Style Morph Ratios in Tristylous Decodon Verticillatus (Lythraceae): Selection vs. Historical Contingency

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STYLE MORPH RATIOS IN TRISTYLOUS *DECODON VERTICILLATUS* (LYTHRACEAE): SELECTION VS. HISTORICAL CONTINGENCY[1]

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Abstract. Tristylous plant populations should exhibit equal frequencies of the three style morphs at equilibrium. In contrast, New England and central Ontario populations of *Decodon verticillatus* (Lythraceae) show a marked deficiency of the mid-styled (M) morph. This pattern was also evident in an independent sample of 30 populations from eastern Ontario; all nine cases of morph loss involved the M morph, and this morph also occurred at low frequencies, especially in large trimorphic populations. The hypothesis that a fitness disadvantage to the M morph accounts for its low frequency was not supported by data from a series of field and glasshouse experiments. Measures of reproductive success from two populations showed no disadvantage to the M morph, but revealed substantially higher seed set in the mid- (M) and short-styled (S) morphs than in the long-styled (L) morph. A pollen addition experiment showed that low seed set in the L morph can only partly be explained by reduced pollen receipt. Comparison of morph frequencies between adult plants and their open-pollinated progeny in three populations failed to reveal any morph-specific fitness differences. Analysis of growth and flowering of open-pollinated progeny from three populations in a 2-yr glasshouse experiment revealed differences among morphs for some parameters in some populations, but no consistent patterns were evident. The deficiency of the M morph may have resulted from an historical accident during post-glacial colonization of parts of the northern range. Computer calculations examining the return of morph frequencies to equilibrium after major perturbations indicated that populations of long-lived clonal species like *D. verticillatus* may preserve skewed morph ratios for >10 000 yr. Moreover, the distribution of fossil seeds indicates that this species has occurred in its post-glacial range in southern Ontario for only ≈5000 yr. It is important to distinguish between ecological and evolutionary time scales when testing selective interpretations of microevolutionary patterns involving clonal taxa in glaciated regions.

Key words: aquatic plants; Decodon; *eastern North America; ecological genetics; glaciation; morph-ratio variation; natural selection; non-equilibrium populations; sexual polymorphisms; tristyly.*

INTRODUCTION

Conspicuous polymorphisms have provided model systems for evolutionary analysis and, in several cases, have been the focus of considerable debate over the relative importance of selective and random evolutionary forces (Wright 1978, Provine 1986, Gould and Woodruff 1990, Bierzychudek and Schemske 1992, Turner 1992). Plant sexual polymorphisms such as dioecy, gynodioecy, and heterostyly are of particular significance because the factors affecting these polymorphisms directly influence mating patterns and consequently the evolutionary dynamics of populations. Also, because sexual polymorphisms are often simply inherited, explicit genetic models may be used to generate testable predictions as to how various evolutionary forces affect mating type frequencies.

Fisher (1941, 1944) first suggested that disassortative mating among style morphs in tristylous plant populations should lead to a single equilibrium with the three morphs at equal frequencies. Further theoretical studies have confirmed this prediction and have shown that it holds under different modes of inheritance (Finney 1952, 1983, Fisher 1965, Spieth and Novitski 1969, Spieth 1971, Heuch 1979*a, b,* Heuch and Lie 1985). In contrast to Fisher's equilibrium prediction, large-scale surveys of morph frequencies have revealed significant deviations from equal frequencies in several tristylous species. These departures from the simple equilibrium are generally interpreted as reflecting the ecology and life history of particular species (Barrett 1992). Yet, in contrast to the burgeoning empirical work on the ecological forces affecting sex ratios in dioecious (reviews in Lloyd and Webb 1977, Cox 1981, Meagher and Antonovics 1982, Bierzychudek and Eckhart 1988, Delph 1990) and gynodioecious species (reviewed in Gouyon and Couvet 1987), detailed investigations of the factors underlying morph-frequency variation in tristylous taxa are few (*Oxalis alpina*: Weller 1979, 1981*a, b,* 1986; *Pontederia cordata*: Barrett et al. 1983, Morgan and Barrett 1988; and *Eichhornia paniculata*: Barrett et al. 1989, Husband and Barrett 1992).

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This paper presents results from a series of studies investigating the causes underlying uneven style morph ratios in tristylous *Decodon verticillatus* (L.) Ell. (Lythraceae), a clonal perennial that inhabits wetland habitats in eastern central North America. (Hereafter the long-, mid-, and short-styled morphs are referred to as the L, M, and S morphs, respectively.) A survey of morph frequencies in four geographical regions indicated striking deviations from equilibrium expectations (Eckert and Barrett 1992). Up to two-thirds of the populations in a given region lacked at least one morph and morph ratios showed wide variation among populations as well as significant overall departures from equality. While genetic drift has probably contributed to some of the observed variation among populations, stochastic forces are unlikely to be responsible for the clear biases in average morph ratios seen in particular regions.

Computer simulations (Heuch 1980) and empirical studies of small populations of tristylous *Eichhornia paniculata* (Husband and Barrett 1992) and *Lythrum salicaria* (Eckert and Barrett 1992) indicate that the stochastic loss of style morphs from populations usually involves the short-styled morph because of a genetic constraint associated with the inheritance of tristyly. This expectation contrasts sharply with the pattern of morph loss observed for *D. verticillatus* in some northern parts of its geographical range. In New England, 82% of the 33 non-trimorphic populations surveyed lacked the M morph, compared to only 30% expected from stochastic morph loss alone. Computer simulations also indicate that stochastic forces should not consistently bias morph frequencies within populations. However, frequent loss of the M morph in New England was also associated with sizeable deficiencies of this morph in populations including all three morphs (average frequency: L, 33%; M, 22%; S, 45%; $n = 17$ populations). Reduced frequencies of the M morph were also evident in a sample of 21 populations from central Ontario, and were reflected in morph ratios derived from herbarium specimens collected in this region (Eckert and Barrett 1992). The failure of the stochastic model to account for the deficiency of the M morph in *D. verticillatus* raises the possibility that selection may play a role in influencing the pattern of morph ratio variation in parts of the species' range.

Deficiencies of the M morph are not restricted to *D. verticillatus* but occur elsewhere in the Lythraceae and in other tristylous families. Ontario populations of related *Lythrum salicaria* exhibit reduced frequencies of the M morph (Eckert and Barrett 1992) and this trend is more exaggerated in geographically marginal populations in northern Sweden (L, 45%; M, 14%; S, 41%; $n = 3156$ plants, 23 populations; J. Ågren, *unpublished data*). Studies in distylous *Pemphis acidula,* the likely sister group to *Decodon* (Graham et al. 1993), suggest that distyly has arisen from tristyly through the evolutionary loss of the M morph (Lewis and Rao 1971, Lewis 1975). Other cases where the M morph is rare or lost altogether include *Oxalis alpina, O. priceae* (Weller 1992), and *Narcissus triandrus* (S. C. H. Barrett, D. G. Lloyd, and J. Arroyo, *unpublished data*). These examples suggest that the M morph may be particularly prone to deficiency in a variety of tristylous taxa, implying that M deficiencies are probably a product of natural selection (Charlesworth 1979).

The first goal of this study was to assess the prevalence of the M deficiency in northern populations of *D. verticillatus.* As discussed, a previous survey revealed striking deficiencies in populations from central Ontario. To determine whether or not this pattern occurs in other parts of the province, we surveyed morph ratios in an independent sample of 30 populations from eastern Ontario. The results revealed a reduced frequency of the M morph, thus confirming patterns seen elsewhere in Ontario and New England.

We then examined two general hypotheses to account for the deficiency of the M morph. First, skewed morph ratios or the consistent loss of a morph from populations could be accounted for by fitness differences among morphs (Charlesworth 1979, Heuch and Lie 1985, Barrett et al. 1989). In *D. verticillatus,* for instance, the deficiency of the M morph might result from a reduction in its viability or reproductive success resulting from pleiotropic effects of the genes controlling the polymorphism and/or the direct effects of the floral polymorphism on mating success. To investigate whether or not the deficiency of the M morph was associated with a fitness disadvantage, we compared components of fitness among morphs in a series of field and glasshouse studies.

An alternative hypothesis for the deficiency of the M morph in *D. verticillatus* involves a stochastic historical reduction in its frequency over a large geographical area. This would not involve contemporary stochastic processes examined in previous studies (Barrett et al. 1989, Eckert and Barrett 1992), but rather historical accidents from which populations of this long-lived clonal plant would be very slow to recover. The possibility of historical contingency was, therefore, explored with computer calculations examining the speed at which morph ratios return to equilibrium after major perturbations.

Methods

Population surveys

Morph frequency variation in eastern Ontario.— During August 1988 and 1989, morph frequencies were estimated for 30 populations of *D. verticillatus* located in the Rideau Lakes area of Leeds and Grenville and Frontenac Counties in eastern Ontario, Canada (Fig. 1; details in Eckert 1993). Our sampling strategy followed methods used in previous surveys (see Eckert and Barrett 1992): A population was defined as a group of plants separated from other groups by ≥ 1 km. Plants

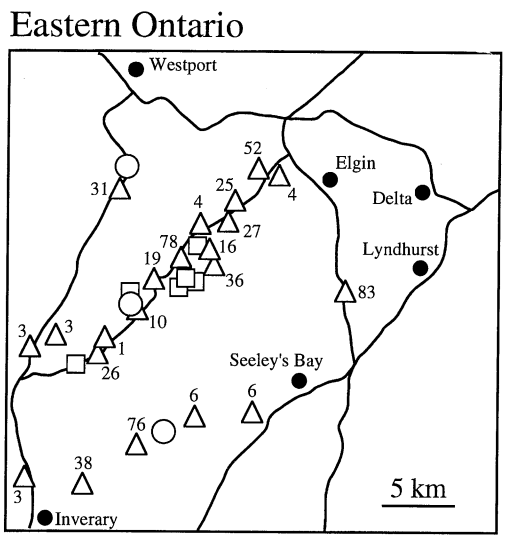

FIG. 1. Location and representation of the M morph in 30 populations of *Decodon verticillatus* sampled from Leeds and Grenville and Frontenac Counties in eastern Ontario. Trimorphic, dimorphic, and monomorphic populations are shown as open triangles, squares, and circles, respectively. The frequency (%) of the M morph is shown beside the symbol for each trimorphic population. All dimorphic and monomorphic populations lack the M morph. Towns (solid circles) are shown as landmarks.

were sampled throughout each population using a spatial interval that maximized the number of ramets sampled while minimizing the possibility of sampling the same ramet more than once. In populations of *D. verticillatus,* individual ramets range in size from those consisting of a single branch to those composed of many branches spreading up to 3 m in diameter. Accordingly, plants were sampled at 3-m intervals. Population size was estimated by inspection as the number of flowering and non-flowering ramets.

Temporal variation in morph frequencies.—The morph frequencies of a given population may fluctuate throughout a growing season and from year to year, even in perennial species (Gilbert and Lee 1980, Barrett et al. 1983). Hence frequencies estimated at one time may not reflect those of the population in general. To assess the importance of these sources of variability, two populations (EO-T3 and EO-T6, where EO = eastern Ontario; T = trimorphic) were sampled in each of three years (1988, 1989, and 1992) and two (EO-T1 and EO-T3) were sampled in 1988 at the beginning and end of the flowering season, which lasts for 2–3 wk in August.

Adult reproductive success

Field experiment.—To compare components of reproductive success among morphs, flower and fruit production as well as plant size and clonal propagation were estimated for 20 plants per morph in each of two populations with a deficiency of the M morph (EO-T6 and EO-T3) in 1988. Plants were selected for study by tagging a randomly chosen plant of the M morph and its nearest L and S morph neighbors. To minimize the likelihood of sampling the same clonal genet more than once, the groups of three were separated by ≥5 m. Data on allozyme variation at the same spatial scale indicated high levels of clonal diversity in both populations (Eckert and Barrett 1993*a*), suggesting that most tagged plants were probably different genotypes. On each tagged plant, we selected one branch coming into flower and followed the fate of all flowers produced on the main branch and all lateral branches. The 120 plants were visited at weekly intervals from the onset of flowering in early August until all fruits had matured in mid-December. At each visit, other flowering and non-flowering branches on the same plant were counted and the production of clonal shoots through adventitious rooting at the branch tips was recorded. Fruits were collected when mature (i.e., splitting along the valves), and seeds were counted for a random subsample of 2–9 fruits (median = 5) per plant. Seeds from each fruit were weighed as a group to 0.1 mg and average seed mass was calculated. In all, 10 variables including total number of branches, number of clonal branches, number of flowering branches, flowers per branch, flowers per plant, fruits per branch, fruits per flower, seeds per fruit, seed mass, and seeds per plant were analyzed using mixed model ANOVA with population (random) and morph (fixed) as main effects.

Other reproductive measures.—Considerable variability among individuals in the onset of flowering was observed in populations of *D. verticillatus.* Consequently, reproductive differences among morphs not detected in a group of plants sampled at the onset of flowering might occur in cohorts flowering later in the season. To assess this possibility, we randomly sampled branches from 15 to 26 flowering ramets per morph (mean = 21) in EO-T3 at both the beginning and end of the flowering season in 1988. For each branch, we counted leaf axils producing flowers, buds, flowers in anthesis (i.e., those retaining petals), and developing fruits. The number of flowers not developing into fruit could also be counted since abscised flowers leave a conspicuous scar in the leaf axil. Leaf axils were classified as 'reproductively active' if they included at least one receptive flower, developing fruit, or flower scar. Reproductive output was calculated for each branch as the number of receptive flowers plus developing fruits and flower scars.

Pollen addition experiment.—Individual flowers of *D. verticillatus* contain ≈100 ovules, but the number of seeds produced is usually much lower (≈45) and may vary widely. Part of this variation may be due to the quantity and quality of pollen received on stigmas (Eckert 1993). To assess the importance of pollen receipt in contributing to potential differences in seed production among morphs, we performed a pollen addition field experiment in two populations (EO-T3 and

EO-T6) during 1988. In each population, we randomly selected 36–46 pairs of flowers (average = 43) from a total of 8–10 plants per morph (an average of 4.7 pairs per each of 55 plants). Members of each pair were located in opposite leaf axils but at the same floral position. Shortly after flowers opened, one was hand-pollinated with excess pollen from an individual of a different morph growing ≈5 m away; the other was left to experience insect pollination (open pollination). All pollinations were performed on two consecutive days (1 d for each population). The weather during this time was warm and sunny.

Viability of open-pollinated progeny

Seed collection.—Germination, growth, and flowering of open-pollinated progeny from the three morphs were compared over a 2-yr period under uniform glasshouse conditions. During the fall of 1988, 21–46 (mean = 38) seed families were randomly collected from each style morph in three trimorphic populations from the eastern Ontario study area (EO-T3, EO-T6, EO-T7). Four dimorphic populations were also sampled in the same fashion and included in the progeny performance experiment. However, for the sake of brevity, only the results from trimorphic populations are presented here. A full analysis including all seven populations is presented in Eckert (1993).

Germination.—In May 1989, after 5 mo of cold storage at 5°C, two lots of 50 seeds per family were weighed to 0.1 mg. Each was sown out on a standard mixture of three parts Pro-Mix (soil-less mix) to one part sandy loam contained in a 5.7-cm plastic pot set in a Perma-Nest tray (37 cm long × 30 cm wide × 8.5 cm deep), 30 pots per tray. In total, 688 pots (representing 344 seed families) were randomly arranged on a single glasshouse bench illuminated 14 h/d by two 400-W high-pressure sodium lamps. Throughout germination, water levels were kept at or just below the soil level, and temperatures ranged between 25° and 35°C. Two months later, emerging seedlings were counted. Average seed mass and proportion of seeds germinated were calculated for each family by averaging the two replicates. These variables were entered in a fixed-effects two-way ANOVA with population and parental morph as main effects. Unlike the field experiment, population was considered a fixed effect in the glasshouse experiment since the choice of populations was not random but represented the array of morph frequencies observed in this region (see Neter et al. 1990: 660).

Growth and flowering.—Growth and flowering of offspring derived from the germination experiment were examined using a factorial randomized block experimental design. One of the drawbacks of glasshouse experiments is that fitness differences among individuals expressed in natural environments may not be expressed in a benign glasshouse environment (e.g., Assouad et al. 1978, Dudash 1990). To assess the importance of environment, the experiment was replicated under both high- and low-nutrient growing conditions. Four seedlings from each of 30 families per morph per population (except in EO-T7, where only 22 families were available for the S morph) were transplanted singly into 5.7-cm pots containing standard soil mix. The four seedlings per family were allocated one into each of four randomized blocks and all 1044 plants were placed on a single glasshouse bench under natural light. Two weeks after transplanting, seedling height (to 0.1 cm) and number of leaves (excluding cotyledons) were recorded for each plant. A week later, and for 2-wk intervals thereafter, plants were fertilized with a solution of 20:20:20 (N:P:K) fertilizer. Two blocks received 80 mg dry mass of fertilizer per plant (hereafter high-nutrient treatment). The other two received half that (low-nutrient). Regular fertilization stopped in early October, at which point plants started to enter dormancy. On 20 October, they were moved to a resting house and kept at 5°–10°C under natural light.

In April the following year, plants emerged from dormancy and on 10 May, they were moved to two glasshouse benches (one block per nutrient treatment per bench), kept under natural light, and fertilized every 2 wk. On 22 May, plants were scored as alive or dead and plant height and number of leaves were recorded. Throughout the summer, individuals were tagged as they flowered, and the number of leaf nodes producing flowers was recorded. On 19 September, after growth and flowering had ceased, the above-soil portion of each plant was harvested, dried at 60°C for 4 d and weighed to 0.1 g.

Pre-nutrient treatment measurements were entered in a fixed-effects three-way ANOVA with population, morph, and block (four blocks) as main effects. None of the interactions with block was significant, hence they were removed from the model (a conservative procedure in a fixed-effects analysis; see Neter at al. 1990: 909–935). Post-nutrient treatment variables were entered in a four-way ANOVA with population, morph, nutrient, and block (two blocks) as main effects. Again, interactions involving block were not significant and were removed from the model. For both pre- and post-nutrient treatment measurements, plant height and number of leaves were highly correlated; thus only plant height was used in the analysis.

Since a considerable proportion of the plants involved in the progeny performance experiment flowered and were scored for style morph, we also examined the effect of progeny morph on growth and flowering. Sample sizes for each combination of population, morph, and nutrient treatment ranged from 15 to 62 (average = 30) and were generally twice as large in the high as in the low nutrient treatment.

Comparison of morph ratios between generations

Differences in male fitness among morphs, occurring as a result of inequalities in pre- or post-pollination

siring ability, may be detected by comparing morph frequencies between parents and their open-pollinated progeny (Fisher and Mather 1943). In populations with low frequencies of the M morph, disassortative mating among morphs gives it a siring advantage, thereby increasing its frequency in the progeny generation. In contrast, a male fitness disadvantage to the M morph will result in an underrepresentation of the M morph in the progeny generation, or will at least maintain skewed parental morph frequencies. Morph frequencies in the progeny generation were estimated in three populations from the open-pollinated families included in the progeny performance experiment. Parental morph frequencies were estimated for each population during the summer before seed collection. To supplement the sample of progeny included in the growth experiment, five to 10 seedlings from a random subset of about 25 families per morph per population were transplanted from the germination experiment and grown under glasshouse conditions. By December 1990, between 398 and 466 offspring per population (1283 in all) had flowered.

Morph frequencies in the progeny generation were estimated by weighting the morph frequencies of progeny from each parental style morph by the frequency of that parental morph in the population (following Weller 1986). For example, L_L, L_M, L_S are the frequencies of L morph progeny in seed families from L, M, and S parents, respectively, and L, M, and S are the parental morph frequencies. Thus the frequency of the L morph in the progeny generation is: $L' = (L_L)(L) + (L_M)(M) + (L_S)(S)$. Parental and progeny morph frequencies were compared for each population by entering raw parental morph counts (i.e., the number of each morph, nL, nM, and nS, from which parental morph frequencies were derived) and adjusted progeny counts (e.g., $nL' = L'$[number of progeny scored]) in two-way contingency tables (Sokal and Rohlf 1981).

Computer calculations

We used a mathematical formulation of mating in tristylous populations modified from Barrett et al. (1989) to examine the effect of life history and mating parameters on the speed at which morph ratios in large populations return to equilibrium after a major perturbation. A vector $\boldsymbol{g}$ contained the frequencies of 10 possible genotypes at the two loci controlling style morph (double heterozygotes in coupling and repulsion were treated as distinct genotypes). Each genotype g_i segregated the j^{th} possible gamete type (*sm, sM, Sm* and *SM*) with probability A_{ij} included in a 10 × 4 matrix $\boldsymbol{A}$. Vectors $\boldsymbol{s}$ and $\boldsymbol{d}$ contained genotype-specific values for the rates of self-fertilization (s_i), and disassortative mating (d_i, where $s_i + (1 - s_i)d_i + (1 - s_i)(1 - d_i) = 1$). The frequency of matings between maternal and paternal gamete types was calculated following Barrett et al. (1989) to give a normalized vector $\boldsymbol{g}_{S'}$ containing genotype frequencies among sexually-produced offspring. A normalized vector of genotype frequencies among individuals surviving from the previous year $\boldsymbol{g}_{I'}$ was equivalent to $\boldsymbol{g}$ (no differences in mortality among genotypes). A normalized vector of genotype frequencies among clonal offspring $\boldsymbol{g}_{C'}$ was also the same as $\boldsymbol{g}$ (no differences in clonal propagation among genotypes). Finally, the genotype frequencies in the next year were calculated as:

$$\boldsymbol{g}' = I\boldsymbol{g}_{I'} + (1 - I)(C\boldsymbol{g}_{C'} + [1 - C]\boldsymbol{g}_{S'}),$$

where I and C are the population-wide rates of year-to-year survival and clonal recruitment, respectively. Note that this modification assumes that all individuals flower each year, regardless of when they were recruited into the population (i.e., no age-specific mortality or reproduction). Hence, this mathematical model may underestimate the time required to reach equilibrium in perennial populations. In the calculations presented hereafter, all genotypes had equal fitness and the style morph loci were unlinked (Eckert and Barrett 1993*b*).

Statistical analyses

In all ANOVA analyses, expected mean squares were derived using the general linear model routines in JMP (version 2.0, SAS Institute 1989), unless otherwise indicated. For significant effects, means were contrasted using Tukey-Kramer multiple comparisons (Tukey 1953, Kramer 1956). Analysis of categorical variables (e.g., probability of flowering) was performed by fitting linear models to a logistic response function using the maximum likelihood routine in JMP. Final models were obtained by eliminating unimportant (i.e., $P > 0.10$) effects based on the significance of the Wald χ^2. A more robust test of significance of the remaining factors was then performed using the likelihood ratio (LR), which is derived by applying the log-linear model both with and without the effect being examined and calculating:

$$LR = 2\{-\text{LogLikelihood[with effect]} - (-\text{LogLikelihood[without effect]})\}.$$

For tests of individual terms, the likelihood ratio is distributed as χ^2 with one degree of freedom. For all types of analyses involving simultaneous tests of significance, the experimentwise error rate (α_{EW}) was held at 5% using Sidák's (1967) correction for non-orthogonal contrasts: error rate per contrast (α_{PC}) = $1 - (1 - \alpha_{EW})^{1/C}$, where C is the number of contrasts or simultaneous tests.

RESULTS

Morph frequencies in eastern Ontario

Results from population surveys indicated a marked deficiency of the M morph in eastern Ontario. Of the 30 populations sampled, 70% were trimorphic, 20% dimorphic, and 10% monomorphic (Fig. 1). All cases of morph loss involved the M morph, resulting in a substantial reduction in its average frequency (20%)

TABLE 1. Average morph frequencies in populations of *Decodon verticillatus* from eastern Ontario. Both average frequencies and frequencies weighted by estimated population size are presented. Of the 30 populations sampled, 21 were trimorphic, 6 were dimorphic and 3 were monomorphic. L, long-styled; M, mid-styled; and S, short-styled morphs.

	Morph frequency (mean ± 1 SE)		
	L	M	S
Average frequencies			
All populations	0.45 ± 0.05	0.20 ± 0.05	0.35 ± 0.05
Trimorphic populations	0.43 ± 0.05	0.28 ± 0.06	0.29 ± 0.04
Dimorphic populations	0.57 ± 0.10		0.43 ± 0.10
Weighted frequencies			
All populations	0.49 ± 0.03	0.16 ± 0.04	0.35 ± 0.03
Trimorphic populations	0.49 ± 0.04	0.18 ± 0.05	0.33 ± 0.03
Dimorphic populations	0.40 ± 0.07		0.60 ± 0.07

compared to the L (45%) and S (35%) morphs (Table 1). Morph loss appears to have occurred independently several times, since dimorphic and monomorphic populations were geographically dispersed throughout the study area (Fig. 1). As in the regions previously surveyed, there was a negative relationship between estimated population size (N) and morph loss (Eckert and Barrett 1992). Trimorphic populations were on average four times larger ($\hat{N}$ = 910 ± 175 (mean ± 1 SE); range 25–3000) than non-trimorphic populations ($\hat{N}$ = 223 ± 268 (mean ± 1 SE); range 15–800; Wilcoxon signed-ranks test: $Z = -2.48$, $P = 0.013$).

In addition to the frequent loss of the M morph, trimorphic populations exhibited major departures from even morph frequencies. Replicated goodness-of-fit tests (Sokal and Rohlf 1981) revealed significant deviations for all populations pooled ($G_{\text{Pooled}} = 523$, df = 2, $P < 0.001$) as well as within individual populations ($G_{\text{Total}} = 2423$, df = 42, $P < 0.001$). Trimorphic populations exhibited, on average, a large excess of the L morph and more or less equal deficiencies of the M and S morphs (Table 1). However, there was significant heterogeneity among populations in the direction of skew ($G_{\text{Heterogeneity}} = 1900$, df = 40, $P < 0.001$). There was no obvious geographic pattern to this variation; neighboring populations differed in the frequency of the M morph as much as did those farther away (Fig. 1). In addition to masking considerable heterogeneity among populations, average morph frequencies obscure a negative relationship between the frequency of the M morph and population size. Of the 10 largest trimorphic populations, seven showed a large deficiency of the M morph (i.e., frequency of the M morph < 20%), compared to only three of the remaining 11 populations. When population frequencies were weighted by estimated population size, average frequencies showed a marked deficiency of the M morph (18%) associated with an excess of the L morph (49%) and a moderate frequency of the S morph (33%).

TABLE 2. Year-to-year and seasonal variation of style morph frequencies in populations of *Decodon verticillatus*. Data are from four populations censused in separate years and two populations censused at different times during the 1988 flowering season (EO, Eastern Ontario; MA, Massachusetts; T, trimorphic). The per-test α was adjusted to 1.69% for three simultaneous tests of year-to-year variation, and to 2.53% for two tests of seasonal variation. Contingency table analysis indicated that morph frequencies varied significantly among years only in EO-T3. This involved variation in the relative frequencies of the L and S morphs but not the M morph.

Population	Census date	Morph frequency L	M	S	Sample size
	Year-to-year variation				
EO-T3	1988	0.48	0.16	0.36	200
	1989	0.51	0.14	0.25	297
	1992	0.45	0.14	0.41	287
EO-T6	1988	0.35	0.12	0.53	245
	1989	0.39	0.11	0.50	357
	1992	0.32	0.17	0.51	379
MA-T4	1963*	0.56	0.41	0.03	114
	1990	0.60	0.38	0.02	440
	Seasonal variation (1988)				
EO-T3	2 August	0.48	0.15	0.37	100
	16 August	0.48	0.18	0.34	100
EO-T1	4 August	0.69	0.01	0.30	123
	22 August	0.58	0.01	0.41	91

* Data from Ornduff (1993).

Substantial variation in morph frequencies among populations was not associated with much seasonal or year-to-year variation within populations (Table 2). Morph frequencies in EO-T1 and EO-T3 did not change during the flowering season in 1988 (EO-T1: 2 × 3 χ^2 = 2.7, df = 2, $P = 0.260$; EO-T3: $\chi^2 = 0.4$, df = 2, $P = 0.819$). Morph frequencies estimated in three successive years also showed no significant variation in EO-T6 (3 × 3 $\chi^2 = 8.3$, df = 4, $P = 0.081$). In EO-T3, frequencies showed small, marginally significant variation among years ($\chi^2 = 11.5$, df = 4, $P = 0.021$). However, this involved variation in the relative frequencies of the L and S morphs ($\chi^2 = 11.0$, df = 2, $P = 0.004$), but no variation in relative frequencies of the L and M morphs ($\chi^2 = 0.01$, df = 2, $P = 0.788$) or the M and S morphs ($\chi^2 = 3.8$, df = 2, $P = 0.150$). In all cases, the frequency of the M morph remained low. There was anecdotal evidence that morph frequencies may be stable over even longer periods of time. Frequencies estimated in 1963 by Ornduff (1993)

TABLE 3. Comparison of reproductive performance among style morphs in two populations of *Decodon verticillatus*. Data are from 20 plants per morph per population (except as noted) monitored for an entire reproductive season in 1988.

Reproductive parameter	Population	Morph performance (mean ± 1 SE)		
		L	M	S*
Flowering branches	EO-T6	3.2 ± 0.6	4.6 ± 1.1	4.7 ± 0.8
	EO-T3	2.4 ± 0.4	2.3 ± 0.4	2.8 ± 0.6
Clonal branches	EO-T6	1.5 ± 0.5	1.9 ± 0.9	1.7 ± 0.7
	EO-T3	0.4 ± 0.1	1.2 ± 0.5	0.9 ± 0.3
Total branches	EO-T6	5.2 ± 0.8	6.5 ± 1.4	7.0 ± 1.2
	EO-T3	4.2 ± 0.9	5.2 ± 1.2	5.1 ± 1.0
Flowers per branch	EO-T6	95 ± 12	127 ± 14	91 ± 8
	EO-T3	75 ± 7	76 ± 7	87 ± 9
Flowers per plant†	EO-T6	318 ± 87	576 ± 133	463 ± 108
	EO-T3	195 ± 45	178 ± 33	329 ± 106
Fruits per branch	EO-T6	64 ± 9	98 ± 10	68 ± 7
	EO-T3	52 ± 6	59 ± 6	61 ± 7
Fruits per flower	EO-T6	0.66 ± 0.03	0.73 ± 0.03	0.74 ± 0.04
	EO-T3	0.69 ± 0.04	0.79 ± 0.03	0.70 ± 0.04
Seeds per fruit‡	EO-T6	30.7 ± 1.2	38.1 ± 1.3	38.2 ± 1.2
	EO-T3	31.3 ± 1.4	42.8 ± 1.4	38.0 ± 1.4
Seed mass (mg)‡	EO-T6	0.81 ± 0.02	0.80 ± 0.02	0.76 ± 0.01
	EO-T3	0.75 ± 0.02	0.68 ± 0.02	0.70 ± 0.02
Seeds produced per plant ($\times 10^{-2}$)	EO-T6	63 ± 17	147 ± 30	144 ± 37
	EO-T3	43 ± 9	62 ± 13	89 ± 31

* Only 19 plants of the S morph were monitored in EO-T6.
† Plant-wide measures were calculated for each plant individually as follows: flowers per plant = flowering branches × flowers per branch; seed production per plant = flowering branches × fruits per branch × seeds per fruit.
‡ Means were calculated from a sample of 2–9 fruits (median = 5) from each plant.

for a population in Massachusetts (MA-T4) were virtually identical to frequencies we obtained for the same population 27 yr later (Table 2, $\chi^2 = 0.6$, df = 2, $P = 0.732$).

Reproductive success of adult plants in natural populations

Field study.—Measures of reproductive success (expressed as mean ± 1 SE) obtained from marked plants in two trimorphic populations did not reveal a reproductive disadvantage to the M morph (Tables 3 and 4). Neither plant size, as estimated by total number of branches (5.5 ± 0.4), or the number of clonal branches per plant (1.3 ± 0.2) varied among morphs or populations. The number of flowering branches, flowers per branch, and flowers per plant were significantly higher in EO-T6 (4.2 ± 0.4; 104.7 ± 6.0; and 452 ± 65, respectively) than in EO-T3 (2.5 ± 0.4; 79.5 ± 6.0; and 233 ± 40, respectively), but did not vary among morphs. The proportion of flowers forming fruit averaged 0.72 ± 0.01 and did not vary among morphs or populations. The pattern of fruit production, therefore, paralleled that of flower production, differing between populations (EO-T6: 73.4 ± 4.4 fruits per branch; EO-T3: 57.4 ± 4.4) but not among morphs. The only significant difference among morphs involved

TABLE 4. Analysis of reproductive parameters in two populations of *Decodon verticillatus*. Values are *F* ratios with probabilities indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The per-test α has been adjusted to 0.5% for the simultaneous evaluation of 10 variables. Significant tests are indicated in boldface type. Response variables have been transformed, as indicated, to satisfy assumptions of ANOVA. Long dashes (—) indicate model terms that do not apply to a particular analysis. Results from nonsignificant ANOVA models obtained for total number of branches ($F_{5,113} = 0.9$, $P = 0.482$), number of clonal branches ($F_{5,113} = 1.0$, $P = 0.445$), and fruits per flower ($F_{5,113} = 1.5$, $P = 0.198$) are not shown. Means are in Table 3.

Source of variation†	Flowering branches	Flowers per branch	Log_{10} (flowers per plant)	Fruits per branch	Seeds per fruit	(Seed mass)$^{1/2}$	Log_{10} (seeds per plant)
Population	**8.8****	**8.7****	**16.5*****	6.7*	0.7	**8.2****	**11.1*****
Morph	1.5	0.6	1.0	1.9	**11.0*****	1.4	6.7
M × P	0.7	2.6	1.1	1.1	0.4	0.6	0.6
Plant [M × P]	—	—	—	—	**3.3*****	**3.9*****	—
R^2	0.10	0.13	0.16	0.11	0.48	0.49	0.16

† All two-way ANOVAs were mixed models with Populations (df = 1) as a random effect. *F* tests for the random effects, therefore, used the MS_{Error} (df = 113) as denominator, and those for Morph (df = 2) used $MS_{\text{M×P}}$ (df = 2) (Neter et al. 1990). In the analysis of seeds per fruit and seed weight, Population, M × P, and Plant[M × P] (df = 111) were random effects. The *F* test for Plant[M × P] used MS_{Error} (df = 503) as denominator. Tests for all other effects used $MS_{\text{Plant(M×P)}}$.

TABLE 5. Comparison of reproductive parameters among style morphs at two times during the 1988 flowering season in a population of *Decodon verticillatus*. One-way ANOVA was used to compare means among morphs for each parameter and sample. The per-test α was adjusted to 0.6% for eight simultaneous tests.

Reproductive parameter	Date of sample*	Morph (mean ± 1 SE) L	M	S	F	P
Total inflorescences	2 August	21.2 ± 2.4	16.8 ± 2.7	14.1 ± 2.2	2.3	0.106
	16 August	14.3 ± 1.3	12.3 ± 1.1	13.6 ± 1.0	0.7	0.513
Active inflorescences	2 August	8.9 ± 0.7	7.6 ± 0.8	6.9 ± 0.6	2.1	0.134
	16 August	14.1 ± 1.3	12.0 ± 1.1	12.7 ± 1.0	0.8	0.439
Flowers in anthesis	2 August	6.3 ± 0.6	6.9 ± 1.1	6.5 ± 1.0	0.1	0.912
	16 August†	2.0 ± 0.8	5.2 ± 1.2	4.0 ± 0.9	1.8	0.190
Reproductive output	2 August	19.9 ± 3.8	17.5 ± 3.0	12.7 ± 1.0	1.6	0.212
	16 August	53.2 ± 5.8	48.8 ± 6.1	47.1 ± 5.4	0.3	0.724

* Samples from 2 August included 20, 15, and 20 branches from the L, M, and S morphs, respectively. Those from 16 August included 26, 19, and 25 branches from the L, M, and S morphs, respectively.
† Only 5 L, 8 M, and 12 S morph branches were still producing flowers in the 16 August sample.

a greater number of seeds per fruit in the M (40.3 ± 1.5 seeds per fruit) and S (37.9 ± 1.5) morphs compared to the L (31.1 ± 1.4) morph (multiple comparison: M = S > L). This pattern was also reflected in large but nonsignificant differences in plant-wide seed production (i.e., the product of seeds per fruit, fruits per branch, and number of flowering branches). Differences in seed number among morphs were not compensated for by corresponding differences in seed size.

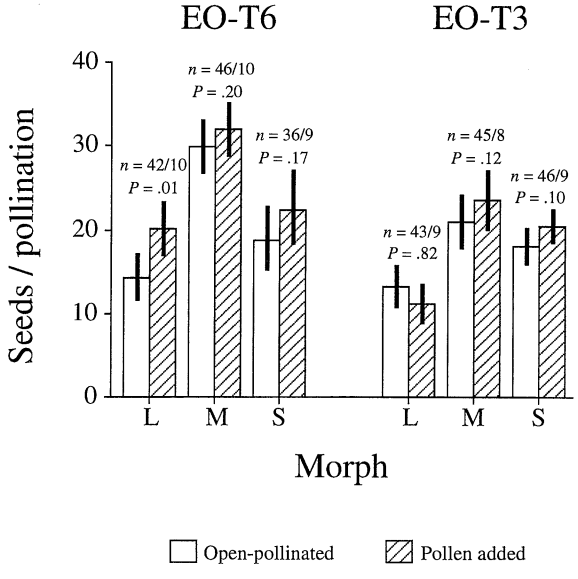

FIG. 2. Experimental investigation of pollen limitation of seed production in two trimorphic populations of *Decodon verticillatus*. Population codes appear above the bars for each. Open bars are mean seed production in open-pollinated flowers. Hatched bars are means for paired flowers supplemented with legitimate pollen. Error bars are ±1 SE. Pollination treatments were contrasted using paired t tests. Probability values (P) and sample sizes (n = pairs of flowers/maternal plants) are given above the bars for each style morph (L, M, and S for the long-, mid-, and short-styled morphs, respectively). The per-test α was adjusted to 0.9% for six simultaneous tests. Hence, the difference between treatments nears significance only for the L morph in EO-T6.

There was a weak but significant negative relationship between number of seeds per fruit and average seed mass in both populations (EO-T6: $F_{1, 318} = 4.5$, $P = 0.034$, $R^2 = 0.01$; EO-T3: $F_{1, 298} = 6.83$, $P < 0.001$, $R^2 = 0.02$). However, the slope of the relationship was not large in either population (EO-T6: $\beta = -0.0018 \pm 0.0008$ SE; EO-T3: $\beta = -0.0020 \pm 0.0008$) and did not lead to a difference in seed mass among morphs. Seed mass varied significantly among populations (EO-T6: 0.79 ± 0.01 mg; EO-T3: 0.71 ± 0.01 mg) and among maternal plants within populations, but not among morphs.

Differences among morphs in flower and fruit production, undetected among the marked plants in EO-T3, did not appear in later-flowering cohorts. Estimates of daily flower production and reproductive output per branch did not differ among morphs in either the early (2 August) or late (16 August) sample (Table 5).

Pollen addition experiment.—Lower seed production of the L morph compared to the M and S morphs could be due to differences in ovule production and/or pollen receipt. The first possibility is unlikely since estimates of ovule production from two populations in this area (including EO-T3) did not differ among morphs (Eckert and Barrett 1994*a*). The addition of outcross pollen to stigmas (Fig. 2) increased fruit set by 10% in EO-T6 (mean fruits per flower, pollen added: 0.75; open pollinated: 0.67; n = 126 flowers) and 2% in EO-T3 (added: 0.73; open: 0.72; n = 134). Pollen addition increased seeds per fruit by 7% in EO-T6 (added: 33.7 ± 1.8 mean ± 1 SE, n = 94 fruits; open: 31.6 ± 1.7, n = 85) and 4% in EO-T3 (added: 25.3 ± 1.3, n = 98; open: 24.4 ± 1.2, n = 95). However, paired analysis of the number of seeds produced per flower indicated that the effect of pollen addition neared significance only for the L morph in EO-T6. Although this does suggest that seed set in the L morph, at least in EO-T6, may be limited by pollen receipt, lower seed set in the L morph was evident even when excess pollen was added. Thus, reduced pollen receipt cannot fully account for lower seed set in the L morph.

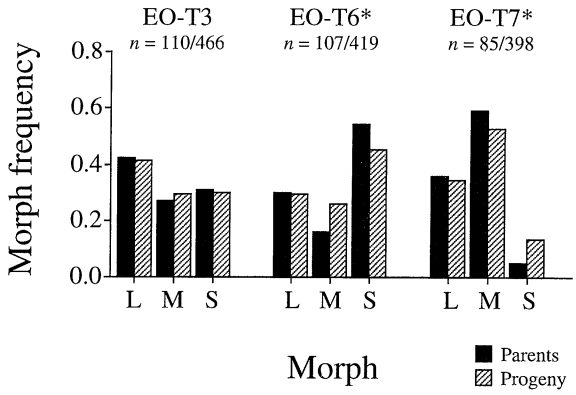

FIG. 3. Comparison of morph frequencies between parental plants and their open-pollinated progeny for three populations of *Decodon verticillatus*. Population codes appear along with sample sizes (n = number of parents/number of progeny) above the bars for each. Populations for which there was a significant difference between parental and progeny morph frequencies are marked with an asterisk. The per-test type I error rate (α) has been adjusted to 1.7% for three different tests.

Comparison of morph frequencies between generations

Progeny morph frequencies estimated for three populations did not differ greatly from parental morph frequencies (Fig. 3) and there was no evidence for reduced contribution to the progeny generation by the M morph. Small but significant differences between generations were apparent in EO-T7 (2×3 $\chi^2 = 10.7$, df = 2, $P = 0.005$) and EO-T6 (2×3 $\chi^2 = 10.2$, df = 2, $P = 0.006$). In both cases, these changes involved a shift toward more even frequencies in the progeny generation.

Viability of open-pollinated progeny—effects of maternal morph

Differences among morphs in growth and flowering of open-pollinated progeny were evaluated in a 2-yr glasshouse experiment. The results indicated that differences in progeny viability among morphs are unlikely to account for the low frequency of the M morph in eastern Ontario. The proportion of seeds germinating was moderate (0.59 ± 0.01, mean ± 1 SE) though once transplanted, most seedlings (98%) survived until the end of the 2-yr period. Survival was, therefore, not entered as a variable in the analyses. During the second summer of the experiment, about half of the plants flowered. While flowering occurred more frequently in the high (70%) than in the low (37%) nutrient treatment, the overall proportion of plants flowering was high enough to be included in the analyses. Means by morph and population are presented in Fig. 4 and results from ANOVA in Table 6. Although main effects were often significant, few of the ANOVA models explained much of the variance in offspring performance; R^2 ranged from 1 to 31% and averaged 14%.

Seed mass and germination.—Open-pollinated seeds from the M morph did not germinate at a reduced frequency compared to those from the L and S morphs. Average seed mass differed among populations but not among morphs. The proportion of seeds germinating varied among populations, but differences among morphs were inconsistent, resulting in a marginally significant interaction between the effects of population and morph. Separate examinations of each population indicated a 19% advantage to seeds from the L morph compared to seeds from both the M and S morphs in EO-T3 ($F_{2, 127} = 5.6$, $P = 0.004$), but the same trend was not apparent in the other two populations.

Growth and flowering.—Analyses of variation in growth and flowering also failed to reveal reduced viability of progeny from the M morph. For height measured in July 1989 (before the nutrient treatment) neither morph nor population explained a significant portion of variation among progeny. After application of the nutrient treatment, plants in the high nutrient treatment outperformed plants under low nutrient conditions for all parameters measured (including probability of flowering: $LR = 114$, df = 1, $P < 0.001$); usually by a factor of two or more. Differences among populations were detected for all variables (including probability of flowering: $LR = 8.6$, df = 1, $P = 0.003$) except number of flowering nodes. Effects of morph approached significance only for final dry mass ($P = 0.047$), and this was confounded by a marginally significant ($P = 0.028$) interaction between morph and population. Analysis of each population separately indicated that significant differences among morphs occurred only in EO-T7 (multiple comparisons: L > M = S), where progeny of the L morph were 15% heavier (6% in the low and 25% in the high nutrient treatment) than progeny of the M and S morphs.

Viability of open-pollinated progeny—effects of progeny morph

Analyses examining the effect of progeny morph on growth and flowering among those offspring scored for style morph did not indicate any disadvantage to the M morph in performance during early life history stages. Means by population and morph are presented in Fig. 5, and statistical analyses in Table 7. The effects of nutrient treatment and population are similar to those reported for maternal morph. Again, most of the variation in offspring performance was not explained by the ANOVA models used; R^2 ranged from 5 to 32% and averaged 16%. The effect of morph was significant only for dry mass. This involved a significant 11% advantage (6% in the low and 15% in the high nutrient treatment) to the M morph over the L morph, with the S morph intermediate (multiple comparisons: S = M > L = S).

Computer calculations

Calculations using a mathematical model of mating and recruitment in tristylous populations indicated that

life history and mating parameters greatly affect the amount of time required for morph frequencies to reach equilibrium (Fig. 6). Varying individual parameters, such as the rate of year-to-year survival (I) or the levels of self-fertilization (s) and disassortative mating (d), delayed equilibrium for a maximum of 75 yr for extreme values of I and 200 yr for extreme values of s (Fig. 6A, B, C). However, varying parameters jointly had a much more pronounced effect. Fig. 6D presents calculations using estimates of self-fertilization and disassortative mating for populations of *D. verticillatus* in eastern Ontario (average s [0.29] corrected for inbreeding depression [relative fitness of selfed progeny ≈ 0.5] = 0.17, Eckert and Barrett 1994*b*; average d = 0.30; Eckert and Barrett 1994*c*). When these parameter values are combined with high rates of year-to-year survivorship and clonal reproduction, populations may maintain skewed morph frequencies for 5000 to 15 000 yr.

Discussion

In plant populations with sexual polymorphisms such as dioecy, gynodioecy, or heterostyly, mating types should eventually attain frequencies which reflect their relative fitness. Population surveys of tristylous *D. verticillatus* in eastern Ontario confirmed the skew in style morph ratios observed in New England and elsewhere in Ontario. The M morph was absent from populations much more often than were the L or S morphs, and usually occurred with reduced frequencies, especially in the larger trimorphic populations. Reduced frequencies of the M morph also appear to be associated with high frequencies of the L morph. If differences in fitness among morphs are responsible for

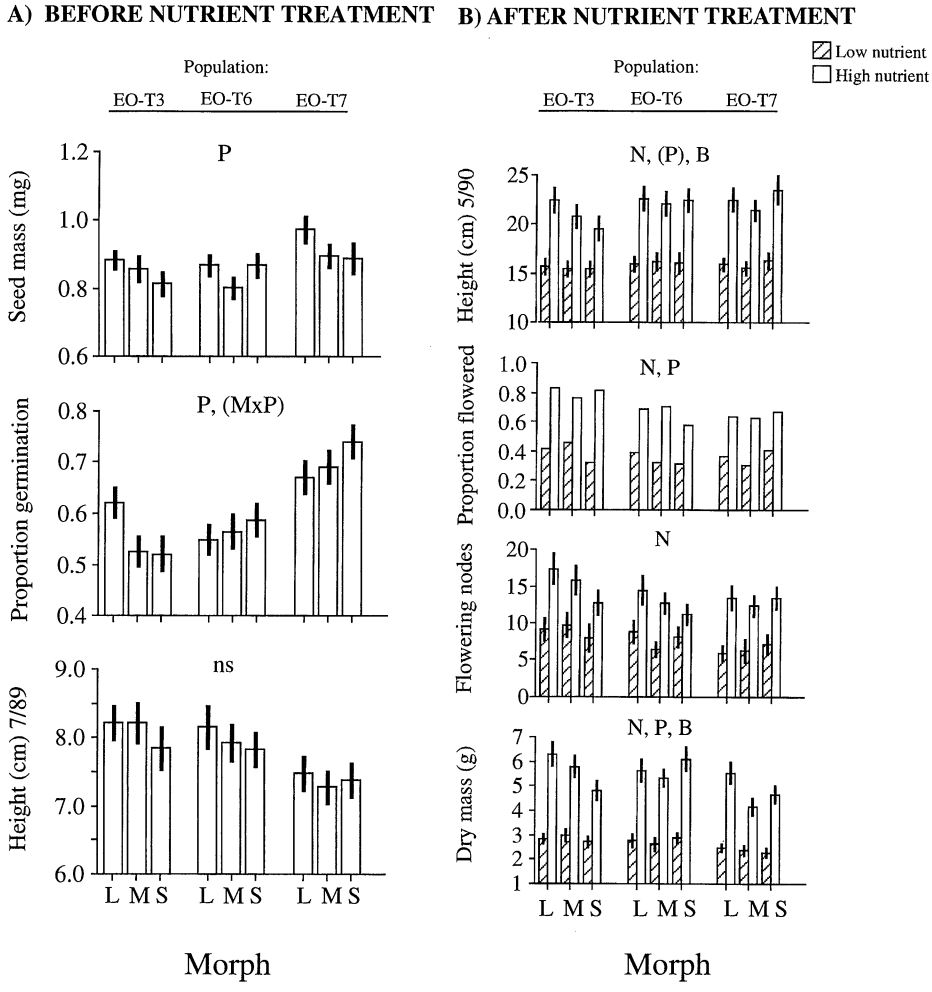

FIG. 4. Comparisons among morphs in the performance of open-pollinated progeny from three populations of *Decodon verticillatus* grown under uniform glasshouse conditions. The parameters estimated are separated into measurements made (A) before, and (B) after, the application of a two-level nutrient treatment. Bars are means. Error bars are ± 1 SE. Significant effects detected in factorial ANOVA appear above the bars (N, nutrient; P, population; M, morph of maternal plant; B, block; see Table 6). Effects in parentheses are only marginally significant; NS, not significant. Sample sizes are given in the text.

TABLE 6. Analysis of the performance of open-pollinated progeny in *Decodon verticillatus* under uniform glasshouse conditions. Values are F ratios with probabilities indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The per-test type I error rate (α) was adjusted to 0.7% for simultaneous assessment of seven variables (including probability of flowering; see text). Significant tests are indicated in boldface type. Variables were transformed, as indicated, to satisfy assumptions of ANOVA. Long dashes (—) indicate model terms that do not apply to a particular analysis. Results from a nonsignificant ANOVA model obtained for Log_{10}(height as of July 1989) ($F_{11, 1023} = 1.5$, $P = 0.134$) are not shown. Means are in Fig. 4.

Source of variation (df)	Average seed mass†	Arcsine (ppn seeds germinated)†	(Height, May 1990)$^{-1}$‡	Log_{10} (flowering nodes)‡	Log_{10} (dry mass)‡
Nutrient (1)	—	—	**128.9*** **	**56.7*** **	**406.1*** **
Population (2)	**5.8****	**23.9*** **	4.7**	0.5	**7.7*** **
P × N (2)	—	—	0.1	0.9	0.5
Morph (2)	3.9*	0.7	0.3	0.4	3.1*
M × N (2)	—	—	0.2	0.3	0.4
M × P (4)	1.4	3.5*	1.5	1.2	2.7*
M × P × N (4)	—	—	0.1	0.2	0.7
Block (1)	—	—	**21.9*** **	1.3	**10.6*** **
R^2	0.07	0.15	0.14	0.11	0.31

† For seed mass and germination, both Population and Morph were fixed effects, thus F tests used MS_{Error} (df = 335) as denominator.

‡ For height, flowering nodes and dry mass, all factors except Block were fixed effects, thus F tests used MS_{Error} (df = 1009 for height as of May 1990, 528 for number of flowering nodes and 1004 for dry mass).

the observed morph frequencies, our results should have revealed a fitness disadvantage to the M morph and a corresponding advantage to the L morph. Neither of these predictions was supported by this study and, therefore, we suggest that, given the large amount of time required for morph frequencies to reach equilibrium after major perturbations, historical contingency may provide an explanation for the reduced frequency of the M morph in certain parts of the northern range of *D. verticillatus.*

Reduced fitness of the M morph?

The results of the experimental studies appear to refute the hypothesis that the M morph is rare because of an inherent fitness disadvantage. None of the approaches used provided evidence for selection against the M morph in any of the populations examined. Several other studies of heterostylous species have also failed to detect fitness differences among the floral morphs. Components of reproductive success measured in the field often do not vary among morphs (Weller 1981*b,* Price and Barrett 1982, Boyd et al. 1990). Similarly, in cases where comparisons of parental and progeny morph frequencies have been made, they have failed to reveal mating asymmetries among morphs in *Pontederia cordata* (Barrett et al. 1983), *Oxalis alpina* (Weller 1986), and *Eichhornia paniculata* (Barrett et al. 1987). Notwithstanding the difficulties in evaluating fitness differences in long-lived organisms (Boyd et al. 1990) and in extrapolating the results of glasshouse experiments to natural habitats (Schemske 1983, Dudash 1990), the diverse approaches used in this study provided no compelling evidence that fitness differences are contributing to the observed deficiency of the M morph in eastern Ontario.

Increased fitness of the L morph?

Further evidence that observed morph frequencies are not the product of selection involves the high observed frequencies of the L morph in trimorphic populations in the absence of any apparent fitness advantage. Some results, when considered individually, were suggestive of an advantage to the L morph (e.g., germination in EO-T3, dry mass in EO-T7). Others, however, showed the opposite trend (e.g., seed set in both EO-T3 and EO-T6; dry mass analyzed by morph of progeny). A similar experimental analysis of offspring viability in four dimorphic populations containing only the L and S morphs also failed to provide any evidence for a fitness advantage to the L morph (Eckert 1993).

Paradoxically, the most striking difference among morphs detected in this study was the low relative seed production of the L morph in two populations. Differences in open-pollinated seed set among morphs are not generally observed in tristylous species (Dulberger 1970, Barrett 1977, Weller 1981*b,* Price and Barrett 1982, Barrett et al. 1989, but see O'Neil 1992), although they have been reported for several distylous species (Richards 1986). In most cases, seed set is usually higher in the L morph than in the S morph. Results from a pollen limitation experiment suggested that part of the difference observed in *D. verticillatus* may be explained by variation among morphs in pollen receipt. While our examination of pollen limitation was limited in scope, such differences among morphs are consistent with observations of the foraging behavior of flower visitors. In most heterostylous species, the inaccessibility of stigmas in flowers of the S morph results in smaller stigmatic pollen loads (Ganders 1979, Glover and Barrett 1983, Wolfe and Barrett 1989) and often lower seed set compared to the L morph (Opler et al.

1975, Ornduff 1976, Lloyd 1979, Wyatt and Hellwig 1979, O'Neil 1992). Preliminary pollinator observations in EO-T3 and EO-T6, however, suggest the opposite pattern for *D. verticillatus*. Flowers of this species are atypical of heterostylous species in being nontubular and actinomorphic, with the long- and mid-level organs projecting well beyond the hypanthium. When insect visitors (usually *Bombus* spp. and *Apis mellifera*) probe a flower to feed on nectar, the L stigma and long-level anthers often fail to contact the insect's body (Fig. 7). This is especially pronounced in the L and S morphs. Whether or not this difference in pollination biology among morphs leads to a reduction of pollen loads on L stigmas will depend on the number of visits a flower receives. It appears that levels of pollinator activity may sometimes be insufficient for full seed set in the L morph.

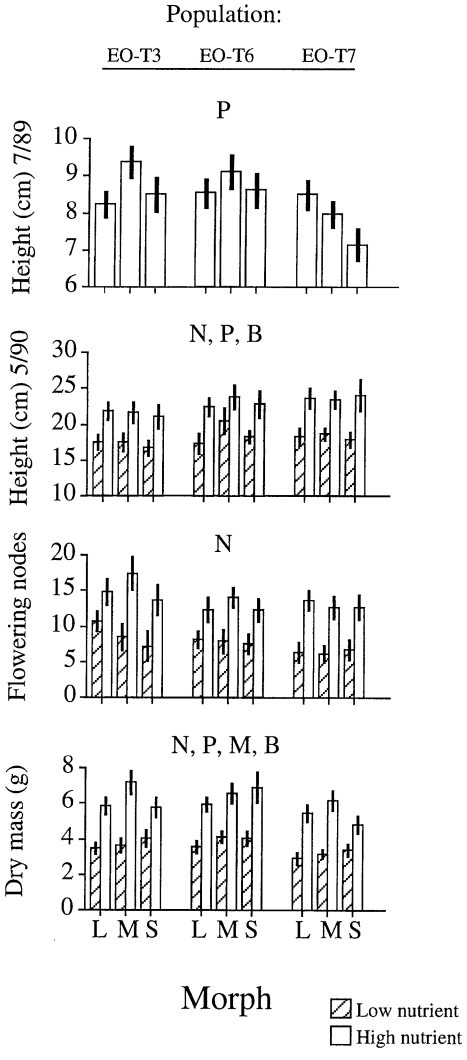

FIG. 5. Comparison of early growth and flowering among morphs of *Decodon verticillatus* in a factorial experiment under uniform glasshouse conditions. These data are from offspring which flowered and were scored for style morph in the progeny performance experiment (see Fig. 4). Bars are means of 15–62 (average = 30) plants per combination of morph, population, and nutrient treatment. Error bars are ± 1 SE. Significant effects detected in factorial ANOVA appear above the bars for each parameter (N, nutrient; P, population; M, morph of progeny; B, block; see Table 7). Height at July 1989 was measured before the application of the nutrient treatment and, therefore, involves about twice as many individuals for each mean.

TABLE 7. Analysis of early growth and flowering of open-pollinated seedlings in *Decodon verticillatus* under uniform glasshouse conditions. The data are from those plants that flowered in the progeny performance experiment (Fig. 4). Values are *F* ratios, with probabilities indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The per-test type I error rate (α) was adjusted to 1.3% for simultaneous assessment of four variables. Significant tests are indicated in boldface type. Variables were transformed, as indicated, to satisfy assumptions of ANOVA. Long dashes (—) indicate model terms that do not apply to a particular analysis. Means are in Fig. 5.

Source of variation (df)	Height, July 1989†	(Height, May 1990)$^{-1}$†	Log_{10} (flowering nodes)†	Log_{10} (dry mass)†
Nutrient (1)	—	**49.0*** **	**57.2*** **	**187.2*** **
Population (2)	**6.3****	**5.4****	0.4	**7.7*** **
P × N (2)	—	0.2	0.6	0.0
Morph (2)	3.7*	1.1	0.8	**4.8****
M × N (2)	—	0.6	2.0	2.3
M × P (4)	2.3	0.4	1.2	0.3
M × P × N (4)	—	0.1	0.7	1.2
Block (3;1)‡	1.1	**13.0*** **	1.2	**8.4****
R^2	0.15	0.14	0.12	0.32

† All factors except Block were fixed effects; thus *F* tests used MS_{Error} (df = 535 for height as of July 1989 and df = 528 for the other variables).

‡ Plants were arranged in four blocks before application of the nutrient treatment (df = 3) and two blocks after application of the nutrient treatment (df = 1).

Differences in pollen receipt cannot fully account for low seed set in the L morph, however, since lower seed number per flower was also observed after addition of excess legitimate pollen. Moreover, similar differences in seed set among morphs were observed after hand pollination in a uniform glasshouse environment (Eckert 1993, Eckert and Barrett 1994*a*). This suggests that low seed set may be a consequence of morphological or physiological characteristics of longer styles rather than strictly ecological factors. Stigma size decreases with style length in *D. verticillatus* (Eckert and Barrett 1994*a*) and it is possible that the size of the stigmatic surface or diameter of the style may restrict the number of pollen tubes reaching the ovary in the L morph. Detailed studies of pollen tube growth and zygote maturation are needed to evaluate the mechanisms responsible for the low seed fertility of the L morph.

Local selection on morph frequencies?

Thus far we have considered a deterministic hypothesis in which fitness differences among morphs are constant in all populations and thus produce biases in

average morph frequencies. However, it is possible that if morph-specific selection occurs in *D. verticillatus,* it would be highly localized and, hence, variable among populations. In this regard, our results revealed several instances in which a particular component of fitness varied among morphs in one population but not in others. For example, seeds from the L morph germinated at a higher rate than those from the M or S morphs in EO-T3, but not in EO-T6 or EO-T7. Variation in morph-specific fitness among populations may occur if local disequilibria between the genes controlling style morph and the genes determining fitness were generated by founder effect and genetic drift (Eckert and Barrett 1992). Also, variation in morph-specific selfing rates may affect variation in progeny viability if inbreeding depression is strong (Eckert and Barrett 1994*b, c*).

Taken together, however, the results of this study provide no clear evidence that local selection can explain the biases in morph frequencies of individual populations. Most of the differences among morphs were not reflected in either population morph frequencies or progeny morph frequencies. Experimental comparisons of offspring viability in four dimorphic populations further support this conclusion (Eckert 1993).

Although the series of experimental and field studies presented here detected significant differences among morphs in some components of fitness, there is little evidence that these differences have shaped morph frequencies at the population level or can explain the marked regional deficiencies of the M morph in Ontario and New England.

The effect of historical contingency

In the absence of major fitness differences among morphs, what is responsible for the marked deficiency of the M morph observed in populations of *D. verticillatus* from New England and Ontario? It is possible that the deficiency of the M morph reflects an historical accident during post-glacial migration from which northeastern populations of this long-lived, highly clonal species have yet to recover. First, results of computer calculations indicated that the return of morph frequencies to equilibrium may be slowed by perenniality, limited sexual recruitment, and clonal reproduction. These factors have been shown to greatly re-

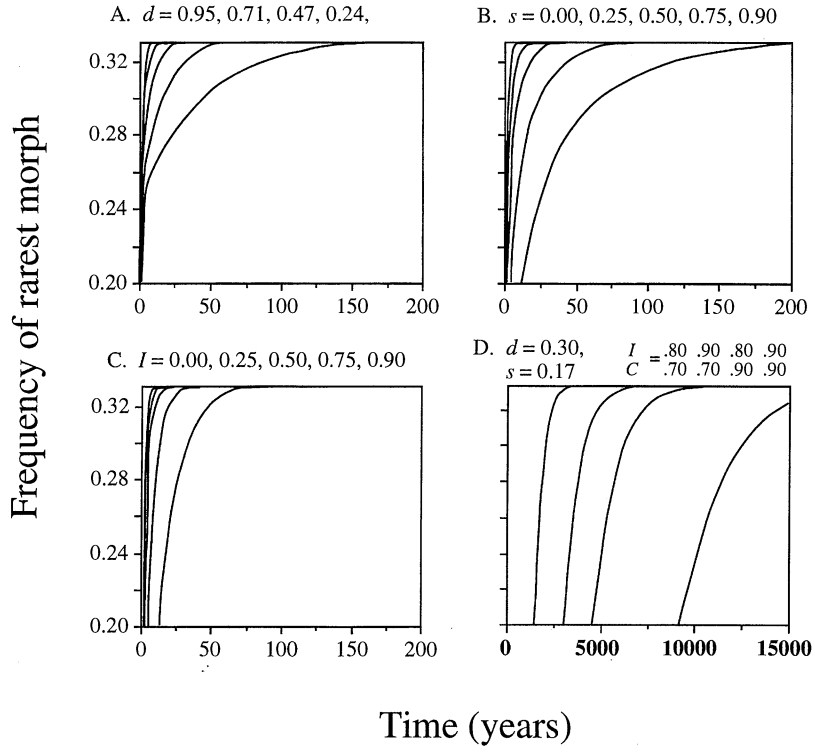

FIG. 6. The effect of life history and mating parameters on the return of morph frequencies to equilibrium in tristylous populations. The top of each panel represents even morph frequencies. The two other morphs (not shown) are always equally common. In three of the four panels, a single parameter has been varied: (A) the rate of disassortative mating (*d*); (B) the rate of self-fertilization (*s*); and (C) the rate of year-to-year survival (*I*). Varying the rate of clonal recruitment (*C*) has the same effect as varying *I*. In the fourth panel (D), mating parameters (*d* and *s*) are held constant while *I* and *C* are varied jointly. Note that the abscissa on this panel is scaled by two orders of magnitude compared to the other three panels. Parameter values as listed correspond to the curves in each panel from left to right.

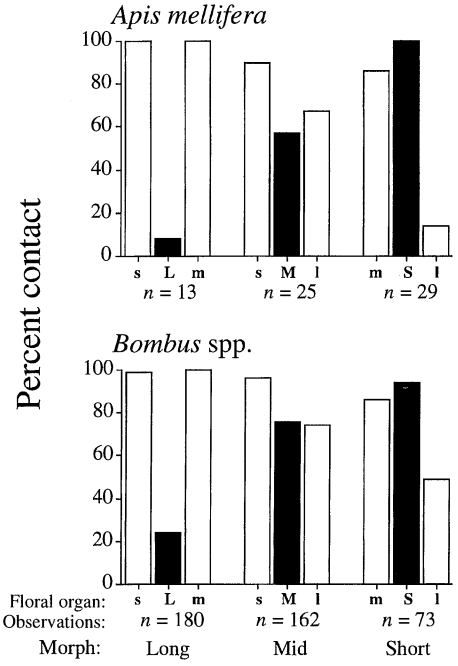

FIG. 7. Differences among floral organs in frequency of physical contact with flower visitors in tristylous *Decodon verticillatus*. Data are shown for the two principle flower visitors: bumble bees (*Bombus* spp.) and honey bees (*Apis mellifera*). Solid bars are contacts with stigmas (for L, M, and S styles); open bars, contacts with anthers (for l, m, and s anther levels). Numbers of observations (*n*) are given below the bars for each morph.

tard the approach of gynodioecious populations toward equilibrium sex ratios (Stevens and Van Damme 1988). In *D. verticillatus,* the combination of reproductive and life history parameters and weak disassortative mating (Eckert 1993, Eckert and Barrett 1993*b,* 1994*b*) may preserve historically skewed morph ratios for very long periods of time. Second, an historical perturbation of morph ratios may be particularly likely to have occurred in *D. verticillatus.* Judging by the distribution of fossils, *Decodon* was distributed widely throughout Europe, Northern Asia, and North America during most of the Tertiary period (Graham and Graham 1971, Dorofeev 1977, Tiffney 1981). Since then, its range has been severely reduced. After the Wisconsin glaciation, *D. verticillatus* returned only to eastern central North America. The oldest fossil seeds deposited after glaciation at seven locations in southern Ontario date from between 2800 and 8000 yr BP (average = 5000 yr BP; McAndrews 1984; J. H. McAndrews, *unpublished data*).

Post-glacial migration has had a large effect on the population structure of many long-lived plant species (Ritchie 1984, Cwynar and MacDonald 1987), and has been suggested as an important factor shaping contemporary patterns of genetic variation in several herbaceous taxa from eastern North America (e.g., Loveless and Hamrick 1988), especially clonal aquatic species (e.g., Les 1986, 1991, Philbrick and Crow 1995). Random historical events also appear to have contributed to the marked heterogeneity in morph frequencies observed in the highly clonal waterweed *Eichhornia crassipes* (Barrett and Forno 1982) and in sex ratios of several dioecious clonal aquatic plants (Sculthorpe 1967, Hutchinson 1975, Barrett et al. 1993). A comparison of morph-frequency variation between northern and southern populations of *D. verticillatus* revealed a reduction in morph diversity in northern populations consistent with the effects of historical factors (Eckert and Barrett 1992). If by chance the M morph were underrepresented among individuals recolonizing deglaciated habitats, then the M deficiency could have become more pronounced through "stepping-stone" colonization (Kimura and Weiss 1964), leading to low frequencies and loss of the M morph from northern populations in Ontario and New England.

The hypothesis that the deficiency of the M morph is due to post-glacial sampling error may be difficult to test directly. Support for the hypothesis would be provided if further population surveys were to show a latitudinal cline in the frequency of the M morph along the eastern coastal plain. If, however, the glacial history of the coastal plain were heterogeneous (Pielou 1991) this pattern might not occur. Additional support might be furnished by comparing morph-frequency variation in the eastern and western parts of the species' range. The distribution of fossil pollen for several aquatic angiosperms suggests that the post-glacial return of aquatic plants to eastern and central North America after the Wisconsin glaciation appears to have occurred via two distinct routes separated by the Appalachian mountains (Vesper and Stuckey 1976). Consequently, regional variation in the genetic structure of aquatic plants, such as *D. verticillatus,* in eastern North America may have arisen from the occurrence of two separate founder events. In this regard, it is worth noting that a sample of 30 populations of *D. verticillatus* in Michigan does not show a similar deficiency of the M morph (Eckert and Barrett 1992), perhaps because these populations were derived from a separate sequence of post-glacial colonization not involving a low frequency of the M morph. Comparisons of large-scale patterns of morph-frequency variation, as well as genetic differentiation in morphological features or molecular markers, may help to substantiate this historical scenario. Interpretations of microevolutionary patterns in clonal plants occurring in glaciated regions must recognize both ecological and evolutionary time scales in evaluating the relative importance of selective and stochastic processes.

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