the 21 studies reporting G_{ST} scores greater than 0.4 reported a P_a value greater than 0.7. Similarly, the high G_{ST} values of *C. demersum* and *C. echinatum* (0.49; 0.48) coincide with low P_a values (67%; 68%). In other instances where interpopulational differentiation is high (relative to the total diversity), populations may vary considerably for both P and P_a values. When asexuality is a major determinant of interpopulational differentiation, P_a should be a fair estimate of sexuality. However, many other factors can influence interpopulational variation, and the effectiveness of P_a values as estimators of relative sexuality will vary considerably.

TABLE 8. *Genetic identities (I) among populations of two* Ceratophyllum *species (computed after Nei, 1972)*

	(*I*)	Range
C. demersum	0.8899	(0.7452–1.0000)
C. echinatum	0.6926	(0.6185–0.7790)
C. demersum × *C. echinatum*	0.1708	(0.0714–0.3076)

Because gene flow among *Ceratophyllum* populations is more likely to occur by transport of vegetative fragments than by pollen or fruit dispersal (Les, 1988a), much of the polymor-

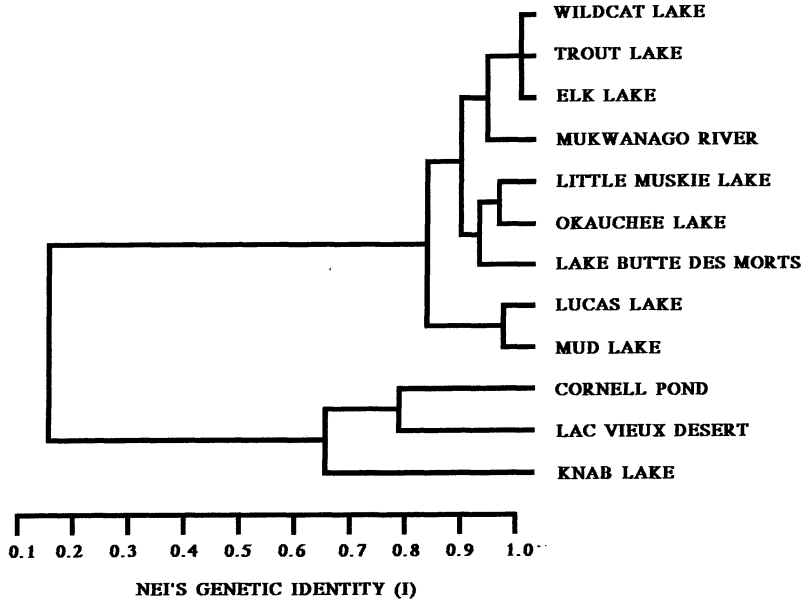

Fig. 2. UPGMA analysis of genetic identities showing relationships of nine *Ceratophyllum demersum* populations (upper cluster) and three *C. echinatum* populations (lower cluster).

phism in populations originates by immigration of vegetative propagules. The high G_{ST} values reported for both *Ceratophyllum* species (Table 4) represent substantially less interpopulational gene flow than levels characteristic of sexual outcrossing species. Only self-pollinating plants typically possess G_{ST} values of this magnitude (Hamrick and Godt, 1990). It is noteworthy that the lowest average G_{ST} (0.099) characterizes outcrossing wind-pollinated plants (Hamrick and Godt, 1990). This observation illustrates that the genetic population structure of anemophiles differs considerably from these hydrophiles.

Despite studies of other water-pollinated species showing a near complete lack of genetic variation (Wain, Haller, and Martin, 1985), a considerable amount of genetic diversity is partitioned within and among *Ceratophyllum* populations (Table 2). Little of the allelic diversity in these populations, however, may represent genetic consequences of sexual recombination. In several populations of *C. demersum* (Little Muskie Lake, Lucas Lake, Mud Lake), only one electrophoretically discernible multilocus genotype was detected despite the presence of several polymorphic loci (Tables 2, 6). All three of these populations were "fixed" for heterozygosity at all polymorphic loci, an obvious result of asexual rather than sexual reproduction. In Elk Lake, six loci were polymorphic, yet the low observed heterozygosity (H_O = 0.007; H_{exp} = 0.076) results from the complete lack of recombination between the two resident genotypes (Tables 2, 3, 6). This conclusion is illustrated by the equal frequency of genotypes and alleles at polymorphic loci. Similarly, the equal frequency of the most common multilocus genotype (0.842) and dominant allele frequency at five polymorphic loci demonstrates the lack of recombination among the three Mukwanago River genotypes (Tables 2, 6). This is an expected result because hydrophily in *Ceratophyllum* essentially precludes fruit formation in habitats of unidirectional currents (Les, 1986c). Two remaining populations with polymorphic loci (Okauchee Lake, Lake Butte Des Morts) contain six and eight genotypes, respectively. Here, discordant allele and genotype frequencies (Tables 2, 6) indicate some degree of sexual recombination, although heterozygosity (Table 3) remains low. The remaining populations of *C. demersum* and all populations of *C. echinatum* were uniclonal. Lack of sexual recombination in two populations of *C. echinatum* was also indicated by fixed heterozygosity at polymorphic loci. There is no apparent relationship between heterozygosity and number of multilocus genotypes in the populations of *Ceratophyllum* studied. Both the highest and lowest heterozygosities occurred in populations containing only one electrophoretic genotype.

The significant deviation from expected heterozygote frequencies occurring in all *Ceratophyllum* populations studied (Table 5) is also a likely consequence of clonal reproduction. The numerous instances where fixation indices were either 1.0 or −1.0 indicate cloning of individuals rather than sexual recombination. Evidence of recombination in all polymorphic populations was negligible. Low heterozygosity also characterized polymorphic loci in two lakes (Okauchee, Butte Des Morts) where the presence of several recombinant genotypes indicates that some sexual reproduction has occurred. Here the positive fixation indices for these localities may indicate some degree of inbreeding as well as clonal reproduction.

These *Ceratophyllum* populations are not typical of "average" clonal terrestrial plant populations as described by Ellstrand and Roose (1987). In contrast to clonal terrestrial plants, populations of *C. demersum* were mostly monoclonal, had fivefold fewer genotypes, the proportion of clones distinguishable was 50% less, and the multilocus genotype diversity was 27% less (Table 7). The low values indicate that these clonal aquatic plants may undergo less extensive sexual reproduction than clonal terrestrial counterparts. Although Silander (1985) summarized that most clonal plants are facultatively sexual, these data indicate that asexuality predominates in *C. demersum* populations studied. Populations of *C. echinatum* were even more extreme. All three consisted of clones from single resident genotypes.

Les (1988a) suggested that annual hydrophiles may possess greater genetic variation than perennials because sexuality is mandatory for their continued survival. A recent electrophoretic study of the dioecious annual hydrophile, *Najas marina,* reported levels of *P, K,* and H_O lower than those given here for *C. demersum* (Triest, 1989). Although described as incapable of asexual reproduction (Triest, 1989), this species commonly fragments during the growing season, giving rise to propagules capable of rooting, and in some instances has the ability to form vegetative turions (Agami, Beer, and Waisel, 1986). Long-distance dispersal in this species, however, is surely by means of sexually derived fruits (Triest, 1989). Regardless, the low level of genetic variation in *Najas* indicates that even dioecious annual hydrophiles possess levels of genetic diversity atypical of outcrossing terrestrial plants. Les (1988a) emphasized that dioecy in hydrophiles

ensures xenogamy, but "outcrossing" is a term misapplied to populations with scarce genetic variation. Triest (1989) suggested that founder effect, drift, and bottlenecks may account for the low genetic variability of *Najas marina* populations.

Consequences of asexuality — Although monoecious abiotically pollinated species are expected to be outcrossed, *Ceratophyllum* populations are constrained by their strong asexuality and vegetative reproduction (Les, 1988a). This conclusion is supported by correlations established between measures of genetic variation and plant breeding systems (Gottlieb, 1977; Hamrick, Linhart, and Mitton, 1979; Loveless and Hamrick, 1984; Hamrick and Godt, 1990). Outcrossing species are typically characterized by higher genetic variation (P_s = 0.50–0.66, K_s = 1.99–2.40, H_O = 0.133) than predominantly selfed species (P_s = 0.42, K_s = 1.69, H_O = 0.032) (Gottlieb, 1977; Hamrick and Godt, 1990). The corresponding P_s and K_s values for *C. demersum* (0.53, 1.82) and *C. echinatum* (0.14, 1.57) are near or below the mean values reported for selfing terrestrial species. The correlations are more dramatic at the population level. Corresponding values of P_p and K_p for *C. demersum* (0.20, 1.22) and *C. echinatum* (0.07, 1.07) are the same or even lower than the average for selfing terrestrial plants (0.20, 1.31), and much lower than the averages for outcrossing terrestrial plants (0.36–0.50, 1.54–1.79).

Also significantly correlated with plant breeding systems are the measures of gene diversity H_S and G_{ST}, with selfing species displaying lower H_S (0.149) and higher G_{ST} (0.510) values than outcrossers (0.243–0.259; 0.099–0.197, respectively) (Hamrick and Godt, 1990). These measures of gene diversity for *C. demersum* and *C. echinatum* (Table 4) are also more similar to selfers. Despite the observation that monoecious species typically have low G_{ST} values averaging 0.092 (Loveless and Hamrick, 1984), the G_{ST} for both *C. demersum* and *C. echinatum* are greater than the average 0.389 reported for hermaphroditic plants (Loveless and Hamrick, 1984). Although outcrossing may predominate in clonal terrestrial plants (Silander, 1985), the low levels in these *Ceratophyllum* populations are not unexpected for perennial hydrophiles (Les, 1988a). Populations of freshwater hydrophiles may better represent a "mixed-selfing" breeding system in which pollen flow is predominantly intraclonal (Silander, 1985).

Why are *Ceratophyllum* species monoecious? A reasonable explanation must consider the evolutionary history of the genus. Understandably, a strong association between dicliny and abiotic pollination has been demonstrated adequately for dioecious species (Muenchow, 1987). Hydrophiles most likely evolved from anemophiles (Les, 1988d), where monoecious species are widespread (Richards, 1986). Monoecy may have promoted outcrossing during the early history of the genus as it acquired a wide range of adaptations facilitating the transition to a submersed aquatic existence. Once adapted to features of relatively stable aquatic habitats, a shift to reduced sexuality and clonal growth may have occurred to maintain coadapted gene complexes. Sexual reproduction would be retained in any event because fruit production provides an alternate dispersal mechanism.

Widespread species are characterized by G_{ST} values averaging 0.210 and high H_T values averaging 0.347; the average G_{ST} of narrow and endemic species is somewhat higher (0.242–0.248) and their average H_T (0.300–0.263) somewhat lower (Hamrick and Godt, 1990). Although *C. demersum* is a widespreead cosmopolitan species, its H_T in this study was lower and its G_{ST} higher than average values reported for endemic species (Table 4). Although the G_{ST} for *C. echinatum* was similarly high, the H_T (Table 4) was surprisingly high for a restricted species. Because of its rarity, the high total diversity in *C. echinatum* may represent genetic drift among clonal populations. Several loci were fixed for different alleles among the three populations studied (Table 2). Overall, however, the genetic bottleneck for this species is apparently less severe than for *Howellia aquatica,* another rare aquatic plant that lacks detectable electrophoretic variation (Lesica et al., 1988).

Patterns of electrophoretic and morphological divergence — High levels of gene flow, a lack of isolating barriers, and associated high mean genetic identities are expected to characterize most conspecific populations. The average of mean genetic identities reported by Crawford (1983) among 38 conspecific (terrestrial) angiosperm populations is 0.95. The value of 0.89 for *C. demersum* populations is within the usual observed range (Giannasi and Crawford, 1986), whereas the value of 0.69 for *C. echinatum* is lower. Gianassi and Crawford (1986) mention that conspecific population genetic identities reduced below 0.90 do occur in certain selfing species. At the subspecific level, the average of genetic identities among 14 comparisons of terrestrial angiosperms is reduced slightly to 0.91 (Crawford, 1983). At the con-

generic species level, the average of genetic identities among 16 comparisons of terrestrial angiosperms is reduced further to 0.79 (Crawford, 1983), although values as low as 0.28 are not unusual (Giannasi and Crawford, 1986). From these comparisons, populations of *C. demersum* exhibit degrees of genetic differentiation similar to most conspecific terrestrial plant populations. For *C. echinatum,* however, the low genetic identity among Wisconsin populations indicates that they are effectively isolated from gene flow. The isolation and divergence of *C. echinatum* populations may reflect a history of repeated bottlenecks consequent of Pleistocene glaciations (Les, 1986b). Indeed, the ability to locate only three of 11 historical state populations for this study attests to the present rarity of this species.

The low genetic identity between *Ceratophyllum demersum* and *C. echinatum* is evidence of a long period of isolation. This low value argues strongly against taxonomic treatments that consider the two species as conspecific varieties (e.g., Gray, 1856; Eyles and Robertson, 1944). The low interspecific genetic identity correlates with morphological, embryological, phytogeographical, and chemosystematic data, further indicating the distinctness of these two species (Les, 1985, 1986b, c, 1988b).

The high degree of morphological similarity among extant populations of *C. echinatum* was initially attributed to sexuality and effective gene flow (Les, 1988b). Although an equal proportion of within- and among-population variation contributes to the total diversity, there is considerable differentiation among populations (Table 4). The pattern of morphological consistency may reflect the loss of variability due to the rarity of this species. Fossils have furnished evidence that *C. echinatum* has experienced considerable morphological stasis since at least the tertiary (Herendeen, Les, and Dilcher, 1990). The slow evolutionary rates associated with morphological features contrast to the high degree of interpopulational genetic differentiation noted at presumably neutral (isozyme) loci. Drift and clonal reproduction act to maintain considerable interpopulational genetic variability in this species. Because different populations (clones) are fixed for alternate alleles and possess fixed heterozygosity, occasional episodes of sexual reproduction among clones could result in periodic "releases" of genetic diversity and heterozygosity.

In the cosmopolitan *C. demersum,* populations contain limited morphological diversity (mostly with respect to quantitative genetic traits) compared to the species as a whole (Les, 1986a). Similarly, a higher proportion of electrophoretic variation occurs among rather than within populations that are highly differentiated (Table 4). As in *C. echinatum,* fixed heterozygosity resulting from vegetative reproduction provides a reserve of genetic variability in some clonal populations. Multiclonal populations are also capable of generating substantial heterozygosity during episodes of sexual reproduction.

Releases of "stored" genetic variability in *Ceratophyllum* clones by sexual reproduction may play an important role in colonization. In *Ceratophyllum* species, sexual reproduction and fruit production increase in drying habitats or during periods of low water levels, a possible cue triggering dispersal to new sites (personal observations). Extensive genetic variability may be adaptive in such instances because of the wide range of potential new habitats that fruits may encounter upon dispersal. When a fruit arrives at a stable new site, conditions may limit sexual reproduction and subsequent colonization may involve the clonal spread of a single genotype. By this scenario, clonal populations of both species are not unusual for this study because they have existed only since the last glaciation (approximately 10,000 years).

It is evident that morphology in *Ceratophyllum* is strongly conserved (Les, 1988a) despite the genetic differentiation of populations resulting from widespread vegetative growth. In other plant species, apomicts reportedly possess more "general purpose" genotypes than their sexual counterparts (Bierzychudek, 1989). Similarly, the stasis of morphological phenotypes in these and other submersed species may relate to the general long-term stability of aquatic habitats (Les, 1988a).

Electrophoretic data document widespread clonal growth in populations of the monoecious hydrophile genus *Ceratophyllum.* Although enzyme polymorphisms exist at both intra- and interpopulational levels, there is little evidence of sexual recombination among genotypes within populations. Asexuality in *Ceratophyllum* results in lower genetic variation, heterozygosity, and outcrossing than may be expected for a monoecious species. *Ceratophyllum* populations differ from terrestrial clonal plants by extensive monoclonal populations, lower genetic diversity, and low levels of outcrossing. Because hydrophily limits pollen gene flow to a specific water body, inbreeding may occur even within sexually reproducing populations. Assumptions that dicliny in hydrophiles relates to widespread outcrossing is not supported in this study of *Ceratophyllum*

species. Further studies of genetic population structure in water-pollinated plants should clarify whether outcrossing is more prevalent in other such groups.

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