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Tristyly, self-compatibility and floral variation in *Decodon verticillatus* (Lythraceae)

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Heterostyly typically involves reciprocal polymorphism in stamen and style lengths, physiological self- and intramorph-incompatibility, and a set of associated polymorphisms of pollen and stigma characters. This study examined floral morphology and compatibility relationships in the monotypic, herbaceous perennial *Decodon verticillatus* (Lythraceae). There have been conflicting reports on the occurrence of tristyly in the species, probably because of frequent loss of style morphs from populations in parts of the species' range. Floral morphology was examined using material collected from natural populations throughout the range. Detailed floral measurements revealed discrete trimorphism in style length and anther positioning in three populations. Data from two dimorphic populations showed similar patterns of floral polymorphism, except that both were missing the mid-styled morph. In one dimorphic population, there was evidence for modification in the length of mid-level stamens. Measurements in three populations indicated pronounced floral variability, including high frequencies of modified phenotypes with reduced stigma–anther separation. Pollen size was only weakly differentiated among anther levels, and there were no differences in pollen production among anther levels or morphs. In contrast, stigma size and papilla length showed a strong negative correlation with style length; a pattern opposite to most heterostylous species. Experimental crosses performed under glasshouse conditions on plants from two populations showed a high degree of both self- and intramorph-compatibility. A comparative analysis of floral morphology showed that *D. verticillatus* is not unusual in terms of the precision and reciprocity of organ positioning compared with 13 other tristylous species.

ADDITIONAL KEY WORDS:—floral morphology – heterostyly – polymorphism – sexual system.

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INTRODUCTION

Heterostylous breeding systems have been regarded as a remarkable example of convergent evolution. This view stems from the observation that, although heterostyly has evolved independently in at least 25 plant families (Darwin, 1877; Vuilleumier, 1967; Ganders, 1979; Barrett, 1992; Lloyd & Webb, 1992a), it almost always involves a suite of correlated polymorphisms featuring the reciprocal arrangement of anthers and stigmas among morphs and an associated physiological self- and intramorph-incompatibility system. In addition, heterostylous morphs usually differ in several 'ancillary' polymorphisms involving pollen size, pollen production, the size of the stigmatic surface and the length of the stigmatic papillae (Dulberger, 1992). Some workers have argued that the recurrent associations between these component traits indicates that they function in an integrated fashion and may have evolved together (Mather & de Winton, 1941; Dulberger, 1975; but see Yeo, 1975; Ganders, 1979), presumably to promote efficient cross-fertilization (Darwin, 1877; Lloyd & Webb, 1992b).

Despite the widely observed character correlations found in most heterostylous taxa, certain components of the syndrome, particularly the heteromorphic incompatibility system and ancillary polymorphisms, are weakly developed or absent in some species (e.g. *Amsinckia*: Ray & Chisaki, 1957; *Eichhornia*: Barrett, 1988; *Oxalis*: Ornduff, 1972). Self-compatible heterostylous species are of interest for at least three reasons. First, a high degree of self-compatibility is often associated with weak expression of ancillary polymorphisms, suggesting a role for these traits in the functioning of heteromorphic incompatibility (Dulberger, 1975, 1992). Second, the absence of incompatibility may greatly affect the maintenance of heterostyly in natural populations (Charlesworth, 1979; Barrett, Morgan & Husband, 1989; Eckert & Barrett, 1992). Finally, the occurrence of floral heteromorphism without incompatibility is of significance to theoretical models concerned with evolution of heterostyly, particularly with regard to the sequence of character evolution in the assembly of the heterostylous syndrome (D. Charlesworth & B. Charlesworth, 1979; Lloyd & Webb, 1992a, b; Barrett & Cruzan, 1994).

In this study, we examine the morphology and compatibility relationships of floral morphs in *Decodon verticillatus* (L.) Ell. (Lythraceae), a monotypic, herbaceous, perennial which occurs in wetland habitats throughout eastern-central North America. There have been conflicting reports as to the nature of the heterostylous syndrome in *D. verticillatus*. Darwin (1877) grew several plants of *Nesaea verticillata* (*D. verticillatus*) from seed and noted clear floral trimorphism. Koehne (1903) also described three floral morphs along with two intermediate phenotypes. East (1940) and Vuilleumier (1967), however, reported *D. verticillatus* as distylous, and Sculthorpe (1967) suggested that the species was largely self-fertilizing. More recent reviews of heterostyly (Ganders, 1979;

Barrett, 1993) and taxonomic treatments of the Lythraceae (Graham, 1964, 1975; Graham *et al.*, 1975) have recognized *D. verticillatus* as tristylous, although the sexual system of natural populations has not been studied in detail.

Some of the earlier confusion over the nature of the sexual system of *D. verticillatus* probably arose because of the wide range of population morph structures found in the species. A survey of morph frequencies in 163 populations from throughout the range revealed that while three floral morphs occur in many populations, more than half were lacking at least one style morph (Eckert & Barrett, 1992; Eckert, 1993). The sexual system could therefore be misinterpreted as dimorphic or monomorphic if only a small number of populations were investigated. Therefore, the first objective of this study was to quantify stamen and style variation in natural populations throughout the species' range to establish the true nature of the sexual system of *D. verticillatus*.

In addition to a high frequency of morph loss, we have observed substantial seed production in monomorphic populations of *D. verticillatus*, suggesting that floral heteromorphism in this species may be accompanied by high levels of self-fertility. The second objective of this study was therefore to examine the compatibility relationships among floral morphs in *D. verticillatus* using experimental hand-pollinations. The relationship between the strength of incompatibility and expression of ancillary polymorphisms was also investigated.

Much of the interest in heterostyly has focused on evolutionary changes in the sexual system (Baker, 1966; B. Charlesworth & D. Charlesworth, 1979; Barrett, 1989; Weller, 1992). In tristylous groups, modifications usually involve a shift to either distyly (Mulcahy, 1964; Lewis & Rao, 1971; Ornduff, 1972; Weller & Denton, 1976; Ornduff, 1979) or increased inbreeding through the evolution of semi-homostyly (Ornduff, 1972; Lewis, 1975; Barrett, 1988). In this regard, *D. verticillatus* appears to possess considerable scope for evolutionary modifications to its tristylous sexual system. Many populations lack floral morphs, providing opportunities for alterations to the floral morphology of remaining morphs, as has been documented in closely related *Pemphis* (Lewis & Rao, 1971). Second, self-compatibility and monomorphism may favour the spread of genes reducing stigma–anther separation; thereby resulting in increased self-fertilization (Barrett, Kohn & Cruzan, 1992). The third part of this study involved documenting the patterns of variability in style and stamen position to determine whether there was any evidence for floral modifications of significance to mating-system evolution in *D. verticillatus*.

MATERIAL AND METHODS

Floral morphology: style-stamen polymorphism

To quantify morphological aspects of the style-stamen trimorphism in *D. verticillatus*, flowers were randomly sampled from about 20 plants per morph in each of three widely separated trimorphic populations (EO-T3, FL-T7 and MI-T3; the first part of the population code indicates location [EO, FL, MI = eastern Ontario, Florida, Michigan] the second part indicates morph structure [T, D = trimorphic, dimorphic]). Floral polymorphism in populations lacking a morph was also investigated in two dimorphic populations from eastern Ontario (EO-D3 and EO-D1). Occasional populations of *D. verticillatus*

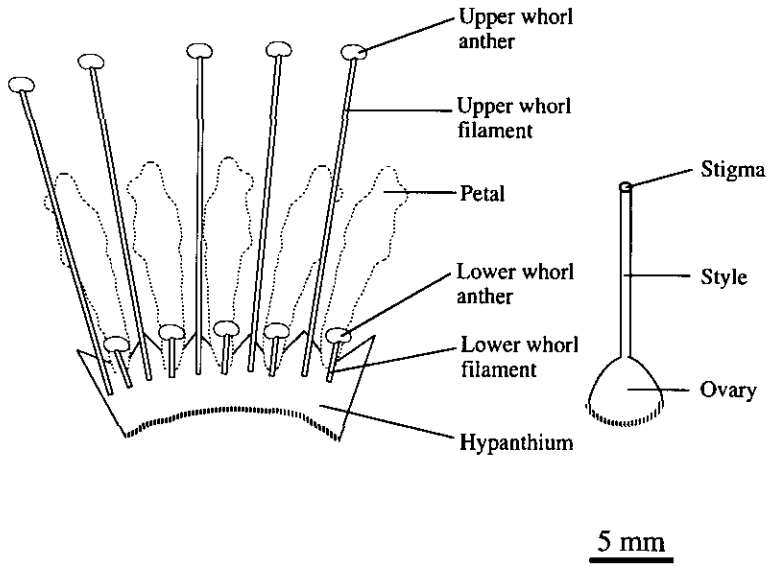

Figure 1. Schematic diagram of a *Decodon verticillatus* flower dissected to display the floral organs. The hypanthium has been removed from the base of the ovary and laid flat. The hatched lines on the hypanthium and ovary base show where they were joined. All organ lengths were measured from this line. The petals are shown flattened beside, and parallel with, the stamens. In reality, they project out from the hypanthium in an irregular radial pattern. The flower in the diagram is mid-styled.

exhibit pronounced floral variability. Patterns of variability were quantified in a highly variable population (MI-T7). Finally, style morph surveys in 163 populations (Eckert & Barrett, 1992; Eckert, 1993) uncovered two populations (MI-T8 and ME-D3) with high frequencies of modified floral phenotypes with greatly reduced stigma–anther separation. Patterns of floral variation were therefore examined in both populations.

In each population sampled, plants were classified by floral morph after inspecting the morphology of several flowers, and one flower from each plant was preserved in 70% ethanol. Each flower was later dissected as shown in Figure 1. Flowers of *D. verticillatus* are actinomorphic, five-sided, with two whorls of stamens (usually five per whorl) and a superior ovary. Where the ovary is attached to the perianth, sepals, petals and stamens are fused basally to form a hypanthium (or 'calyx tube', Richards & Barrett, 1992). Petals and stamens arise from the hypanthium 2 to 3 mm from the base of the ovary. Stamens of the lower whorl are attached just beneath the petals and alternate with stamens of the upper whorl, attached on the sepals. Floral measurements were made at 10 × magnification using a dissecting microscope equipped with a drawing tube mounted over a digitizing tablet. Eight measurements were made on each flower. First, the length and width of the hypanthium was traced. Then the hypanthium was removed from the base of the overy and spread flat. The number of stamens per whorl was recorded and the occurrence and position of extra stamens noted. Hypanthium circumference was measured by tracing a line through the origin of each stamen. The length of the longest and shortest stamen in each whorl was measured from the origin of the filament to the point of

attachment with the anther. Finally, style length was measured from the base of the ovary to the tip of the stigma.

Floral morphology: ancillary polymorphisms

Pollen size

Differences in pollen size among anther levels were examined in samples of two anthers per whorl for each of 10 to 20 flowers per morph in each of four trimorphic (EO-T11, EO-T7, EO-T3 and GA-T3) and two dimorphic (EO-D3 and EO-T1) populations. Anthers were kept dry in glassine envelopes stored in an air-tight box filled with desiccant. The size (in μm) of 100 dry pollen grains per anther type was measured under 1000 × magnification. For EO-T3, both the longitudinal and equatorial axes of each grain were measured. In other populations, only the longitudinal axis was measured.

Pollen and ovule production

The number of pollen grains in each type of anther whorl was estimated following Lloyd (1965). Mature buds were collected from plants in two populations (EO-T11 and EO-T3) and preserved in 70% ethanol. For each population, anthers were dissected from each whorl of stamens in two samples of five buds per morph. The number of anthers per sample was recorded and each sample was ground in 0.4 mL of a 3 : 1 mixture of glycerine and lactic acid. Each sample received one drop of cotton blue in lactophenol and was resuspended by vortexing for 30 sec before pollen counts were made. A small volume was immediately removed and pollen grains were counted in a 0.0001 mL counting chamber under 400 × magnification. Six replicate counts were made for each sample. Although the number of anthers in any given whorl varied between four and six, there were no significant differences among populations, morphs or anther levels. Each replicate count was therefore standardized to the number of grains in a whorl of five anthers. The number of ovules per flower was estimated by dissecting the ovaries of 20 flowers per morph, collected from the same two populations. Each ovary was cut in half longitudinally and ovules were counted in one half under a dissecting microscope. Ovule number per flower was then calculated by doubling these counts.

Stigma size and papilla length

The style from one recently opened flower per plant was collected and preserved in 70% ethanol for each of about 20 plants per morph in two populations (FL-T7 and EO-T3). The width and height of the stigma on each style was measured to 0.1 mm at 100 × magnification. For five styles per morph, the stigma was removed, stained for 2 sec in 0.05% basic fuschin, and gently pressed under a cover slip. Five stigmatic papillae per stigma were measured to 0.025 mm under 400 × magnification.

Compatibility relationships

Compatibility relationships among the floral morphs were examined by performing hand-pollinations on plants grown from open-pollinated seed collected in two eastern Ontario populations. One population included all three morphs (EO-T6), the other (EO-D1) lacked the M morph (hereafter the long-,

mid- and short-styled morphs will be called the L, M and S morphs, respectively). Maternal plants were grown in a 3:1 mixture of Pro-Mix® (soilless mix) and sandy loam and fertilized twice a month with a weak solution of 20:20:20 (N:P:K) fertilizer; about 200 mg dry weight of fertilizer per plant. High-pressure sodium lamps were used as required to maintain at least 13 h of light per day and temperatures ranged between 20–30°C. Flowers were emasculated and pollinated with fine forceps within 5 h of the beginning of anthesis.

Each plant received several pollination treatments in a random order. For both populations, treatments included: (1) self pollen, (2) illegitimate outcross pollen from a donor of a different morph (between-morph), or (3) legitimate outcross pollen. Plants from the trimorphic population also received illegitimate outcross pollen from the same morph (within-morph). In species with heteromorphic incompatibility, only legitimate pollinations result in full seed set. The dimorphic population received two additional treatments: (1) between-morph illegitimate pollen of a sib from the same open-pollinated family, and (2) legitimate pollen from the same sib donor. These additional treatments were used to assess reductions in seed set due to inbreeding depression.

On average, 10 flowers were pollinated per treatment per recipient plant during April and May, 1990. Ripe fruits were collected six to eight weeks after pollination and seeds were counted for an average of eight fruits per treatment per recipient. Seeds from each fruit were weighed as a group to 0.1 mg and average seed weight was calculated.

The effect of pollination treatment on probability of fruit set was assessed with log-linear categorical models using the maximum likelihood routine in JMP (version 2.0, SAS Institute, 1989). Morph of recipient and pollination treatment were entered as main effects. The importance of each term was initially assessed using the Wald X^2 statistic. Effects significant at the 10% level were further tested using the more exact likelihood ratio test. Non-significant terms were continually dropped from the model. The likelihood ratio (LR) 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 X^2 with one degree of freedom.

Seed number per fruit and average seed weight were entered in a factorial ANOVA. For the trimorphic population, replication of pollination treatments on individual recipients was unbalanced. Data were therefore pooled across recipients and analysed with morph of recipient and pollination treatment as main effects. For the dimorphic population, replication was relatively balanced so that recipient nested within morph could be added to the ANOVA model.

RESULTS

Floral morphology: style-stamen polymorphism

Trimorphic populations

The three trimorphic populations of *D. verticillatus* each showed a clear style-stamen trimorphism with non-overlapping distributions of style length and a

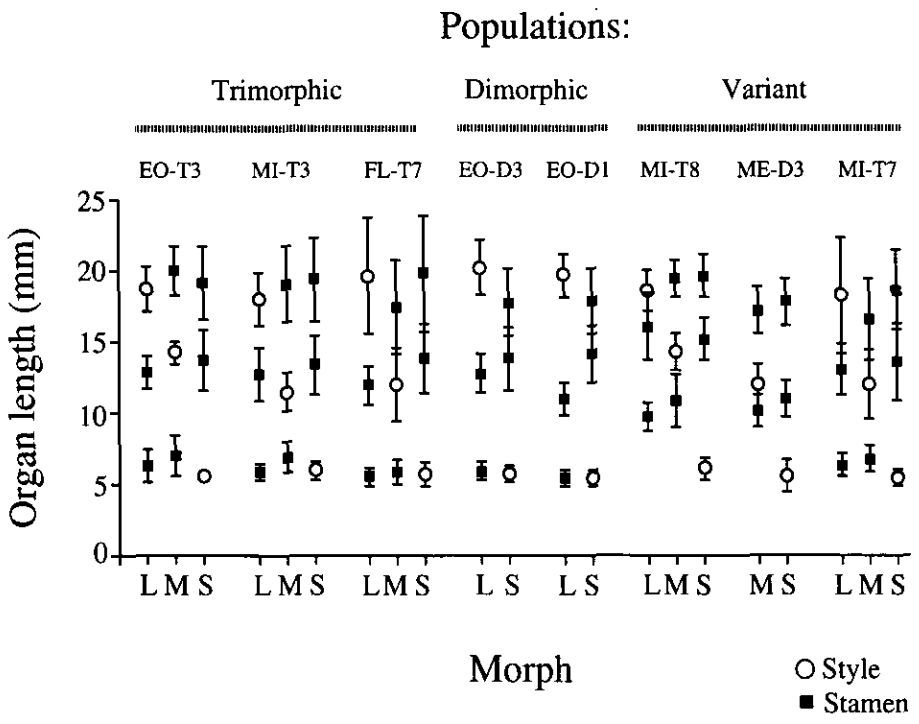

Figure 2. Mean organ lengths by style morph in eight populations of *Decodon verticillatus*. Styles are open circles; stamens, solid squares. Points are mean organ length. Bars are 1 *SD*. Two *SD* around each mean includes almost all of the flowers in any given sample. Populations are identified by a code shown above data points for each, with the first part of the code denoting location (EO, FL, ME and MI = eastern Ontario, Florida, Maine and Michigan, respectively) and the second part indicating the number of morphs present (T and D = trimorphic and dimorphic, respectively).

reciprocal polymorphism in stamen length indicating reciprocity of anther whorls among morphs (Figs 2 and 3). Differences in stigma height among morphs were due to differences in style length only. Ovary height did not differ among morphs, except in MI-T3, where ovaries of the L morph were significantly smaller (2.71 ± 0.05 *SE* mm) than those of the M (3.19 ± 0.05) or S (3.15 ± 0.10) morphs ($F_{2,57} = 14.3$, $P < 0.001$). Similarly, differences in stamen height among whorls within morphs was due to differences in filament length, not height of filament insertion on the hypanthium.

In all three trimorphic populations examined, there was considerable variation in the length of both styles and stamens among individuals of a given morph. This was particularly striking in L and M flowers from FL-T7. The average coefficient of variation for organ length was 12% in EO-T3, 13% in MI-T3 and 17% in FL-T7 (Fig. 2). In addition to variation among individuals, there was significant variation in stamen length within the anther whorls of individual flowers (Fig. 3). The mean proportional range of stamen lengths within whorls (i.e. difference in length between longest and shortest stamen in a whorl divided by the average stamen length in that whorl) was 10% in EO-T3, 12% in MI-T3 and 11% in FL-T7. There were a few flowers (5% overall) in each sample in which the style and at least one of the stamens were almost equal

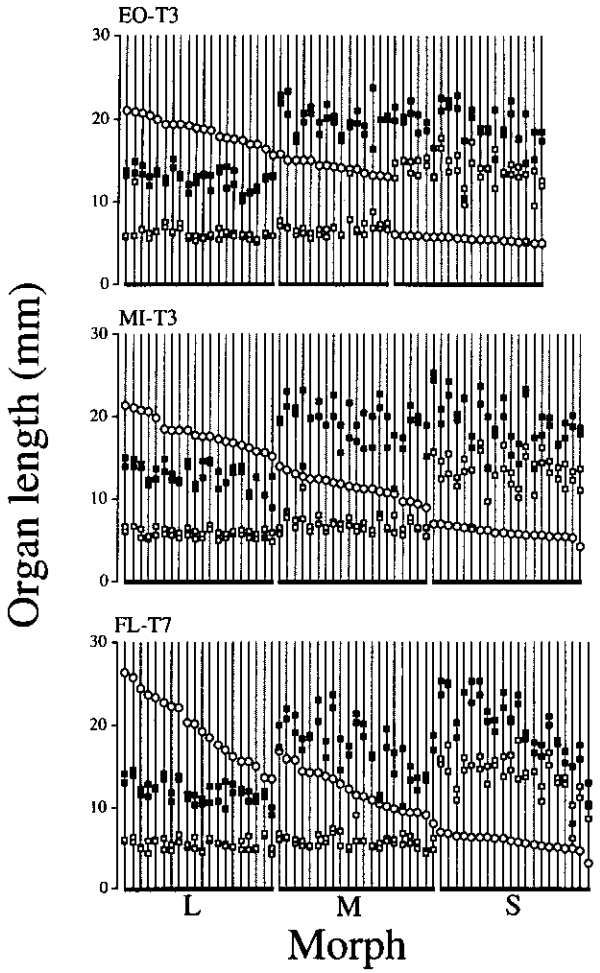

Figure 3. Patterns of variation in style and stamen length in three trimorphic populations of *Decodon verticillatus*. Stigmas are open circles; the longest and shortest stamen of the lower whorl of anther are represented by solid squares, the longest and shortest of the upper whorl by open squares. For each population, flowers classified as the same morph (usually 20 per morph) are joined by a thick line at the bottom of each panel, and have been ranked by style length. Note that organ lengths have been collapsed along one linear axis. In reality, stamens project away from the centrally-positioned style at about a 45° angle. Means by morph and population are in Fig. 2.

in length. This condition occurred more often in the M morph (11%) than the L (0%) or S morphs (5%).

Floral measurements from MI-T7 revealed the most pronounced floral variability of the populations surveyed, with considerable overlap in style length between flowers of the L and M morphs (Figs 2 and 4). Ten of the 38 L and M morph flowers sampled were difficult to classify by morph on the basis of these measurements alone. An additional four of 20 M flowers had style lengths equivalent to the length of at least one short-level stamen. Overlap in organ position within and among morphs was associated with more overall variability compared to the other trimorphic populations. Coefficients of variation for organ lengths averaged 16% and the proportional range of stamen lengths within whorls averaged 13%.

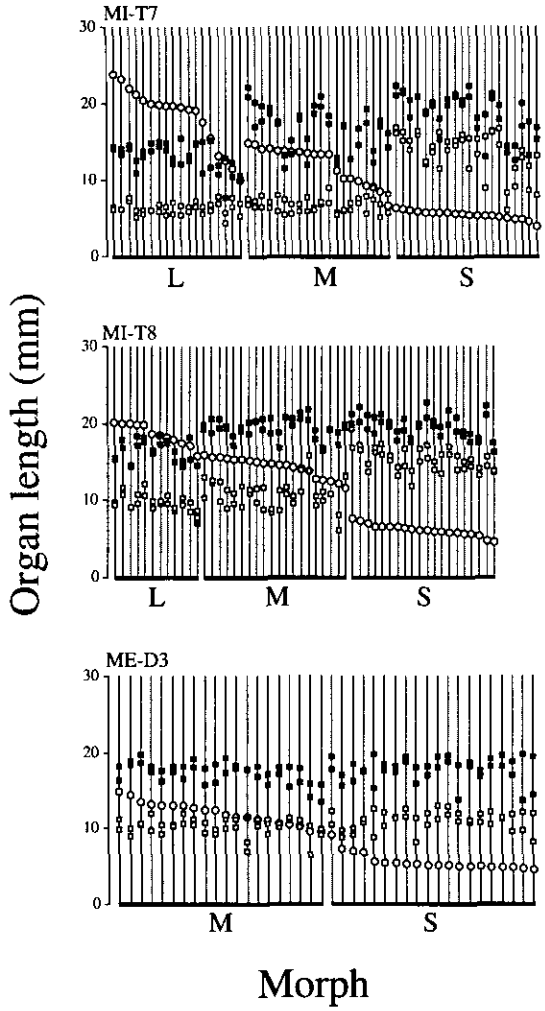

Figure 4. Patterns of variation in style and stamen length in three populations of *Decodon verticillatus* which show unusual patterns of floral variation. See Fig. 3 for details. Means by morph and population are in Fig. 2.

Dimorphic populations

Floral measurements in two dimorphic populations revealed patterns of style-stamen polymorphism similar to those observed in the four trimorphic populations, except that the M morph was missing. There was no evidence for major rearrangements of organ positions in the remaining morphs. In both populations, however, mid-level stamens of the S morph were significantly longer than mid-level stamens of the L morph (Figs 2 and 5; EO-D3: $t = 2.6$, $df = 78$, $P = 0.011$; EO-D1: $t = 8.7$, $df = 78$, $P < 0.001$). However, the same difference was evident in the three trimorphic populations examined (Fig. 2). Differences in length between the mid-level stamens of the S morph and those of the L morph ranged from 1.90 ± 0.43 mm ($\pm SE$ of the difference) in FL-T7, through 0.85 ± 0.38 in EO-T3, to 0.68 ± 0.45 in MI-T3. In EO-D1, however, the

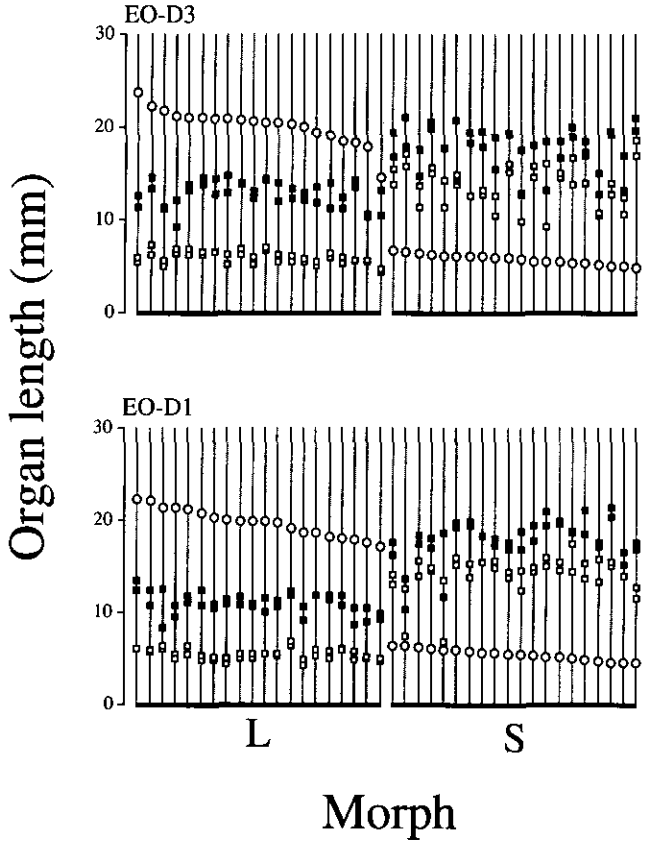

Figure 5. Patterns of variation in style and stamen length in two dimorphic populations of *Decodon verticillatus*. See Fig. 3 for details. Means are in Fig. 2.

difference in mid-level stamen length (3.17 ± 0.36) was much greater than in trimorphic populations (EO-D3: 1.07 ± 0.41).

Modified floral phenotypes

Reduced stigma–anther separation in a trimorphic population from northern Michigan (MI-T8) involved convergence between the length of styles and mid-level stamens in the L morph, and styles and short-level stamens in the M morph (Figs 2 and 4). Although there was considerable variation among individuals in this regard, both the L and M morphs exhibited greatly reduced average stigma–anther separation. Minimum difference in length between mid-level stamens and styles in the L morph averaged 2.3 ± 0.4 mm, 39% of that in the other trimorphic populations (Fig. 2; MI-T3: 4.8 ± 0.4; FL-T7: 7.2 ± 0.8; EO-T3: 5.5 ± 0.3). Similarly, the difference in length between short-level stamens and styles in the M morph averaged 3.0 ± 0.3, 54% of that in other trimorphic populations (MI-T3: 4.1 ± 0.3; FL-T7: 5.8 ± 0.6; EO-T3: 6.8 ± 0.6). Short-level stamens in the L morph were also slightly elongated. In both morphs, reductions in stigma–anther separation involved modification of stamen length rather than style length and modifications involved all anthers in a whorl.

Flowers of the S morph appeared similar to those measured in other trimorphic populations, except for slight elongation of mid-level stamens (Fig. 2).

Floral modifications were expressed to an even greater degree in a dimorphic population from Maine that included the M and S morphs (ME-D3). Reduced stigma–anther separation involved an increase in the length of short-level stamens in all individuals of the M morph (Figs 2 and 4). Again, this involved all stamens in the short-level whorl. There was some variation in stigma–anther separation associated with variation in style length; however, the average difference in length between short-level stamens and styles in the M morph was 1.6 ± 0.3 mm, 28% of that in trimorphic populations. Short-level stamens of the M morph were only slightly shorter, on average, than mid-level stamens of the S morph (Fig. 2).

Floral morphology: ancillary polymorphisms

Pollen size

In the floral morphs of most heterostylous species, pollen grains from upper anther levels are usually larger than those from lower levels. Data on pollen grain length in three trimorphic and two dimorphic populations of *D. verticillatus* showed the same trend; however, there was much overlap in pollen size among all anther levels in each population (Fig. 6). The degree of pollen size differentiation also varied among populations, as indicated by the significant population by anther type interaction (Table 1). A detailed analysis of pollen grain size in EO-T3 gave similar results (Fig. 7). Grain length, width and volume differed significantly among anther levels (Length: $F_{5,594} = 51.0$, $P < 0.001$; Width: $F_{5,594} = 33.0$, $P < 0.001$; Volume: $F_{5,594} = 46.1$, $P < 0.001$), with a significant contrast between short-level anthers and the other anther levels. Again, there was much overlap in pollen size among levels.

Pollen and ovule production

Pollen heteromorphism is typically associated with differences in pollen production among anther levels. Upper anthers in a floral morph generally

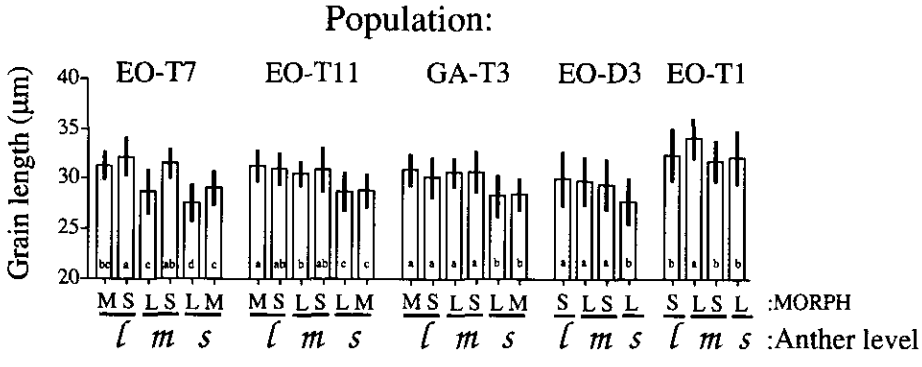

Figure 6. Differences in pollen grain length (μm) among anther levels in five populations of *Decodon verticillatus*. Each bar is the mean of 100 measurements. Error bars are 1 *SE*. Pair-wise comparisons performed for each population separately are shown as letters at the base of each bar. Bars not sharing a letter are significantly different. Analysis of these data is in Table 1. Populations are identified by a code above the bars (see Fig. 2).

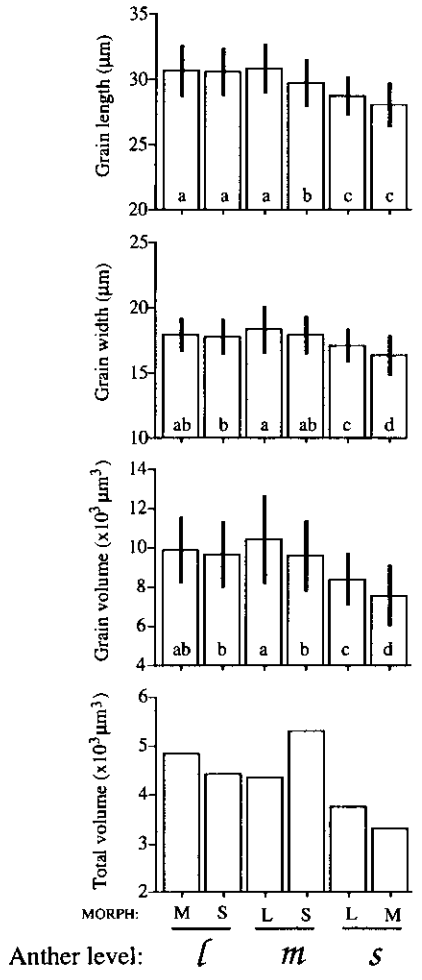

Figure 7. Differences in pollen length, width, volume and total pollen volume among anther levels in a trimorphic population (EO-T3) of *Decodon verticillatus*. Grain volume = (grain length)(grain width)^2. Each bar in the top three panels is the mean of 100 measurements. Error bars are 1 *SE*. Total volume = (grain volume)(pollen production per flower) (see Fig. 11). Multiple comparisons are shown at the base of each bar; those not sharing a letter are significantly different.

produce a small number of large pollen grains compared to lower levels which produce a larger number of smaller pollen grains. Weak pollen size polymorphism observed in *D. verticillatus* was not, however, associated with differences in pollen production among anther levels (Fig. 8A). There were significant differences in pollen production between populations but not among anther levels (Table 2). The lack of a size-number trade-off in pollen production was also reflected by significant differences in anther sac size among anther levels (Fig. 8B, Table 2). Multiple comparisons showed that the effect of anther type was due to reduced size of short-level anthers compared to long- and mid-level anthers.

There were no significant differences in ovule number between populations or morphs (EO-T3: L = 84.3±4.4 ovules/flower, M = 89.7±4.0, S = 83.1±4.3; EO-T7: L = 87.3±3.7, M = 92.1±3.0, S = 91.8±4.0; $F_{5,107} = 1.0$, $P = 0.400$).

TABLE 1. Analysis of pollen grain length (μm) by anther type in three trimorphic and two dimorphic populations of *Decodon verticillatus*. Significant effects are indicated with asterisks. Main effects are abbreviated by their first letter. Sample variances were highly heteroscedastic and could not be transformed to meet the assumptions of ANOVA. Consequently, ranked data were entered in a mixed model with Population as a random effect. The results of this analysis agree closely with those obtained using raw data. Means are in Fig. 6

Populations	Source of variation	*df*	*SS*	*F*[1]	*P*
Trimorphic $R^2 = 0.39$	Model	17	1978×10^5	68.7	<0.001
	Population*	2	29×10^5	8.7	<0.001
	Anther*	5	1631×10^5	10.1	0.001
	A × P*	10	321×10^5	19.0	<0.001
	Error	1803	3053×10^5		
Dimorphic $R^2 = 0.38$	Model	7	163×10^5	70.2	<0.001
	Population*	1	124×10^5	374.7	<0.001
	Anther	3	27×10^5	2.3	0.256
	A × P*	3	12×10^5	11.8	<0.001
	Error	792	263×10^5		

[1] *F*-tests for Anther used $MS_{A \times P}$ as the denominator; those for Population and A × P used MS_{Error}.

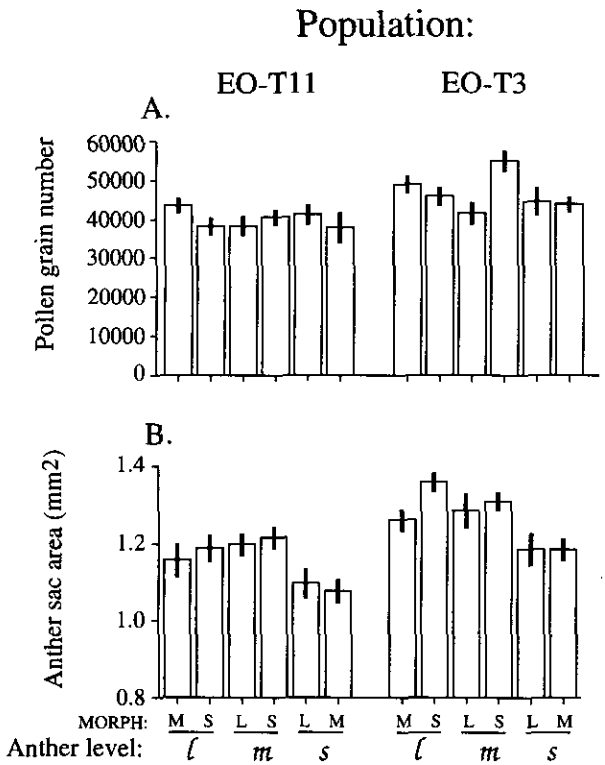

Figure 8. Comparison of pollen production (A) and anther sac size (B) among anther types in two trimorphic populations of *Decodon verticillatus*. For pollen production, bars are the means of pollen counts standardized to a single whorl with five anthers. For anther sac size, bars are the means of 20 anthers per anther type. Error bars are 1 *SE*. Tukey–Kramer multiple comparison of mean anther sac area indicated that *s*M = *s*L < *m*S = *m*L = *l*S = *l*M. Analysis of these data is in Table 2.

TABLE 2. Analysis of pollen production and anther sac area (mm^2) by anther type in two trimorphic populations of *Decodon verticillatus*. Adjusted pollen production per whorl was entered in a partially-nested, mixed model ANOVA with Population, and Sample[Anther × Population] as random effects. In the analysis of anther sac area, population is a random effect and anther is fixed. Significant effects are emphasized with asterisks. Means are in Fig. 8

Source of variation	*df*	*SS*	*F*	*P*
Pollen production[1] ($R^2 = 0.50$)				
Model	23	499×10^7	5.3	<0.001
Population*	1	158×10^7	15.0	0.011
Anther	5	114×10^7	2.2	0.209
A × P	5	53×10^7	0.7	0.617
Sample[A × P]*	12	174×10^7	3.5	<0.001
Error	120	490×10^7		
Anther sac area[2] ($R^2 = 0.34$)				
Model	11	1.51	10.5	<0.001
Population*	1	0.72	55.0	<0.001
Anther*	5	0.74	15.2	0.005
A × P	5	0.05	0.7	0.590
Error	228	2.98		

[1] The *F*-tests for Population and Anther used $MS_{A \times P}$ as the denominator; those for A × P used $MS_{Sample(A \times P)}$; those for Sample[A × P] used MS_{Error}.

[2] The *F*-test for Anther used $MS_{A \times P}$ as the denominator; those for Population and A × P used MS_{Error}.

TABLE 3. Analysis of the stigma characteristics of morphs in two trimorphic populations of *Decodon verticillatus*. Characteristics include papilla length (μm), stigma width (mm) and stigma height (mm). Population and Stigma[M × P] are random effects. Significant effects are emphasized with asterisks. Some response variables have been transformed as indicated to satisfy assumptions of ANOVA. Means are in Fig. 9

Response variable	Source of variation	*df*	*SS*	*F*[1]	*P*
Log_{10} (papilla length)	Model	29	2.33	42.9	<0.001
$R^2 = 0.91$	Population*	1	1.75	3728.2	<0.001
	Morph*	2	0.38	402.4	0.002
	M × P	2	0.00	0.1	0.944
	Stigma[M × P]	24	0.20	4.4	<0.001
	Error	120	0.22		
(Stigma width)[2]	Model	5	0.150	7.2	<0.001
$R^2 = 0.25$	Population*	1	0.058	13.9	<0.001
	Morph*	2	0.081	27.4	0.035
	M × P	2	0.003	0.3	0.705
	Error	108	0.449		
Stigma height	Model	5	0.181	13.8	<0.001
$R^2 = 0.39$	Population*	1	0.059	22.7	<0.001
	Morph*	2	0.112	47.0	0.021
	M × P	2	0.002	0.4	0.639
	Error	108	0.283		

[1] In the analysis of papilla length, *F*-tests for Population and Morph used $MS_{M \times P}$ as the denominator; the test for M × P used $MS_{Stigma(M \times P)}$; and the test for Stigma[M × P] used MS_{Error}. In the analysis of stigma width and stigma height, *F*-tests for Morph used $MS_{M \times P}$ as the denominator; those for Population and M × P used MS_{Error}.

On average, flowers of *D. verticillatus* contained 88 ovules and produced 86 610 pollen grains.

Stigma size and papilla length

Stigma size and stigmatic papilla length usually increase with style length in heterostylous species. In contrast, both features decreased with increasing style length in the two populations of *D. verticillatus* that were examined (Fig. 9, Table 3). Tukey–Kramer multiple comparisons (Tukey, 1953; Kramer, 1956) showed strong differentiation among morphs in papilla length (S > M > L) and, to a lesser extent, stigma height and width (S > M = L). However, within populations there was much variation in stigma size and shape among styles of a given morph. This was also apparent in papilla length which showed significant

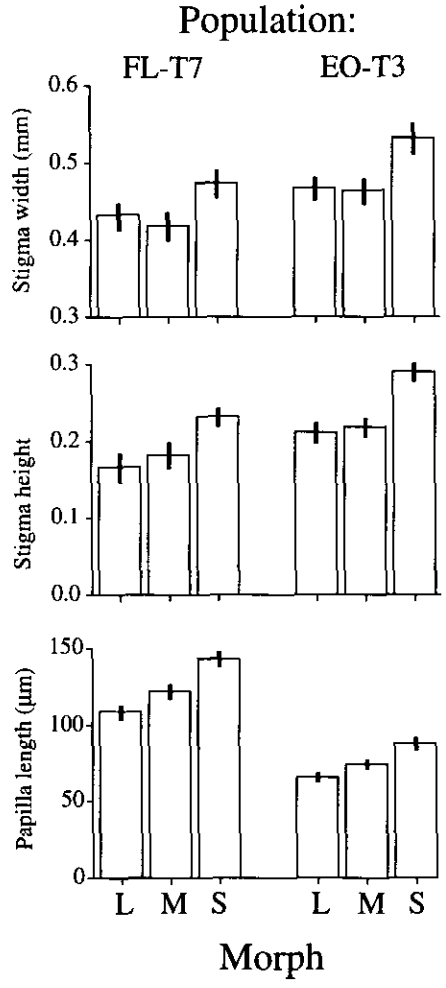

Figure 9. Comparison of stigma size and stigmatic papilla length among floral morphs in two trimorphic populations of *Decodon verticillatus*. Bars are means. Error bars are 1 *SE*. Multiple comparisons showed that S > M = L for both stigma dimensions, and that S > M > L for papilla length. Analysis of these data is in Table 3.

variation among stigmas within a given morph. All three characters varied significantly among populations, with the population from eastern Ontario possessing stigmas of greater width (EO-T3 = 0.49±0.01 mm; FL-T7 = 0.44±0.01) and height (EO-T7 = 0.24±0.01; FL-T7 = 0.19±0.01) but with much shorter papillae (EO-T3 = 75.8±2.0 μm; FL-T7 = 124.7±2.0) than the population from Florida.

Compatibility relationships

Experimental crosses in both populations of *D. verticillatus* provided no evidence for heteromorphic incompatibility or any other form of self-incompatibility. Although some small differences in fruit and seed set following self- and intramorph-pollination were detected, values were similar to those from legitimate pollination (Figs 10 and 11).

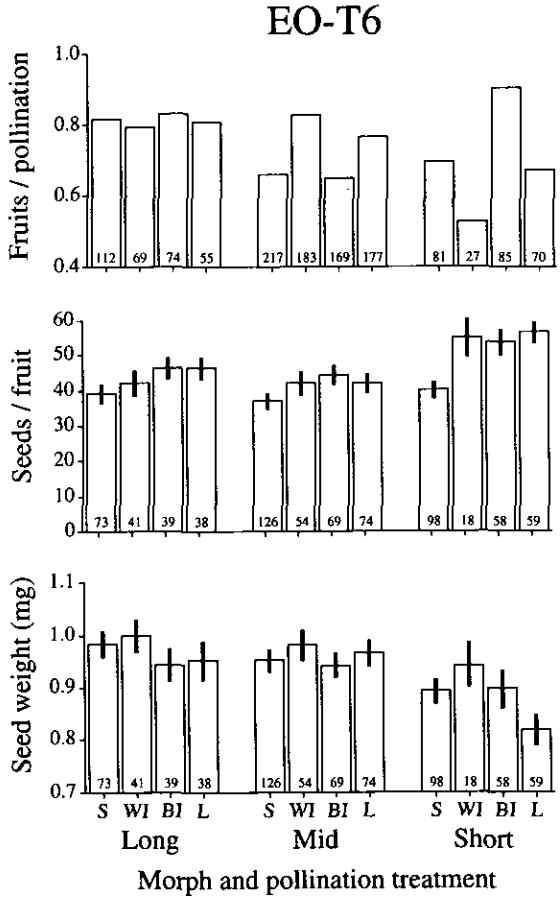

Figure 10. Differences in fruit set, seed set and seed weight among pollination treatments in a trimorphic population of *Decodon verticillatus*. Pollination treatments are: S = self-pollination, WI = within-morph illegitimate pollination; BI = between-morph illegitimate pollination; L = legitimate pollination. Means are for all plants pooled. Sample sizes are shown at the base of each bar. Error bars are 1 *SE*. Analysis of these data is in Tables 4 and 5.

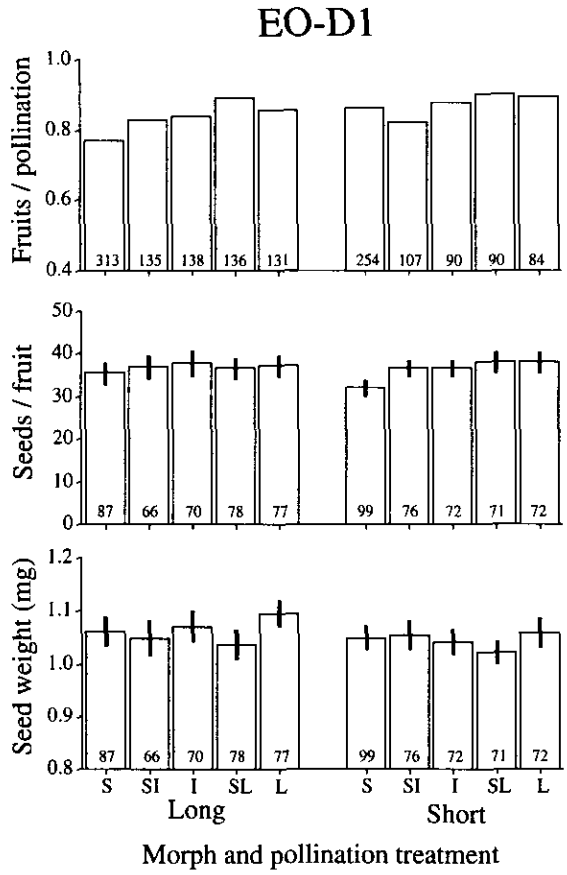

Figure 11. Differences in fruit set, seed set and seed weight among pollination treatments in a dimorphic population of *Decodon verticillatus*. Pollination treatments are: S = self-pollination, SI = illegitimate sib pollination; I = illegitimate outcross pollination; SL = legitimate sib pollination; L = legitimate outcross pollination. Means are for all plants pooled. Sample sizes are shown at the base of each bar. Error bars are ± *SE*. Analysis of these data is in Tables 4 and 5.

Categorical analysis of fruit set revealed significant effects of morph and pollination treatment in both populations (Table 4). In the trimorphic population (average fruit set = 74%), these main effects were complicated by a significant interaction. Examining each morph separately revealed significant heterogeneity in fruit set among treatments for the M (mean = 72%, $X^2 = 28.0$, $df = 3$, $P < 0.001$) and S (mean = 72%, $X^2 = 29.1$, $P < 0.001$) morphs only (L morph: mean = 81%, $X^2 = 0.9$, $P = 0.930$). Treatment effects were not, however, consistent between morphs (M morph: within-morph illegitimate = legitimate > self = between-morph illegitimate; S morph: between-morph illegitimate > self = legitimate > within-morph illegitimate). In the dimorphic population, differences among treatments were small but statistically significant, and involved a 6% reduction in fruit set after self- and illegitimate sib-pollination (mean = 82%; mean for other treatments = 87%). Fruit set was also 5% higher in the S (87%) than the L morph (82%).

TABLE 4. Effects of pollination treatment and morph on fruit set in two populations of *Decodon verticillatus*. The Wald X^2 was used to eliminate nonsignificant (i.e. $P_{Wald} > 0.10$) terms from the model. The significance of the remaining terms was confirmed using a likelihood ratio test (LR). Because populations were analysed separately, the per-test type I error rate (α) has been adjusted to 2.53% using Sidák's (1967) correction to hold the experiment-wise α at 5%. Means are in Figs 9 and 10

Population	Source	*df*	Wald X^2	P_{Wald}	LR[1]	P_{LR}
EO-T6	Morph	2	9.65	0.008	10.23	0.001
	Cross	3	7.28	0.063	7.92	0.005
	C × M	6	44.34	0.000	50.89	<0.001
EO-D1	Morph	1	6.03	0.014	6.17	0.013
	Cross	4	11.13	0.025	11.73	<0.001

[1] The likelihood ratio (LR) is distributed as X^2 with one *df*.

Pollination treatment had a significant effect on the number of seeds per fruit in both populations (Table 5; mean seed number $\pm SE = 43.9 \pm 0.6$ for EO-T6 and 36.5 ± 0.6 for EO-D1). Multiple comparisons indicated that the treatment effect involved a 9% (in EO-D1) and 18% (in EO-T6) reduction in seed set in selfed fruits (EO-T6 = 38.7 ± 0.9; EO-D1 = 33.9 ± 1.2) compared with fruits from outcross-pollinations (EO-T6 = 47.3 ± 0.9; EO-D1 = 37.4 ± 0.7). There was also a significant effect of morph in the trimorphic population, with the S morph setting 17% more seeds per fruit than the M or L morphs.

Seed weight was relatively unaffected by pollination treatment in both populations (Table 5). In the trimorphic population (mean seed weight = 0.94 ± 0.19 mg) treatment effects were marginally significant ($P = 0.066$), with seeds from within-morph illegitimate pollinations slightly heavier than seeds from the other treatments. There was also a significant effect of morph, with seeds from the L and M morphs (0.96 ± 0.01) averaging 10% heavier than seeds from the S morph (0.88 ± 0.01). Morph effects were the reverse of those observed for seed number, and may have arisen through a trade-off between seed size and number. This is suggested by a significant negative regression of seed weight on seed number, overall ($F_{1,740} = 164.0$, $P < 0.001$, $\beta = -0.0046 \pm 0.00036\ SE$) and for each morph and treatment combination separately. The effect of treatment, however, did not reflect a size-number trade-off. In the dimorphic population, seed weight (1.05 ± 0.001) only varied among recipient plants, and this was associated with a small, marginally significant ($P = 0.035$) plant by treatment interaction.

DISCUSSION

Most populations of *D. verticillatus* exhibit clear trimorphism in style and stamen lengths with reciprocal positioning of anthers and stigmas among the floral morphs. Despite a high frequency of morph loss in the species, there was no evidence of any fundamental differences in floral morphology of morphs between dimorphic and trimorphic populations as has been reported in *Pemphis* and *Oxalis* (reviewed in Weller, 1992). In one of the two dimorphic populations examined there was evidence for small modifications to the length of the stamens corresponding to the absent morph. However, a larger sample of populations

TABLE 5. Analysis of seed number and seed weight by pollination treatment in two populations of *Decodon verticillatus*. Significant effects are emphasized with asterisks. Because populations were analysed separately, the per-test type I error rate (α) has been adjusted to 2.53%. Response variables have been transformed as indicated to meet the assumptions of ANOVA. Means are in Figs 9 and 10

Response	Population[1]	Source of variation	*df*	*SS*	*F*	*P*
$\sqrt{\text{Seed number}}$	EO-T6	Model	11	150	7.9	<0.001
$R^2 = 0.11$		Morph*	2	54	15.6	<0.001
		Cross*	3	80	15.4	<0.001
		C × M	6	18	1.7	0.109
		Error	730	1268		
$\sqrt{\text{Seed number}+\sqrt{\text{Seed number}+1}}$	EO-D1	Model	99	3533.6	10.0	<0.001
$R^2 = 0.60$		Morph	1	3.3	0.0	0.881
		Plant[M]*	18	2818.6	26.8	<0.001
		Cross*	4	66.2	2.9	0.025
		C × M	4	13.6	0.6	0.663
		C × P[M]*	72	428.3	1.7	0.001
		Error	665	2362.4		
Seed weight	EO-T6	Model	11	1.6	4.4	<0.001
$R^2 = 0.06$		Morph*	2	0.7	10.2	<0.001
		Cross(*)	3	0.2	2.4	0.066
		C × M	6	0.2	1.3	0.267
		Error	735	25.4		
1/Seed weight	EO-D1	Model	99	9.880	4.0	<0.001
$R^2 = 0.37$		Morph	1	0.003	0.0	0.924
		Plant[M]*	18	6.645	11.1	<0.001
		Cross	4	0.108	0.8	0.509
		C × M	4	0.017	0.1	0.971
		C × P[M](*)	72	2.414	1.3	0.035
		Error	665	16.543		

[1] Data for EO-T6 were entered in a fixed effects two-way ANOVA. Those for EO-D1 were analysed using a partially-nested, mixed model, with Plant[M] and C × P[M] as random effects. In this model, *F*-tests for all effects, except C × P[M], used a synthetic denominator consisting mostly of $MS_{plant(M)}$ plus a much smaller component (4–12%) of MS_{error}. The *F*-tests for C × P[M] used MS_{error}.

should be examined before this can be considered a general feature of dimorphic populations.

Tristyly in *D. verticillatus* is apparently not associated with physiological barriers to self- or intramorph-fertilization (see also Ornduff, 1993). Controlled crosses in two populations revealed high levels of self-compatibility with only small reductions in seed set upon selfing. Evidence from experimental studies showing significant inbreeding depression at most life-history stages in this species (Eckert & Barrett, 1994a) suggests that the reduced seed set of self-pollinated flowers probably results from the expression of genetic load during seed development, and not weak incompatibility. Although floral heteromorphism with self-compatibility occurs in several heterostylous groups (reviewed in Barrett & Cruzan, 1994) including the tristylous Oxalidaceae (Ornduff, 1972) and Pontederiaceae (Barrett, 1988), among the heterostylous members of the Lythraceae, self-compatibility has been reported only for *D. verticillatus* (Darwin, 1877; Stout, 1923; Dulberger, 1970; Lewis & Rao, 1971; Ornduff, 1993). The apparent lack of strong incompatibility in *D. verticillatus*

raises two questions. First, is this condition derived or ancestral among heterostylous members of the Lythraceae? Second, what does the expression of ancillary features in *D. verticillatus* indicate about functional relationships between floral polymorphism and heteromorphic incompatibility? The range of floral variation revealed in this study also raises questions concerning the functioning of heterostyly and the potential for evolutionary modifications of the sexual system. Below, we discuss each of these issues in turn.

Evolution of heteromorphic incompatibility in the Lythraceae

Self-compatibility has usually been interpreted as a derived character state in heterostylous groups, primarily because the vast majority of heterostylous species exhibit strong incompatibility (Lewis, 1954; Ornduff, 1972; Yeo, 1975; Ganders, 1979). Moreover, theoretical models have suggested that the most likely scenario for the evolution of heterostyly consists of incompatibility evolving first as an anti-selfing mechanism in response to strong inbreeding depression, and floral heteromorphism evolving later to promote efficient pollen transfer among the small number of mating types (D. Charlesworth & B. Charlesworth, 1979; Ganders, 1979). Hence, self-compatible heterostylous taxa have been generally viewed as arising through secondary loss of incompatibility. A recent model proposed by Lloyd & Webb (1992a, b), however, suggests that floral polymorphism could evolve independently of incompatibility solely to promote efficient pollen transfer. Accordingly, self-compatible species need not be derived from self-incompatible ancestors.

Phylogenetic analysis of the sequence of character evolution in groups including both self-compatible and self-incompatible taxa may provide some insights into these alternative models (Donoghue, 1989; Barrett, 1993). Interpreting the evolutionary relationship between floral heteromorphism and incompatibility in the Lythraceae is, however, hampered by a lack of comparative data on the reproductive biology of most heterostylous members; especially, key groups such as *Nesaea*, which includes monomorphic, dimorphic and trimorphic species (Ornduff, 1979), or *Lagerstroemia* and *Adenaria* which may possess incipient or vestigial heterostylous characters (Darwin, 1877; S. A. Graham, personal communication).

Recent phylogenetic analysis of the Lythraceae (Graham, Crisci & Hoch, 1993) has suggested that a clade consisting of *Decodon* and distylous *Pemphis* differentiated before other heterostylous taxa in the family. Accordingly, it is possible that if heterostyly is monophyletic in the family, floral polymorphism combined with self-compatibility might be the ancestral condition. *Pemphis*, however, is strongly self-incompatible and appears to have arisen from a tristylous ancestor (Lewis & Rao, 1971; Lewis, 1975). Hence, it is just as likely that the common ancestor of *Decodon* and *Pemphis* possessed heteromorphic incompatibility.

Interpreting the evolution of floral heteromorphism and incompatibility in this family is further complicated by evidence for the possible existence of cryptic incompatibility in *D. verticillatus*. Experiments using genetically-marked mixed pollen loads indicated that *D. verticillatus* possesses a post-pollination mechanism independent of inbreeding depression that gives outcross pollen a significant siring advantage over self pollen. However, unlike other heterostylous taxa with

cryptic incompatibility (Weller & Ornduff, 1977; Casper, Sayigh & Lee, 1988; Cruzan & Barrett, 1993), the siring differences among pollen types in *D. verticillatus* are not consistent with the operation of a weakened version of heteromorphic incompatibility (Eckert & Barrett, 1994b). Because incompatibility may be quantitative in expression rather than always operating in a categorical manner (see Barrett & Cruzan, 1994), phylogenetic analysis of sexual-system evolution in heterostylous groups will require close examination of the nature of incompatibility systems before character states can be assigned with any confidence.

Relationships among heterostylous characters

Stamen-style polymorphism in heterostylous species is usually associated with ancillary polymorphisms in several traits, especially pollen size, pollen production and stigmatic characters. Yet despite the ubiquity of ancillary polymorphisms among heterostylous taxa, their role in the functioning of heterostyly remains unclear (Dulberger, 1992). The expression of ancillary features in *D. verticillatus* underscores the difficulties in interpreting their functional significance.

There was strong differentiation among morphs in stigmatic characters; however, the relationships with style length were the reverse of that reported in most species including related *Lythrum* spp. (Ornduff, 1979). Rather than stigma size and papilla length increasing with style length, the opposite trend was revealed. This unusual relationship has been reported in several taxa including distylous *Pemphis* (Gill & Kyauka, 1977), the probable sister-group to *Decodon* (Graham *et al.*, 1993). *Pemphis* is strongly self-incompatible, yet the degree of differentiation in papilla length is roughly the same (30% and 36% difference between L and S morphs in *D. verticillatus* and *P. acidula*, respectively). Polymorphism in stigmatic characters is, therefore, not correlated with the strength of incompatibility in these two species but is likely associated with some aspect of the pollination process.

In heterostylous groups including both self-compatible and self-incompatible taxa, the former often show weaker differentiation in pollen size among anther levels than the latter (e.g. Ornduff, 1972; Barrett, 1988). The data from *D. verticillatus* support this trend. Among strongly self-incompatible taxa in the Lythraceae, pollen size differentiation expressed as the length of long-level pollen divided by that of short-level pollen is 1.67 and 1.72 in tristylous *Lythrum salicaria* (Darwin, 1877) and *L. junceum* (Dulberger, 1970), respectively, and 1.41 in distylous *P. acidula* (Lewis & Rao, 1971). Pollen size differentiation in *D. verticillatus* ranged from 1.01 to 1.12 (average = 1.07) among the six populations examined in this study. Weak expression of pollen trimorphism in *D. verticillatus* contrasts with the striking differences in stigmatic characteristics among morphs, again highlighting the often conflicting relationships among component characters of the heterostylous syndrome.

Floral variation and the functioning of heterostyly

Heterostyly is generally thought to have evolved because it promotes efficient cross-pollination (Darwin, 1877; Lloyd & Webb, 1992b). Its functioning in this

regard should depend upon precise removal of pollen from anthers and its subsequent deposition on stigmas by insect vectors. This process should be enhanced by *reciprocity* in organ levels among morphs as well as *precision* in the placement of organs within a given level. Besides the exact positioning of organs, efficiency appears to be enhanced in most taxa by tubular or funnel-shaped corollas that restrict the positioning of insect visitors while probing for nectar (Ganders, 1979; Lloyd & Webb, 1992a). Tubular flowers are usually considered to be more effective in controlling the deposition of pollen on an insect's body (Faegri & van der Pijl, 1979).

Decodon verticillatus possesses unusual flowers for a heterostylous species. Floral whorls are fused to form a tubular hypanthium; however, this structure is very short and only encloses short-level organs. Mid- and long-level organs project well beyond the hypanthium and spread out in a radial pattern, giving the impression of a brush blossom (*sensu* Faegri & van der Pijl, 1979: 106, Table 3). Whether this atypical floral structure is associated with precise pollen transfer is unclear and will depend upon the morphology and behaviour of insects visiting the flowers. Preliminary observations of pollinator behaviour in the northern half of the species' range (Ontario and Michigan), where *Bombus* spp. and *Apis mellifera* are the most common visitors, suggest that pollen transfer is unlikely to be very precise (Eckert, 1993). In the southern portion of the range (South Carolina, Georgia and Florida), however, butterflies comprise a substantial component of the visitor fauna and the floral syndrome of the species shares many features in common with other butterfly-pollinated plants.

Geographic variation in the pollinator fauna may be associated with variation in patterns of pollen transfer in tristylous species (Price & Barrett, 1984; Barrett, 1985). In the absence of strong incompatibility, geographic variation in pollinator service may have important mating-system consequences. Reduced levels of disassortative mating resulting from imprecise pollen transfer makes populations more vulnerable to stochastic morph-frequency variation (Barrett *et al.*, 1989; Eckert & Barrett, 1992). This may explain, in part, the higher frequency of morph loss in northern than southern populations of *D. verticillatus*.

In addition to the atypical floral structure of *D. verticillatus*, there is considerable variation in the position of reproductive organs, both between morphs (Fig. 2) and among individuals within a morph (Figs 3, 4 and 11). To assess whether this floral variability is associated with reduced reciprocity and precision in *D. verticillatus* compared to other tristylous species, we have developed indices for both these parameters (see Appendix; following Lloyd, Webb & Dulberger, 1990; Richards & Koptur, 1993; J. H. Richards, D. G. Lloyd & S. C. H. Barrett, unpublished). The index of reciprocity (*RI*) summarizes deviations from perfect reciprocal positioning of organs among morphs (i.e. perfect reciprocity = 0). The precision index (*PI*) reflects the degree of between-morph variation in organ position at a given level and is the coefficient of variation within each level (with three morphs, $n = 3$ for each level), averaged over the three levels. A *PI* of zero indicates perfect precision.

In Figure 12, we have plotted *PI* over *RI* for 30 populations of 13 tristylous species, including the five trimorphic populations of *D. verticillatus* examined in this study. This analysis suggests three things. First, there is a significant positive correlation between reciprocity and precision ($r_s = 0.42$). Second, both reciprocity and precision together tend to vary as much among populations of a

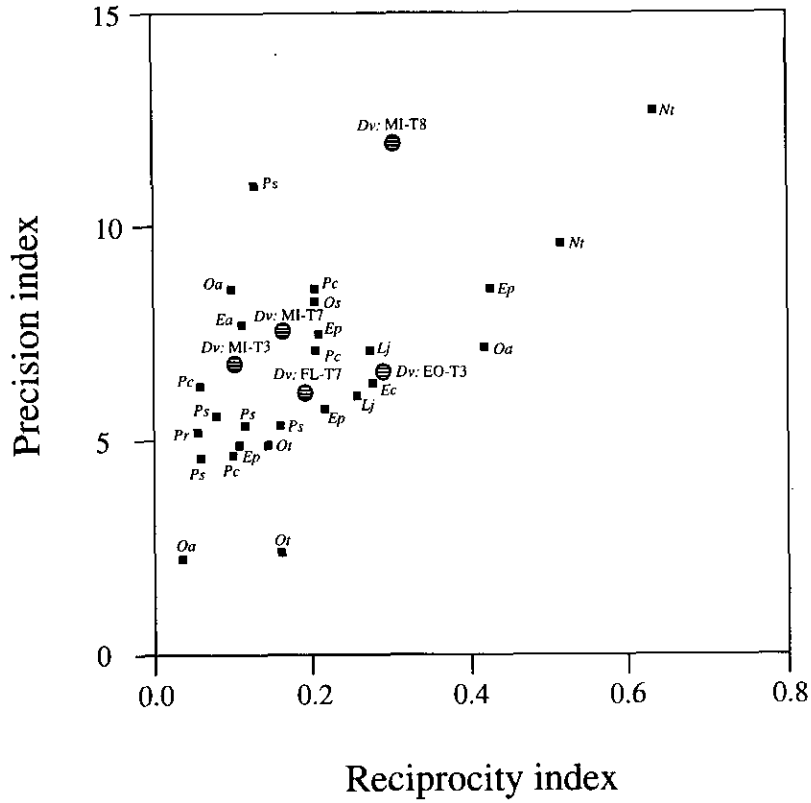

Figure 12. Indices of floral precision and reciprocity for 30 tristylous populations involving 13 species from four plant families. Populations of *Decodon verticillatus* are shown as hatched circles, other species by solid squares. Species are indicated by the following codes: Dv = *D. verticillatus* (present study), Lj = *Lythrum junceum* (Dulberger, 1970; Ornduff, 1975); Oa = *Oxalis alpina* (Weller, 1976, 1979); Os = *O. suksdorfii* (Ornduff, 1964); Ot = *O. tuberosa* (Gibbs, 1976); Ea = *Eichhornia azurea* (Barrett, 1978); Ec = *E. crassipes* (Barrett, 1977a); Ep = *E. paniculata* (Barrett, 1985); Pc = *Pontederia cordata* (Price & Barrett, 1982); Pr = *Pontederia rotundifolia* (Barrett, 1977b); Ps = *Pontederia sagittata* (Glover & Barrett, 1983); Nt = *Narcissus triandrus* (S. C. H. Barrett, unpublished). These indices are derived in the Appendix.

given species as between different species. Though some species, for example *Narcissus triandrus* ($RI = 0.55$–0.65, $PI = 9$–13%) and *Oxalis tuberosa* ($RI = 0.15$–0.16, $PI = 2.5$–5%), clearly differ for both parameters, most show overlapping distributions. Finally, despite its unusual floral morphology and observations of substantial floral variability in some populations, *D. verticillatus* does not show significantly less precision or reciprocity compared to other tristylous species. An exception is population MI-T8 which showed reduced stigma–anther separation in both the L and M morphs. Aside from this population, *D. verticillatus* generally falls in the middle of the distribution for both indices (ranked between 8th and 26th for *RI* and between 13th and 22nd for *PI*).

This analysis does not take into account another component of precision and reciprocity: the variation in organ position at a given level among individuals, within morphs. To address this problem we calculated the coefficients of

variation (*CV*) for each organ on each morph from standard deviations available in the literature and averaged the coefficients of variation across organ levels and morphs for each of 11 tristylous species. The mean within-morph, within-level *CV* ranges from 4.0% for *Eichhornia crassipes* to 36.3% for *Oxalis tuberosa*. *Decodon verticillatus* ranks 8th among the 11 species ($CV = 13.7\%$). Interestingly, there is a negative relationship between the rank order of species in terms of within-organ *CV* and their ranks in terms of either *RI* ($r_s = -0.52$) or *PI* ($r_s = -0.40$). These negative correlations arise because three of the species ranked lowest in terms of *RI* and *PI* (i.e. most reciprocal, most precise: *Oxalis tuberosa*, *O. alpina* and *Pontederia rotundifolia*) are ranked highest in terms of within-organ *CV*. Among the remaining species, there is no correlation. Clearly, the results of this analysis will be affected by the method individual investigators used to make floral measurements. Nevertheless, from these two analyses it appears that *D. verticillatus* is not particularly unusual among tristylous species in its lack of floral reciprocity or precision.

Evolutionary implications of floral variation

Certain patterns of floral variation observed in *D. verticillatus* are suggestive of the two major trends in breeding-system modification observed among tristylous taxa (Weller, 1992). Repositioning of organ levels in one of the two dimorphic populations examined suggests that widespread dimorphism in this species could potentially set the stage for the evolutionary transition of tristyly to distyly if appropriate ecological conditions prevailed. Similar evolutionary trends have received detailed study in *Oxalis* spp., especially in *O. alpina* (Weller, 1979). In *Oxalis*, however, it is possible that the spread of modifier genes affecting floral modification are the cause rather than effect of morph loss (Charlesworth, 1979). In this regard, it is worth noting that in *D. verticillatus*, divergence in the length of mid-level stamens between the L and S morphs was also observed in trimorphic populations. The shift from tristyly to distyly appears to have occurred repeatedly in the Lythraceae (Ornduff, 1979), including closely related *Pemphis acidula* (Lewis & Rao, 1971; Lewis, 1975). In some cases, the anther whorl corresponding to the missing morph has been lost entirely. In other cases, both whorls are retained. Little, however, is known about the reproductive biology or patterns of floral variation in these taxa or their trimorphic relatives.

The evolutionary shift from outcrossing to predominant self-fertilization via homostyle formation is thought to have occurred in many heterostylous families (Darwin, 1877; Baker, 1959; Ganders, 1979; Barrett, 1989). The modified phenotypes with reduced stigma–anther separation that were observed in *D. verticillatus* are of interest in this regard. The evolutionary significance of these floral modifications will depend upon the degree to which they are heritable as well as the fitness consequences of the floral variation.

Heritable floral variation involving modified style and stamen positions has been shown to occur in several heterostylous taxa (Mather, 1950; Ernst, 1955; Shore & Barrett, 1990; Seburn, Dickinson & Barrett, 1990). Observations of floral variation in *D. verticillatus* suggest that floral modifications are heritable in a broad sense, since genotypes transplanted from the field maintain their original patterns of floral variation under uniform glasshouse conditions. The expression of floral modifications governed by recessive genes may be enhanced by partial

self-fertilization (Robertson, 1952; Falconer, 1980; Barrett, 1985). Some of the floral variability observed in *D. verticillatus* may represent the release of genetic variation through self-fertilization (Eckert, 1993). However, inbreeding may also lead to increased variability through developmental instability associated with homozygosity (Mather, 1950; Lerner, 1954; Jinks & Mather, 1955). Determining the relative importance of these two sources of variation in *D. verticillatus* will require formal morphogenetic analysis (Seburn *et al.*, 1990; Barrett & Harder, 1992; Richards & Barrett, 1992).

Regardless of the mechanisms responsible for floral variability in *D. verticillatus*, it could potentially affect mating patterns. Small differences in organ positioning (i.e. a few mm) greatly affect patterns of pollen flow in distylous *Lithospermum californicum* (Ganders, 1979). Larger changes in organ position associated with a reduction in stigma–anther separation are likely to have correspondingly larger effects (e.g. *Mitchella repens* Ganders, 1975a; *Primula vulgaris* Piper & Charlesworth, 1986). Reduced herkogamy is associated with reduced disassortative mating in *Amsinckia spectabilis* (Ganders, 1975b) as well as lower levels of outcrossing in *Eichhornia crassipes* (Barrett, 1979), *Amsinckia* spp. (Ganders, Denny & Tsai, 1985), *Eichhornia paniculata* (Glover & Barrett, 1986) and *Turnera ulmifolia* (Barrett & Shore, 1987). Whether reduced herkogamy has a significant impact on mating patterns in populations of *D. verticillatus* will depend upon the relative importance of floral morphology compared to ecological factors such as population density, and clonal structure as well as physiological mechanisms involving discrimination against self pollen (Eckert, 1993).

Floral modifications which increase the selfing rate may have large effects on fitness. Unless there is a strong fitness disadvantage to self-fertilization, mutations increasing the selfing rate are expected to spread quickly to fixation in outcrossing populations (Fisher, 1941). Evidence of inbreeding depression in *D. verticillatus*, however, suggests that selfing variants may experience a strong fitness disadvantage and this may explain, in part, the rarity of modified phenotypes in most populations (Eckert & Barrett, 1994a). The fitness consequences of particular floral phenotypes may, however, be difficult to surmise from their relative frequencies since populations of *D. verticillatus* may still be in a non-equilibrium state after post-glacial migration (Eckert, 1993). Limited recruitment of sexually produced offspring in this long-lived, highly clonal species is likely to greatly slow evolutionary modification of its sexual system. At the very least, populations of *D. verticillatus*, perhaps by virtue of their sluggish evolutionary tempo, exhibit patterns of floral variation that presage the major trends in the evolutionary modification of tristylous sexual systems.

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APPENDIX

Reciprocity

The measures of reciprocity and precision for tristylous flowers presented below were derived from similar indices for distylous flowers developed by D. G. Lloyd (unpublished), Richards & Koptur (1993) and Lloyd, Webb & Dulberger (1990). In distylous species, perfect reciprocity is achieved when anthers of one morph are positioned at the same level as the stigma of the other. That is, when the long-level stigma (L) is at the same position as the long-level anther of the S morph (lS): L = lS. Also, S = sL. Reciprocity may, therefore, be summarized as:

$$(\mathrm{L}-\mathrm{S}) = (\mathrm{lS}-\mathrm{sL}) \quad \text{or} \quad \frac{(\mathrm{lS}-\mathrm{sL})}{(\mathrm{L}-\mathrm{S})} = 1.$$

In tristylous species, there is three-way reciprocity (i.e. L stigmas receive pollen from long-level anthers of both the M and S morphs, and M stigmas receive pollen from mid-level anthers of both the L and S morphs and so on). Accordingly, reciprocity can be summarized by an equality involving three terms which, when reciprocity is perfect, should sum to one:

$$\frac{1}{3}\left[\frac{(\mathrm{lS}-\mathrm{sL})}{(\mathrm{L}-\mathrm{S})} + \frac{(\mathrm{lM}-\mathrm{mL})}{(\mathrm{L}-\mathrm{M})} + \frac{(\mathrm{mS}-\mathrm{sM})}{(\mathrm{M}-\mathrm{S})}\right] = 1.$$

A problem with this equality is that each of the three terms may show either positive or negative deviations from reciprocity, and thus may cancel each other

out. To remedy this, each of the three terms can be expressed as an absolute deviation from one:

$$\frac{1}{3}\left[\left|1-\frac{(\mathrm{lS}-\mathrm{sL})}{(\mathrm{L}-\mathrm{S})}\right|+\left|1-\frac{(\mathrm{lM}-\mathrm{mL})}{(\mathrm{L}-\mathrm{M})}\right|+\left|1-\frac{(\mathrm{mS}-\mathrm{sM})}{(\mathrm{M}-\mathrm{S})}\right|\right]=1.$$

The left-hand side of the equality above may, therefore, be used as an index of reciprocity. It is worth noting three problems with this index. First, from (L−S) = (lS−sL) it does not necessarily follow that L stigmas are positioned at the same height as long-level anthers of the S morph. This index, therefore, measures equality of inter-organ distances rather than true reciprocity *per se*. However, it will only misrepresent reciprocity if mean anther heights are consistently higher or consistently lower than the height of their target stigmas. If variation in mean organ height is random, the index should reflect reciprocity.

Second, most of the data which can be used for comparative purposes (including the data for *D. verticillatus* presented here) are organ lengths, not organ heights. If there is two- or three-dimensional structure to the flower, length will not properly reflect height (Richards & Barrett, 1992). This alternative approach will, therefore, tend to overestimate deviations from reciprocity in species with more than a one-dimensional relationship between styles and stamens.

The third problem with this index involves the use of relative versus absolute deviations from reciprocity. The three deviations from reciprocity are scaled to remove the effect of absolute distance between organs. That is, one might expect greater absolute deviations in reciprocity between the L and S morphs than between the L and M morphs simply because (L−S) ≈ 2(L−M). Dividing (lS−sL) by (L−S) removes size effects to a greater extent than, for instance, taking the absolute value of the difference between (L−S) and (lS−sL) as a measure of reciprocity. Whether the effects of general size should be removed is debatable, since the absolute effect of developmental variation in large flowers may be a major constraint on the evolution of heterostyly (Richards & Barrett, 1992).

Precision

The accuracy of pollen transfer in heterostylous species should be influenced by the precision of organ positioning at a given level. L stigmas should be at the same height as both long-level anthers of the M and S morphs. If the organ height of one morph is out of line with those of the others, the pick-up and delivery of pollen should be less precise. To index precision, we have used the coefficient of variation of organ lengths at a given level [$CV = 100(SD$ of 3 organ lengths/mean of 3 organ lengths)]. A precision index (PI) is calculated for a given population by averaging the CV's for each organ level:

$$PI=\frac{(CV_{\mathrm{L}}+CV_{\mathrm{M}}+CV_{\mathrm{S}})}{3}.$$

Again, by using coefficients of variation, the deviations at a given organ level are scaled to remove the effects of general size. Note also that the problem with respect to the alignment of stigmas and anthers discussed above applies here too.

Species with three-dimensional flower structure will tend to have higher precision indices simply because organ length is a relatively poor indicator of organ height.