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CLONAL REPRODUCTION AND PATTERNS OF GENOTYPIC DIVERSITY IN *DECODON VERTICILLATUS* (LYTHRACEAE)[1]

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Most perennial plants combine sexual reproduction with some form of clonal propagation. These mixed strategies may produce considerable variation among populations in levels of clonal diversity in response to ecological factors limiting one or other reproductive mode. Surveys of style morph frequencies in 163 populations of the eastern North American, clonal, tristylous aquatic, *Decodon verticillatus* (L.) Ell. (Lythraceae) suggested a wide range of clonal diversity among populations. Populations consisting of a single style morph were most common at the northern margin of the species' range and could have arisen through severe founder events followed by exclusive clonal propagation. Here, we test this hypothesis by comparing allozyme variation in populations monomorphic and polymorphic for style length located in Ontario and Michigan. Each of the four populations monomorphic for style length were fixed for a single three-locus allozyme genotype while the seven trimorphic and five dimorphic populations contained an average of 26 multilocus genotypes each. Measures of genotypic diversity were high in polymorphic populations (average $D = 0.93 \pm 0.02$ standard error; $D = 0.00$ for all populations monomorphic for style length). Three of the populations monomorphic for style length were fixed for a heterozygous genotype at one of the loci surveyed, suggesting that each consists of a single clone. In contrast, genotype frequencies in polymorphic populations conformed to Hardy-Weinberg proportions indicative of sexual reproduction. The range of clonal diversity found in *D. verticillatus* is the largest reported for a clonal plant species, although the literature is too limited to determine whether this is truly unusual. Clonal diversity in *D. verticillatus* is likely to be regulated largely by ecological factors affecting seed production and establishment. However, genetically based sexual sterility also occurs in some populations.

Most perennial plants possess some capacity for asexual reproduction; either by vegetative propagation or the formation of asexual seed (Gustafsson, 1946, 1947; Abrahamson, 1980; Richards, 1986). In some species, such as sterile polyploids, asexual reproduction is the sole mode of regeneration. In most taxa, however, it is facultative and combined with some degree of sexual reproduction (Stebbins, 1950; Fryxell, 1957). Despite the widespread occurrence of clonal reproduction in flowering plants, the genetic and evolutionary consequences of mixed clonal and sexual reproductive strategies have not been studied in much detail (Silander, 1985). A high degree of asexuality is generally thought to be associated with limited recombination and genetic monomorphism (Williams, 1975; Harper, 1977). However, a growing body of data indicates that populations of clonal plants can maintain considerable amounts of genetic diversity.

Ellstrand and Roose (1987) and Hamrick and Godt (1990) recently surveyed the literature on allozyme variation in clonal plants and found that although clonal taxa may not be as variable as their sexual counterparts, widespread genetic monomorphism is the exception, not the rule. While these comparative surveys have refined our views on patterns of genetic diversity in clonal plants, the classification of taxa as either clonal or sexual ignores intraspecific variation in reproductive mode that commonly occurs among populations. Studies examining the patterns of genetic diversity associated with variation in the intensity of clonal reproduction would supplement comparative surveys in improving our understanding of the genetic and ecological factors affecting clonal vs. sexual reproduction, as well as the genetic consequences of facultative asexuality.

There is abundant evidence from interspecific comparisons that clonal reproduction is favored under certain ecological conditions (Salisbury, 1942; Abrahamson, 1980). For example, plants of aquatic environments are notorious for their capacity to reproduce clonally (Sculthorpe, 1967; Hutchinson, 1975). Similarly, ecological studies have shown that within a species the importance of clonal vs. sexual reproduction can vary among habitats in response to biotic and abiotic factors (e.g., Barrett, 1980; Douglas, 1981; Low, Cook, and Manlove, 1983; reviews in Abrahamson, 1980; and Richards, 1986). Few studies, however, have examined whether ecological variation in the occurrence of clonal vs. sexual reproduction has a significant impact on the genetic diversity of populations. In this study, we describe geographical patterns of genotypic diversity in the emergent aquatic, *Decodon verticillatus* (L.) Ell. (Lythraceae), and investigate the ecological and genetic causes and consequences of clonal reproduction.

Decodon verticillatus is a self-compatible, tristylous, perennial that inhabits swamps, bogs, and marshes in eastern North America from north-central Ontario to central Florida. In addition to producing seed, *D. verticillatus* forms clonal offspring through adventitious rooting by branch tips that descend into the water. When the deciduous foliage senesces during the fall and winter, the connections between parental plants and their clonal offspring are severed. Although both seed and clonal progeny are produced in most populations, the relative contribution

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TABLE 1. Location of 16 populations of *Decodon verticillatus* examined in this study. An estimate of population size is also presented along with the level of polymorphism for style length.

Population	County	Latitude (N)/ longitude (W)	Estimated size	Morphs present[a]
Ontario				
EO-T3	Leeds & Grenville	44°36′/76°18′	2,000	LMS
EO-T6	Leeds & Grenville	44°35′/76°19′	1,000	LMS
EO-T11	Frontenac	44°30′/76°25′	500	LMS
EO-D1	Frontenac	44°33′/76°23′	800	LS
EO-D3	Leeds & Grenville	44°33′/76°20′	200	LS
EO-D5	Leeds & Grenville	44°35′/76°20′	75	LS
ON-M1	Haliburton	45°00′/78°40′	10,000	L
ON-M5	Haliburton	44°50′/78°50′	400	S
ON-M6	Haliburton	45°06′/78°55′	300	S
Michigan				
MI-T3	Mecosta	43°41′/84°41′	3,000	LMS
MI-T7	Kalamazoo	42°00′/85°27′	1,000	LMS
MI-T8	Cheboyan	45°30′/84°37′	500	LMS
MI-D1	Lake	43°58′/85°02′	1,000	LM
MI-D2	Grand Traverse	44°44′/84°52′	500	LS
MI-M11	Benzie	44°39′/85°07′	100	S
Georgia				
GA-T3	Lanier	30°56′/83°00′	1,000	LMS

[a] L, M, and S = long-, mid-, and short-styled morphs, respectively.

of the two reproductive modes to the genetic structure of populations is not known.

Surveys of style morph frequencies in 163 populations from four regions of the species' range provided evidence that the recruitment of sexual vs. clonal progeny may vary widely both within and among geographical regions (Eckert and Barrett, 1992; Eckert, 1993). Populations from three regions in the northern half of the range often consisted of only one of the three morphs (Ontario: ten of 42 populations were monomorphic for style length; Michigan: 12 of 30; New England: 22 of 50). Moreover, *within* each of the regions populations consisting of a single style morph were most frequent at the northern limit of the range. In contrast to these northern regions, floral monomorphism was rare among populations (one of 32) surveyed in Florida, Georgia, and the Carolinas. These results suggested that much of the floral monomorphism is due to severe founder events followed by exclusive clonal propagation.

Here, we examine this hypothesis by comparing levels of allozyme variation in populations monomorphic and polymorphic for style length. If floral monomorphism is maintained by clonal propagation, populations monomorphic for style length should consist of a single allozyme genotype, whereas populations that are polymorphic for style length are expected to show higher levels of genotypic diversity. Determining the genetic significance of floral monomorphism should involve comparisons among populations in the same geographic region. Such comparisons avoid the possibility of confounding differences in genetic diversity arising from variation in reproductive mode with regional differences in diversity caused by historical processes such as glaciation. Accordingly, we compared patterns of genotypic diversity in populations monomorphic and polymorphic for style length that are located within two northern regions where the transition from floral polymorphism to floral monomorphism is the steepest.

MATERIALS AND METHODS

Population samples—Genotypes of mature plants were sampled from 15 populations in Ontario and Michigan during the fall of 1988 and 1990 (Table 1). Four populations monomorphic for style length (ON-M1, ON-M5, ON-M6, and MI-M11) and one trimorphic population (MI-T8) were located at the northern limit of the species' range. Five trimorphic (EO-T3, EO-T6, EO-T11, MI-T3, and MI-T7) and five dimorphic populations (EO-D1, EO-D3, EO-D5, MI-D1, and MI-D2) were located 100 to 200 km further south, in areas where populations were commonly trimorphic (Eckert and Barrett, 1992). A single trimorphic population from the southern portion of the species' range in Georgia (GA-T3) was also included for comparison. Population size was estimated by inspection as the total number of ramets, both flowering and nonflowering. Polymorphism for style length was assessed by randomly sampling about 200 flowering branches from throughout each population (mean sample size = 225; range = 95–387; Eckert, 1993). In small populations (EO-D5 and MI-M11), all flowering ramets were sampled.

In each population, cuttings or seed families were collected from 16 to 88 ramets sampled from throughout the population at 4-m intervals. The style morph of each ramet was determined by examination of withered floral organs persisting on the ripe fruits. Electrophoretic genotypes were assayed directly from cuttings or inferred from progeny arrays from about ten seedlings following Brown and Allard (1970) with estimates of the outcrossing rate and allele frequencies obtained following Ritland (1986). This inferential method tends to assign the most common multilocus genotype in ambiguous cases, thus providing conservative estimates of genotypic diversity.

Electrophoretic methods—Variation at isozyme loci was resolved using horizontal starch gel electrophoresis. Leaves or seedlings were crushed in a small volume of buffer

consisting of 0.4% w/v bovine serum albumin, 6 mM diethyldithiocarbamic acid (DIECA, Na salt), 3 mM DL-dithiothreitol (DTT), 1 mM edetate disodium (EDTA), 5% w/v polyvinylpyrolidine-40 (PVP-40), 200 mM sucrose, and 0.1% v/v Tween-80, all dissolved overnight in 0.05 M Na_2HPO_4 buffer (pH 7.0), with 0.1% v/v β-mercaptoethanol added just before use. Homogenate was absorbed on 2 × 10-mm chromatography-paper wicks (Whatman 3 mm), that were inserted 3.5 cm from the cathodal end of 11% starch gels (15 cm long × 20 cm wide × 1.5 cm deep, 375 ml). A histidine-citrate tray buffer (0.056 M L-histidine with 0.006 M anhydrous citric acid, pH 6.1) was used, with gels made from a 1:3 dilution of tray buffer and distilled water (modified from Cardy, Stuber, and Goodman, 1981). Gels were run under constant current (45 mA, about 200 V) at 5 C until a food coloring marker (Blue Ribbon® green) had run 10 cm from the origin (about 4 hours). Gels were cut horizontally into 2-mm slices and stained for enzyme activity following recipes in Wendel and Weeden (1991). An initial survey of 25 enzyme systems using plant material from across the species' range revealed variability for seven systems. Of these seven, phenotypes could be clearly resolved for acid phosphatase (ACP, EC 3.1.3.2), isocitric dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), and phosphoglucomutase (PGM, EC 2.7.5.1). Mendelian inheritance of allozyme bands was confirmed for all putative loci by examining the segregation of allozyme phenotypes in progeny from controlled crosses. Because variability at *Pgm* appeared to involve null alleles, this enzyme was excluded from further study.

Measures of genotypic diversity—Genotypic diversity was estimated using three-locus isozyme genotypes with style morph added as a fourth genetic marker. For each population, we calculated the number of distinct multilocus genotypes observed (G) and the maximum number possible (G_{max}), given the number of possible genotypes at each locus and style morphs in the population:

$$G_{max} = \left(\prod_{i=1}^{m} a_i(a_i + 1)/2\right)M,$$

where a_i is the number of alleles detected at the i^{th} locus, m is the number of loci, and M is the number of style morphs in the population. In some populations, G_{max} was greater than the number of ramets sampled (N_r). Since G cannot exceed N_r, G_{max} was set at N_r rather than the number of possible genotypes, in these cases. Three measures of genotypic diversity were calculated following Ellstrand and Roose (1987). The first is the "proportion distinguishable" measure calculated as G/N_r. The second is Pielou's (1969) corrected version of the Gini index:

$$D = 1 - \frac{\sum_{i=1}^{G} n_i(n_i - 1)}{N_r(N_r - 1)},$$

where n_i is the number of the i^{th} genotype observed. D ranges from zero in a population composed of a single clone to unity in a population where every individual sampled is a different genotype (i.e., $G = N_r$). This measure is influenced by the number of polymorphic loci, the number of alleles at each locus, and the number of morphs in populations. Accordingly, this parameter was scaled to the level of polymorphism in the population following Fager (1972) to produce the third measure of clonal diversity, genotypic evenness (E):

$$E = \frac{D_{observed} - D_{minimum}}{D_{maximum} - D_{minimum}},$$

where

$$D_{minimum} = \frac{(G - 1)(2N_r - G)}{N_r(N_r - 1)},$$

and

$$D_{maximum} = \frac{N_r(G - 1)}{G(N_r - 1)}.$$

Genotypic evenness ranges from zero for a population in which all individuals possess the same genotype to unity in a population with completely uniform genotype frequencies.

In completely clonal populations, the presence of many genotypes may result from large founder groups, multiple founder events, and/or recurrent gene flow. The frequency of a given genotype may be a complex function of its initial frequency among founders, its genotype-specific rate of clonal proliferation, and the amount of time it has existed in the population. Consequently, genotypic frequencies in clonal populations may be rather idiosyncratic. Genotypic frequencies in random-mating populations, however, should conform to Hardy-Weinberg proportions. To determine whether the observed patterns of genotypic diversity are consistent with sexual reproduction, genotypic frequencies were tested for departure from Hardy-Weinberg proportions using the algorithms in BIOSYS-1 (Swofford and Selander, 1981). Observed heterozygosity at allozyme loci was calculated directly from observed genotypic counts. Expected heterozygosity was calculated following Nei (1978). Deviations from Hardy-Weinberg proportions were assessed using goodness-of-fit tests. Because such tests are sensitive to low frequencies in some genotypic classes, genotypes involving rare alleles for loci with more than two alleles were pooled. Genotype frequencies for diallelic loci were also highly uneven in some cases. Accordingly, deviations from Hardy-Weinberg proportions were evaluated using an analog of Fisher's exact test. This test avoids problems associated with low expected values stemming from uneven allele frequencies and small sample sizes (Weir, 1990). The experiment-wise type I error rate (α_{EW}) was held at 5% for C different tests using Sidák's (1967) correction for nonorthogonal contrasts, where the per contrast error rate (α_{PC}) is: $\alpha_{PC} = (1 - (1 - \alpha_{EW}))^{1/C}$.

RESULTS

The populations of *D. verticillatus* examined in this study showed a wide but discontinuous range in genotypic diversity (Table 2). Populations were either fixed for a single three-locus allozyme genotype or contained considerable genotypic diversity. As predicted, the levels of allozyme variation matched patterns of style length polymorphism. All four populations monomorphic for style length consisted of a single allozyme genotype; the seven

TABLE 2. Genotypic frequencies for three allozyme loci in 16 populations of *Decodon verticillatus*. The electrophoretic migration distances of alleles are denoted by alphabetical codes (i.e., A is the fastest migrating allele), except for *Acp* where the rare allele D was intermediate in migration between B and C. The number of ramets sampled (N_r) was the same for all loci. For those samples marked with an asterisk, genotypes were inferred from progeny arrays of about ten progeny per family.

	Locus:	*Mdh*			*Idh*			*Acp*								
Population	N_r	AA	AB	BB	AA	AB	BB	AA	AB	BB	AC	BC	CC	AD	BD	DD
Trimorphic populations																
EO-T3	88*	0.02	0.28	0.69	0.01	0.26	0.73	0.38	0.36	0.26	0.00	0.00	0.00	0.00	0.00	0.00
EO-T6	80*	0.13	0.34	0.54	0.13	0.33	0.55	0.06	0.28	0.29	0.11	0.21	0.05	0.00	0.00	0.00
EO-T11	81*	0.20	0.46	0.35	0.02	0.14	0.84	0.32	0.53	0.15	0.00	0.00	0.00	0.00	0.00	0.00
MI-T3	81*	0.00	0.31	0.69	0.04	0.30	0.67	0.17	0.58	0.25	0.00	0.00	0.00	0.00	0.00	0.00
MI-T7	83*	0.17	0.55	0.28	0.27	0.40	0.34	0.19	0.25	0.01	0.01	0.00	0.00	0.40	0.11	0.02
MI-T8	83	0.16	0.73	0.11	0.00	0.00	1.00	0.00	0.04	0.96	0.00	0.00	0.00	0.00	0.00	0.00
GA-T3	25	0.04	0.36	0.60	0.56	0.36	0.08	0.08	0.72	0.00	0.16	0.00	0.04	0.00	0.00	0.00
Dimorphic populations																
EO-D1 (LS)	57*	0.19	0.47	0.33	0.00	0.40	0.60	0.56	0.14	0.00	0.21	0.05	0.04	0.00	0.00	0.00
EO-D2 (LS)	60*	0.07	0.28	0.65	0.10	0.40	0.50	0.12	0.08	0.05	0.33	0.17	0.25	0.00	0.00	0.00
EO-D5 (LS)	58*	0.00	0.21	0.79	0.02	0.40	0.59	0.12	0.14	0.36	0.09	0.24	0.05	0.00	0.00	0.00
MI-D1 (LM)	58*	0.02	0.31	0.67	0.02	0.10	0.88	0.40	0.50	0.10	0.00	0.00	0.00	0.00	0.00	0.00
MI-D2 (LS)	53*	0.00	0.17	0.83	0.00	0.00	1.00	0.15	0.45	0.40	0.00	0.00	0.00	0.00	0.00	0.00
Monomorphic populations																
ON-M1 (L)	32	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ON-M5 (S)	32	0.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ON-M6 (S)	33	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
MI-M11 (S)	20	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00

trimorphic and five dimorphic populations consisted of many genotypes. Combining allozyme and style length polymorphisms (Table 3), the number of genotypes observed (G) per population averaged 29.6 (±6.1 SE) and 20.4 (±3.9) for trimorphic and dimorphic populations, respectively.

Similar patterns were revealed using other measures of genotypic diversity (Table 3). The number of observed genotypes expressed as a proportion of the sample size (G/N_r) ranged from 0.03 to 0.63 and was always much higher in trimorphic (0.41 ± 0.06) and dimorphic populations (0.35 ± 0.06) than in those monomorphic for style length (0.04 ± 0.006). The number of genotypes relative to the maximum number possible averaged 0.44 (±0.04) and 0.43 (±0.05) for trimorphic and dimorphic populations, respectively. Estimates from populations monomorphic for style length were inflated (range = 0.33–1.00) due to low values of G_{max} as a result of reduced levels of allozyme polymorphism. Genotypic diversity (D) and evenness (E) were uniformly high ($\bar{D} = 0.93 \pm 0.02$; $\bar{E} = 0.91 \pm 0.02$) in populations polymorphic for style length and did not differ between trimorphic ($\bar{D} = 0.92 \pm 0.04$; $\bar{E} = 0.90 \pm 0.04$) and dimorphic ($\bar{D} = 0.93 \pm 0.02$; $\bar{E} = 0.94 \pm 0.01$) populations ($D = 0.00$ for all populations monomorphic for style length). Diversity was lowest in the trimorphic population from an area in northern Michigan (MI-T8) where populations were typically monomorphic for style length (Eckert and Barrett, 1992). The population from Georgia (GA-T3) did not show particularly high levels of diversity compared to populations polymorphic for style length in Ontario or Michigan. It ranked 9th for D and 11th for E.

Genotypic uniformity in populations monomorphic for style length appears to be the result of exclusive clonal propagation. In two of four cases, the fixed genotype was heterozygous at one allozyme locus (Table 2). In another case (ON-M6), the fixed genotype was homozygous at all

TABLE 3. Estimates of clonal diversity in 16 populations of *Decodon verticillatus*. Number of maternal genotypes sampled (N_r) is given along with the number of distinct multilocus genotypes observed (G), the maximum number of genotypes possible (G_{max}), the proportion of distinct genotypes (G/N_r), the proportion of possible genotypes observed (G/G_{max}), clonal diversity (D), and genotypic evenness (E).

Population	N_r	G	G_{max}	G/N_r	G/G_{max}	D	E
Trimorphic populations							
EO-T3	88	33	81	0.37	0.41	0.97	0.97
EO-T6	80	45	80	0.56	0.56	0.98	0.97
EO-T11	81	32	81	0.39	0.39	0.96	0.95
MI-T3	81	26	81	0.32	0.32	0.95	0.94
MI-T7	83	52	83	0.63	0.63	0.98	0.93
MI-T8[a]	47	8	27	0.17	0.30	0.71	0.70
GA-T3[b]	25	11	25	0.44	0.44	0.90	0.83
Dimorphic populations							
EO-D1	57	25	57	0.44	0.44	0.96	0.94
EO-D3	60	31	60	0.52	0.52	0.97	0.96
EO-D5	58	21	58	0.36	0.36	0.95	0.95
MI-D1	58	15	54	0.26	0.28	0.90	0.90
MI-D2	53	10	18	0.19	0.56	0.88	0.93
Monomorphic populations							
ON-M1	32	1	1	0.03	1.00	0.00	—
ON-M5	32	1	3	0.03	0.33	0.00	—
ON-M6	33	1	1	0.03	1.00	0.00	—
MI-M11	25	1	3	0.04	0.33	0.00	—

[a] This is a subset of a larger sample of 83 ramets, of which only 47 were scored for style morph. The total sample, without style morph as a genetic marker, consists of four two-locus genotypes ($G/N_r = 0.05$) of a total of nine possible genotypes ($G/G_{max} = 0.44$) with $D = 0.48$ and $E = 0.59$.

[b] The 25 ramets collected in this population were not scored for style morph; hence these estimates are based on three polymorphic isozyme loci.

TABLE 4. Heterozygosity at three allozyme loci in 12 populations of *Decodon verticillatus*. Observed (H_{obs}) and expected (H_{exp}) heterozygosities have been averaged over loci for each population. The deviation of genotypic frequencies to Hardy-Weinberg expectations is shown by exact probability values for each locus. The experimentwise error rate has been held at 0.05 for 34 tests using Sidák's (1967) correction for nonorthogonal contrasts ($\alpha_{PC} = 0.0015$). Both cases of significant departure from expectations involved excess heterozygosity.

Population	No. of loci	H_{obs}	H_{exp}	Deviation from Hardy-Weinberg		
				Mdh	*Idh*	*Acp*
EO-T3	3	0.303	0.338	1.000	1.000	0.017
EO-T6	3	0.421	0.477	0.106	0.097	0.826
EO-T11	3	0.374	0.381	0.649	0.097	0.826
MI-T3	2	0.395	0.353	0.197	1.000	0.181
MI-T7	3	0.574	0.534	0.374	0.078	0.004
MI-T8	2	0.257	0.179	<0.001	—	1.000
GA-T3	3	0.533	0.447	1.000	0.640	<0.001
EO-D1	3	0.427	0.411	0.791	0.096	0.497
EO-D2	3	0.422	0.455	0.255	0.759	1.000
EO-D5	3	0.356	0.373	1.000	0.269	0.109
MI-D1	3	0.305	0.290	1.000	0.288	0.575
MI-D2	2	0.310	0.312	1.000	—	0.775

allozyme loci, but controlled crosses on plants collected from this population provided evidence of heterozygosity at one of the style morph loci (C. G. Eckert and S. C. H. Barrett, unpublished data). The fixation of a single-locus heterozygous genotype is a reliable indicator of clonal monomorphism since any amount of segregation would produce homozygous genotypes. The remaining monomorphic population (ON-M1) was fixed for the long-styled morph, and thus homozygous at style morph loci as well as at the three isozyme loci.

In trimorphic and dimorphic populations, single-locus genotype frequencies showed only minor deviations from Hardy-Weinberg proportions, suggesting significant levels of sexual reproduction. Departures from Hardy-Weinberg proportions occurred for *Mdh* genotypes in MI-T8 and *Acp* in GA-T3. Both cases involved a significant excess of heterozygotes. However, excess heterozygosity was not a general feature of populations. Average observed heterozygosity exceeded the average expected heterozygosity in six of 12 populations (Table 4).

DISCUSSION

Populations of *D. verticillatus* exhibit a wide range of clonal diversity, suggesting substantial variation in the relative importance of clonal and sexual reproduction. Most populations at the northern limit of the species' range consisted of a single genotype, whereas more southerly populations in each region consisted of many multilocus genotypes. This result supported inferences about genetic structure in *D. verticillatus* based on patterns of style morph polymorphism. Populations monomorphic for style length located at the northern limit of the range probably result from severe founder events followed by exclusive clonal reproduction. These results raise two questions concerning the ecology and genetics of clonal reproduction in *D. verticillatus*: Is the wide range of observed values for genotypic diversity in *D. verticillatus* unusual among clonal plants? What ecological and genetic factors are likely to limit the frequency of sexual reproduction in the species?

Comparison with other clonal taxa—There is a wealth of natural history observations implying that clonal plants exhibit wide variation in genotypic diversity among populations (Abrahamson, 1980). Two of the most dramatic examples involve the sexually polymorphic aquatic weeds *Elodea canadensis* (Sculthorpe, 1967) and *Eichhornia crassipes* (Barrett and Forno, 1982). Contrasting patterns of genotypic diversity between native and adventive portions of the range of the species are suggested by patterns of mating-type diversity. In these and other similar cases, allozyme data required to confirm this interpretation are largely unavailable, reflecting a general paucity of allozyme studies of aquatic plants (Barrett, Eckert, and Husband, 1993). Quantitative investigation of allozyme variation in terrestrial plants, on the other hand, has involved several hundred species (Hamrick and Godt, 1990). Few of these studies, however, have specifically addressed the problem of clonal diversity in species with well-developed vegetative reproduction. A review of the available data on allozyme variation in 27 taxa by Ellstrand and Roose (1987) revealed that measures of genotypic diversity vary widely both within and among species of clonal plants. However, only four of the species surveyed exhibited a range of genotypic diversity similar to that observed in *D. verticillatus* (range of $D = 0.00$–0.98; $E = 0.00$–0.97).

Determining whether the range of genotypic diversity seen in *D. verticillatus* is unusual is hampered by sampling problems at several levels. First, the taxonomic sample summarized by Ellstrand and Roose (1987) is limited; one-third of the taxa surveyed were species (or microspecies) of *Taraxacum* and *Oenothera*. Second, estimates of clonal diversity at the species or population level are particularly sensitive to the scale and intensity of sampling (Hebert, Ward, and Weider, 1988). Of the 27 clonal taxa summarized by Ellstrand and Roose (1987) most were represented by a small sample of populations (<5); six taxa were represented by a single population. At the population level, estimates of genotypic diversity will depend on the number and spatial distribution of ramets sampled and the number and variability of the genetic markers assayed (Ellstrand and Roose, 1987; Hebert, Ward, and Weider, 1988). In this study, these two aspects of the sampling strategy may have had opposite effects. On the one hand, genotype number (G) was probably underestimated in *D. verticillatus* because of the small number of polymorphic loci available. On the other hand, genotypic diversity (as measured by D and E) may have been overestimated in dimorphic and trimorphic populations, since ramets were sampled at a coarse spatial scale (4-m intervals). Clonal spread resulting in reduced genotypic diversity at finer spatial scales (e.g., Silander, 1979; Murawski and Hamrick, 1990; Aspinwall and Christian, 1992; Parker and Hamrick, 1992) would not have been detected, and genotype number (G) would appear proportionally larger for a given sample size (N_r).

Factors limiting the occurrence of sexual reproduction—The patterns of genotypic diversity found in *D. verticillatus* suggest that clonal reproduction predominates at the northern margin of the species' range. While geographic

marginality is often assumed to be associated with harsh environments, it may also be associated with genetic isolation and stochastic effects on genetic variability (Brown and Marshall, 1981; Barrett and Husband, 1990). Hence, both ecological and genetic factors may underlie variation among populations in clonal diversity.

Ecological factors—Abrahamson (1980) provides examples of a variety of biotic and abiotic factors that influence the production and establishment of sexual and clonal progeny. In *D. verticillatus,* the relative importance of the two reproductive modes is probably determined by the factors that limit sexual reproduction. Clonal propagation occurs in most populations, whereas fruit production and seedling establishment are more variable. Indeed, seedlings have rarely been observed under field conditions. Of ten populations of *D. verticillatus* that we visited regularly over a 3-year period, seedlings were seen in only two, and in both cases they were restricted to small, sheltered patches of bare mud. Infrequent seedling establishment is commonly reported in demographic studies of clonal populations (Sarukhan and Harper, 1973; Lovett Doust, 1981; Ashmun and Pitelka, 1985; Cook, 1985). There is also evidence that harsh conditions reduce the establishment of sexual progeny to a greater extent than vegetative offspring (Harmer and Lee, 1978; Abrahamson, 1980). In northern populations of *D. verticillatus,* the growing season is likely to be too short for seedlings to become large enough to overwinter. Clonal progeny, on the other hand, usually attain large size and develop extensive root systems before their first winter.

Ecological conditions may also affect the production of seed as well as its establishment. Severe environmental conditions may reduce pollinator activity (Salisbury, 1942; Gustafsson, 1946, 1947; Hagerup, 1951; Barrett, 1980) and may inhibit successful fertilization and/or seed development (Mooney and Billings, 1961; Barrett, 1980; Douglas, 1981; Garbutt, Bazzaz, and Levin, 1985; Weis and Hermanutz, 1988; Hermanutz, Innes, and Weis, 1989). Low levels of seed set are typical of northern populations of *D. verticillatus,* including the four examined in this study. In the fall of 1990, these populations were thoroughly searched without finding a single fruit. In contrast, high levels of seed production were observed in the southern dimorphic and trimorphic populations included in this study (Eckert, 1993). Glasshouse studies indicate that low seed set in monomorphic populations usually results from environmental rather than genetic factors. Self- and cross-pollination of ramets from ON-M1, ON-M5, and MI-M11 grown under glasshouse conditions resulted in seed production comparable to glasshouse-grown plants from dimorphic and trimorphic populations.

The results of this study suggest a latitudinal trend in clonal diversity among populations of *D. verticillatus.* We compared populations monomorphic for style length located at the extreme northern margin of the range in Ontario and Michigan with dimorphic and trimorphic populations further south within each region. If, however, floral monomorphism is a reliable indicator of clonal monomorphism, the distribution of populations monomorphic for style length found in a larger geographical survey (Eckert and Barrett, 1992) suggests that conditions discouraging successful sexual reproduction in *D. verticillatus* may not always covary with latitude. Trimorphic populations occur in areas of widespread monomorphism (e.g., MI-T8) and vice versa. Hence, the ecological conditions underlying differences in clonal diversity may often involve several environmental parameters rather than a single variable such as temperature. This problem underscores the tenuous relationship between ecological and geographical marginality (Brussard, 1984).

Genetic factors—Marginal plant populations often consist of small numbers of sparsely distributed individuals. These demographic characteristics have important population genetic consequences, especially with respect to the level of inbreeding and the random fixation of deleterious genes (Levin, 1970; Glover and Barrett, 1987). In clonal populations founded by a single genotype, sexual reproduction necessarily involves self-fertilization. Strongly self-incompatible or dioecious species will fail to set seed entirely (examples in Sculthorpe, 1967; Hutchinson, 1975; Cook, 1988). In self-compatible species such as *D. verticillatus,* seed can be produced, but its germination, establishment, and survival may be reduced by inbreeding depression (Charlesworth and Charlesworth, 1987). This may be particularly important in *D. verticillatus,* as studies of inbreeding depression indicate that offspring derived from self-fertilization make a negligible contribution to the adult population (Eckert, 1993). Hence, the harmful effects of inbreeding may act in concert with harsh environmental conditions to reduce the frequency of sexual reproduction in northern populations.

Founder events and genetic drift in marginal populations may also lead to the chance fixation of deleterious genes (Pollack, 1987). In clonal populations, deleterious mutants affecting sexual reproduction may be perpetuated as long as vegetative growth is not compromised. It has also been proposed that sexual function may be lost *gradually* through the accumulation of somatic mutations in clonal lineages (Klekowski, 1988). Many workers have commented on the apparent association between clonal reproduction and sexual sterility in aquatic plants (Arber, 1920; Sculthorpe, 1967; Hutchinson, 1975; Les, 1988), although the basis of this sterility is usually unknown. Two examples from *D. verticillatus* suggest that genetic factors may reduce the ability of clonal populations to reproduce sexually. Sexual sterility has been shown to occur in one of the monomorphic populations included in this study (ON-M6). No seed production has been observed in this population for 3 different years. Evidence that this sterility has a genetic basis was obtained from controlled crosses conducted under glasshouse conditions. Both self- and cross-pollinations produced few seed; however, pollinations on plants from nearby monomorphic populations (ON-M1 and ON-M5) set abundant seed (Barrett, Eckert, and Husband, 1993; C. G. Eckert and S. C. H. Barrett, unpublished data). The second example involves a population in Connecticut (CT-M1, surveyed in 1990) with a high frequency (30%) of individuals that produce only sterile, petaloid flowers. Again, this condition is maintained under glasshouse conditions indicating that the petaloid phenotype is under genetic control (Eckert, 1993). Herbarium specimens collected from this population by J. H. Bishop in 1877 (specimen from GH) and H. S. Clark in 1900 (specimen from DAO) indicate

that this phenotype has persisted at this location for more than a century. While such genotypes may enjoy the extreme longevity documented for other clonal taxa (Cook, 1985), their potential evolutionary significance is limited.

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