INBREEDING DEPRESSION IN PARTIALLY SELF-FERTILIZING
DECODON VERTICILLATUS (LYTHRACEAE):
POPULATION-GENETIC AND EXPERIMENTAL ANALYSES

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Abstract. — Inbreeding depression is a major selective force favoring outcrossing in flowering plants. Some self-fertilization, however, should weaken the harmful effects of inbreeding by exposing genetic load to selection. This study examines the maintenance of inbreeding depression in partially self-fertilizing populations of the long-lived, herbaceous wetland plant, *Decodon verticillatus* (L.) Ell. (Lythraceae). Estimates from ten populations indicate that 30% of offspring are produced through self-fertilization. Population-genetic estimates of inbreeding depression ($\delta = 1 -$ relative mean fitness of selfed progeny) based on changes in the inbreeding coefficient for the same ten populations were uniformly high, ranging from 0.49 to 1.79 and averaging 1.11 ± 0.29 SE. Although confidence intervals of individual population estimates were large, estimates were significantly greater than 0 in six populations and greater than 0.5 in four. Inbreeding depression was also estimated by comparing growth, survival, and flowering of experimentally selfed and outcrossed offspring from two of these populations in a 1-yr glasshouse experiment involving three density regimes; after which offspring were transplanted into garden arrays and two field sites and monitored for two consecutive growing seasons. Overall δ for survival averaged 0.27 ± 0.01 in the glasshouse, 0.33 ± 0.04 in the garden, and 0.46 ± 0.04 in the field. The glasshouse experiment also revealed strong inbreeding depression for growth variables, especially above-soil dry weight ($\delta = 0.42 \pm 0.03$). The fitness consequences of inbreeding depression for these growth variables approximately doubles if survival to maturity is determined by severe truncation selection. Despite substantial selfing, inbreeding depression appears to be a major selective force favoring the maintenance of outcrossing in *D. verticillatus*.

Key words. — *Decodon verticillatus*, genetic load, inbreeding, inbreeding depression, natural selection, self-fertilization.

The harmful effects of close inbreeding constitute one of the major selective forces in the evolution of plant mating systems (Darwin 1876; Wright 1977; Charlesworth and Charlesworth 1987). Recent theoretical work has emphasized that inbreeding depression is not a static property of a species or population but is expected to co-evolve with the mating system (Lande and Schemske 1985; Uyenoyama 1986; Charlesworth and Charlesworth 1987; Holsinger 1988). When genetic load is determined primarily through mutation-selection balance, interactions between inbreeding depression and the mating system should lead to one of two evolutionarily stable endpoints: predominant outcrossing associated with strong inbreeding depression and predominant self-fertilization with weak inbreeding depression (Lande and Schemske 1985; Charlesworth et al. 1990, 1991, 1992). The threshold between these alternative evolutionary states depends on the relative fitness of selfed (ω_s) versus outcrossed (ω_x) progeny. When ω_s is less than one-half of ω_x, outcrossing should usually be maintained. Otherwise selfing should evolve. Partial selfing may reduce the strength of inbreeding depression by exposing recessive mutations to selection but should not be evolutionarily stable on either side of this threshold.

Growing evidence that many plants practice intermediate levels of self-fertilization presents a challenge for these theoretical models (Schemske and Lande 1985; Waller 1986; Barrett and Eckert 1990). Unfortunately, our present understanding of the evolutionary significance of partial selfing is limited by a general lack of data on inbreeding depression and other fitness consequences of mixed mating systems in natural populations for which selfing rates have been estimated (e.g., Ritland and Ganders 1987a, 1987b; Holtsford and Ellstrand 1990; Barrett and Charlesworth 1991; Latta and Ritland 1993; Dole and Ritland 1993). A major issue here is what level of inbreeding depression is maintained in partially selfing populations given the purging effects of self-fertilization on genetic load? Whereas mutational load

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involving relatively few strongly deleterious alleles should be greatly reduced by a little selfing (Lande and Schemske 1985; Charlesworth and Charlesworth 1987), inbreeding depression caused by mildly deleterious mutations at many loci may be maintained in the face of substantial self-fertilization (Charlesworth et al. 1990, 1991, 1992). In this paper, we investigate this issue by determining the strength of inbreeding depression in natural populations of partially self-fertilizing *Decodon verticillatus* (L.) Ell. (Lythraceae). The species is a long-lived, clonal, herbaceous perennial that inhabits marshes, swamps, and bogs throughout eastern central North America. It possesses a tristylous breeding system, however, unlike most heterostylous taxa *D. verticillatus* is highly self-compatible (Eckert and Barrett 1994a). Estimates of the frequency of self-fertilization in ten populations from the northern half of the range showed that 30% of all offspring are the products of selfing (Eckert and Barrett 1994b). Here, we use a population-genetic approach to estimate the strength of inbreeding depression in these ten populations. For two of these, we also present an experiment analysis of inbreeding depression conducted in both the field and glasshouse.

The experimental approach to measuring inbreeding depression has been used with a variety of plant species (e.g., Darwin 1876; reviewed by Wright 1977; Charlesworth and Charlesworth 1987; Barrett and Kohn 1991) and entails comparing the survival, growth, and reproduction of selfed and outcrossed offspring produced by controlled crosses. Usually it is desirable to create both types of progeny on each maternal parent such that differences in offspring performance are not obscured by maternal effects (Lynch 1988). It is also important to compare offspring viability for as many life-history stages as possible, because differences in offspring fitness may vary substantially throughout the life cycle (Charlesworth and Charlesworth 1987). Finally, the expression of inbreeding depression may depend on the experimental environment in which fitness comparisons are made (Lerner 1954; Lloyd 1980). Hence, where feasible, experiments should be conducted in natural habitats (e.g., Schemske 1983; Schoen 1983; Mitchell-Olds and Waller 1985; Kohn 1988) or across a range of artificial conditions such that the response of inbreeding depression to environmental conditions may be assessed (e.g., Schemske 1983; Waller 1984; Dudash 1990; Schmitt and Ehrhardt 1990; Wolfe

1993). In this study, we estimated the strength of inbreeding depression throughout 1 yr of growth in a glasshouse experiment incorporating three density regimes. Survival during two consecutive growing seasons was monitored in natural habitats and an outdoor garden.

A population-genetic approach to measuring inbreeding depression based on changes in the inbreeding coefficient (F) between life-history stages has been developed by Ritland (1990). In partially self-fertilizing populations, the inbreeding coefficient increases from adult to zygote as a result of selfing, and subsequently declines throughout the life cycle as a result of selection against inbred zygotes. The magnitude of this decline, therefore, reflects the strength of inbreeding depression. The decline in F may be measured directly, or may be inferred from estimates of the parental F and the selfing rate (s) by assuming inbreeding equilibrium. At equilibrium, F does not change between generations, thus the progeny inbreeding coefficient [$F' = s(1 + F)/2$] is assumed to drop to the parental F by maturity. Whereas this observational approach lacks some of the control and precision of the experimental approach, it produces estimates of inbreeding depression that include most life-history stages and are free from the biases introduced by experimental manipulations in an artificial environment. The approach is particularly useful for long-lived species, such as *D. verticillatus*, where experimental estimates of inbreeding depression for the entire life cycle may be impossible to obtain. The two different approaches provide complementary information leading to a more robust assessment of the strength of inbreeding depression in natural populations.

MATERIALS AND METHODS

Population-Genetic Estimates

Estimates of inbreeding depression ($\hat{\delta}$) were obtained for ten populations of *Decodon verticillatus* located in Michigan and Ontario from progeny array data using Ritland's (1990) equilibrium estimator:

$$\hat{\delta} = 1 - \frac{2(1 - \hat{s})\hat{F}}{\hat{s}(1 - \hat{F})},$$

where $\hat{s}$ and $\hat{F}$ are estimates of the selfing rate and parental inbreeding coefficient, respectively. The data for each population involved ten progeny from each of 53 to 88 randomly sampled

seed families, screened for three polymorphic isozyme loci (*Mdh*, malate dehydrogenase; *Idh*, isocitric dehydrogenase; and *Acp*, acid phosphatase) as part of a study of mating-system variation in *D. verticillatus* (details and population locations in Eckert 1993; electrophoretic methods in Eckert and Barrett 1993). For each population, $\hat{s}$ and $\hat{F}$ were found as minimum variance averages of single-locus estimates (Ritland 1986) and $\hat{\delta}$ was subsequently calculated using the equilibrium estimator. Comparison of single locus (s_s) and multilocus (s_m) selfing rates revealed very low levels of biparental inbreeding in all populations (mean $[s_s - s_m] = 0.026 \pm 0.005$ SE); hence, the estimator was not corrected for crossing among relatives (Dole and Ritland 1993). Standard errors of the three statistics were derived as the standard deviation of 1000 bootstraps with the progeny array as the unit of resampling. The distribution of bootstrap values for $\hat{\delta}$ was roughly normal for each population. Statistical departures of estimates from 0 and 0.5 were assessed by deriving 95% confidence limits from the upper and lower percentiles of the bootstrap distribution (Eckert and Barrett 1994b).

Experimental Analysis

Maternal plants were collected as open-pollinated seed from each of two populations of *D. verticillatus* in eastern Ontario, Canada (population EO-T6: Leeds and Grenville County, 44°35′N, 76°19′W; population EO-D1: Frontenac County, 44°33′N, 76°23′W). Experimental self- and outcross pollinations were conducted in the glasshouse during the summer of 1990. A maternal plant was randomly selected from each of 18 seed families per population and randomly matched with a single pollen donor from a different family in the same population. Self- and cross-pollinations were performed on emasculated flowers (mean = 30 pollinations per treatment per plant). Seeds were counted in six to ten fruits per treatment per plant. The seeds from each fruit were weighed as a group to 0.1 mg, and average seed weight was calculated.

In September 1991, groups of 20 seeds from each of six fruits per treatment per plant were sown on a mixture of three parts Pro-Mix® (soil-less mix) to one part sandy loam contained in 5.7-cm plastic pots set in Perma-Nest® trays, 30 pots per tray. Two pots per treatment per family were randomly positioned in each of three blocks (432 pots in total) located on a single glasshouse bench illuminated for 14 h per day by two 400-watt, high-pressure sodium lamps. Throughout germination, water levels were kept at or just below the soil level, and temperatures generally ranged between 25°C and 35°C. Emerging seedlings were counted after 2 ("early germination") and 5 wk ("late germination"). Six seedlings per treatment per family were allocated to each of three blocks for a total of 1296 plants. Plant height was measured 2 and 6 wk after transplanting.

Because the expression of inbreeding depression may depend upon the experimental conditions, further comparisons of growth and flowering were conducted in three density regimes. When the seedlings had grown to the size limit of their 5.7-cm pots, each of the three blocks was randomly assigned as a group to either high-density [5.7-cm pots ($\approx$ 75 ml of soil), 30 per tray], medium-density [7.5-cm pots ($\approx$ 200 ml of soil), 20 per tray] or low-density [10-cm pots ($\approx$ 325 mL of soil), 11 per tray] growing conditions. At 2-wk intervals, plants were fertilized with a solution of 20:20:20 (N:P:K) fertilizer (about 80, 120, and 240 mg dry weight of fertilizer per plant at high, medium, and low densities, respectively). Plants comprising each density regime remained together on separate glasshouse benches. This spatial clustering did not, however, confound the effect of density, because differences in plant growth among benches observed before the application of the density treatment and in other growth experiments were negligible in comparison (Eckert 1993). After 4 mo of growth under these conditions, most plants showed signs of entering dormancy, and the above-soil portion of each was harvested, dried at 60°C for 4 d, and weighed to 0.1 g. Throughout the growing period, plants were tagged as they came into flower, and the number of flowering leaf axils was recorded. After harvest, plants were moved outdoors where they remained dormant until the following summer.

In early June 1992, year-old plants from the low-density treatment were transplanted into two natural wetlands located within 5 km of their native populations in eastern Ontario. One of the sites was a small cattail and sedge marsh in which *D. verticillatus* was already growing ("inhabited" site). The other was very similar in appearance but was not inhabited by *D. verticillatus* ("uninhabited" site). Five blocks of plants were planted at each site. Each block consisted of 10 selfed and 10 outcrossed offspring from each population (EO-T6 and EO-D1), planted among undisturbed native vegetation in two parallel lines

of 20 with population and treatment alternating. Owing to a shortage of selfed offspring from EO-T6, the fifth group at the inhabited site consisted of 13 plants per treatment from EO-D1 plus 13 outcrossed plants from EO-T6. In all, we transplanted 399 plants, consisting of an average of 5.4 selfed and 5.9 outcrossed progeny from each of the original 36 maternal families. Survival was recorded in September of the following year (1993). Plants from the medium- and high-density treatments (five to six progeny per density from each of the same 36 families) were arranged in a random array in an outdoor garden in Toronto, Ontario and were regularly watered but not fertilized throughout the following two summers. Plants overwintered in the garden and survival was recorded in October 1993.

The effects of pollination treatment (self/outcross), population, density, and maternal family were evaluated using partially hierarchical linear models (family nested within population). Family and interactions involving family were considered random effects; other factors were fixed. Within each family, outcrossed progeny were full sibs; hence, "family effects" potentially include genetic and nongenetic maternal effects plus variation among maternal plants in genetic load as well as variation in complementarity between maternal and paternal genomes (Falconer 1980; Lyons et al. 1989). Statistical analysis of continuous variables used the factorial ANOVA routines in JMP (version 2.0, SAS Institute 1989). Aptness of the ANOVA models was assessed by inspecting the residuals for both normality and independence from predicted values and by testing for heterogeneity of sample variances. Variables were transformed as required (see tables 2 to 4 for specific transformations). When sample variances could not be successfully equalized by transformation, the analysis was conducted on the ranks of the raw data. This procedure is the most robust approximation of a nonparametric test in cases such as partially hierarchical designs for which formal nonparametric tests are not available (Conover 1980, p. 337). In all cases, analyses based on ranks did not differ greatly from those based on raw data. Analysis of categorical variables (survival and 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 using the Wald χ^2 to eliminate unimportant (i.e., $P > 0.10$) effects. Significance of the remaining effects was evaluated using the likelihood ratio test (LR), which is performed by applying the 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, LR is distributed as χ^2 with 1 df. Because the glasshouse experiment involved simultaneous examination of 11 variables, the experimentwise type I error rate was held at 5% by adjusting the per-test error rate to 0.5% following Sidàk (1967).

The strength of inbreeding depression is usually summarized by $\delta = 1 - \omega_s/\omega_x$, where ω_s and ω_x are the fitness of selfed and outcrossed offspring, respectively. We estimated δ for each maternal family by using the family means for each survival and growth variable. The overall effect of inbreeding on survival was estimated from the multiplicative fitness (fruit set × seed set × late germination × survival) of selfed and outcrossed progeny in each family. Data on survival from the field and garden arrays were also incorporated into these multiplicative estimates. Because no evidence existed for variation in inbreeding depression between populations, families from the two populations were pooled and standard errors calculated from the combined sample of 36.

We also estimated inbreeding depression for growth variables using a percentile-based measure of relative fitness. In studies of long-lived plants, like *D. verticillatus*, ultimate survival and fecundity are often extrapolated from some measure of plant vigor (e.g., dry weight or plant height: Schemske 1983; Stevens and Bougourd 1988; Karron 1989). This substitution assumes, however, that measures of vigor are linearly related to ultimate fitness. In contrast, our population-genetic estimates of δ (see below) suggested that selection may act on plant vigor in a nonlinear fashion; perhaps through severe truncation selection. In this case, only individuals in the upper percentiles of the trait distribution survive or reproduce; hence, inbreeding depression would be reflected by the relative frequency of selfed offspring in these upper percentiles. Accordingly, if only the top 10% of a distribution survive, then inbreeding depression could be calculated as

$$\delta_{10\%} = 1 - \frac{\text{frequency of selfed progeny in top 10\%}}{\text{frequency of outcrossed progeny in top 10\%}}$$

TABLE 1. Population-genetic estimates of inbreeding depression for ten populations of *Decodon verticillatus* from Ontario (EO) and Michigan (MI). Estimates of the selfing rate (s), inbreeding coefficient (F), and inbreeding depression (δ) are presented with standard errors (in parentheses) derived from 1000 bootstrap values. All estimates are based on the same three loci. except those for MI-D2 in which only two of the loci were polymorphic. Estimates of δ significantly greater than 0.0 and 0.5 are marked with * and **, respectively.

Population	Families	Progeny	$\hat{s}$	$\hat{F}$	$\hat{\delta}$
EO-T3	88	872	0.13 (0.05)	0.021 (0.076)	0.49 (1.65)
EO-T6	80	799	0.32 (0.05)	0.081 (0.060)	0.59 (0.32)
EO-T7	81	776	0.39 (0.05)	0.025 (0.084)	0.88 (0.32)*
MI-T3	81	697	0.31 (0.06)	−0.119 (0.072)	1.48 (0.31)**
MI-T7	83	742	0.37 (0.05)	−0.127 (0.062)	1.39 (0.21)**
EO-D1	57	568	0.26 (0.05)	−0.105 (0.088)	1.50 (0.43)**
EO-D3	60	600	0.40 (0.05)	0.003 (0.075)	0.96 (0.25)*
EO-D5	58	540	0.23 (0.05)	0.007 (0.084)	0.86 (0.72)
MI-D1	58	511	0.39 (0.07)	−0.039 (0.100)	1.10 (0.32)**
MI-D2	53	340	0.21 (0.07)	−0.150 (0.136)	1.79 (2.15)
Mean	699	6445	0.30 (0.02)	−0.040 (0.027)	1.11 (0.29)**

We considered that truncation selection may be particularly likely during seedling establishment (see Dole and Ritland 1993) and winter dormancy and therefore applied this measure of δ to data on seedling height and above-soil dry weight.

RESULTS

Population Genetic Estimates

Estimates of the selfing rate (s), parental fixation index (F), and inbreeding depression (δ) are presented in table 1. All ten populations exhibited substantial levels of self-fertilization (mean $\hat{s} = 0.30 \pm 0.02$ SE, range $= 0.13–0.41$). However, in none of the populations did the parental fixation index differ significantly from zero (mean $\hat{F} = -0.040 \pm 0.027$). Accordingly, estimates of inbreeding depression were high, ranging from 0.49 to 1.79 (mean $\hat{\delta} = 1.11 \pm 0.29$). As is usually the case (see Ritland 1990), standard errors associated with these estimates were large (especially for EO-T3 and MI-D2). Nevertheless, six of ten estimates were significantly greater than zero, and four were significantly greater than 0.5. The 95% percent confidence interval (CI) around the mean of the ten populations was 0.504–1.540. Excluding two populations with particularly large standard errors (EO-T3 and MI-D2), the 95% CI was 0.806–1.357. In both cases, the mean inbreeding depression was significantly greater than 0.5. Estimates for the two populations used in the experimental study (EO-T6 and EO-D1) were not significantly different.

TABLE 2. Analysis of variation in seed set, seed weight, and germination after experimental self- and cross-fertilization of *Decodon verticillatus*. Values are F-ratios. F-tests for population used $MS_{M(P)}$ as the denominator; those for T × M(P) and block used MS_{Error}; all others used $MS_{T \times M(P)}$. MS_{Error} df was 606 for seeds/fruit and seed weight and 360 for germination. Means are in figure 1.

Source of variation (df)	Response variable			
	Seeds/fruit[†] ($r^2 = 0.34$)	Seed weight[‡] ($r^2 = 0.61$)	Early germination[§] ($r^2 = 0.35$)	Late germination[§] ($r^2 = 0.28$)
Population (1)	3.2[NS]	5.9*	1.4[NS]	1.7[NS]
Maternal plant(P) (34)	3.7**	19.2***	3.2**	1.1[NS]
Treatment (1)	18.5***	1.4[NS]	10.4**	6.2*
T × P (1)	2.1[NS]	3.6[NS]	0.3[NS]	1.9[NS]
T × M(P) (34)	1.6*	1.2[NS]	1.2[NS]	1.6*
Block (2)			4.1*	5.4*

NS $P > 0.05$; * $P < 0.05$ (marginally significant); ** $P < 0.005$; *** $P < 0.0005$; **** $P < 0.00005$.

† Log[10] transformed.

‡ Analysis based on ranked data.

§ Proportion of seeds germinating was arcsine transformed ($y = 2\sin^{-1}\sqrt{x}$).
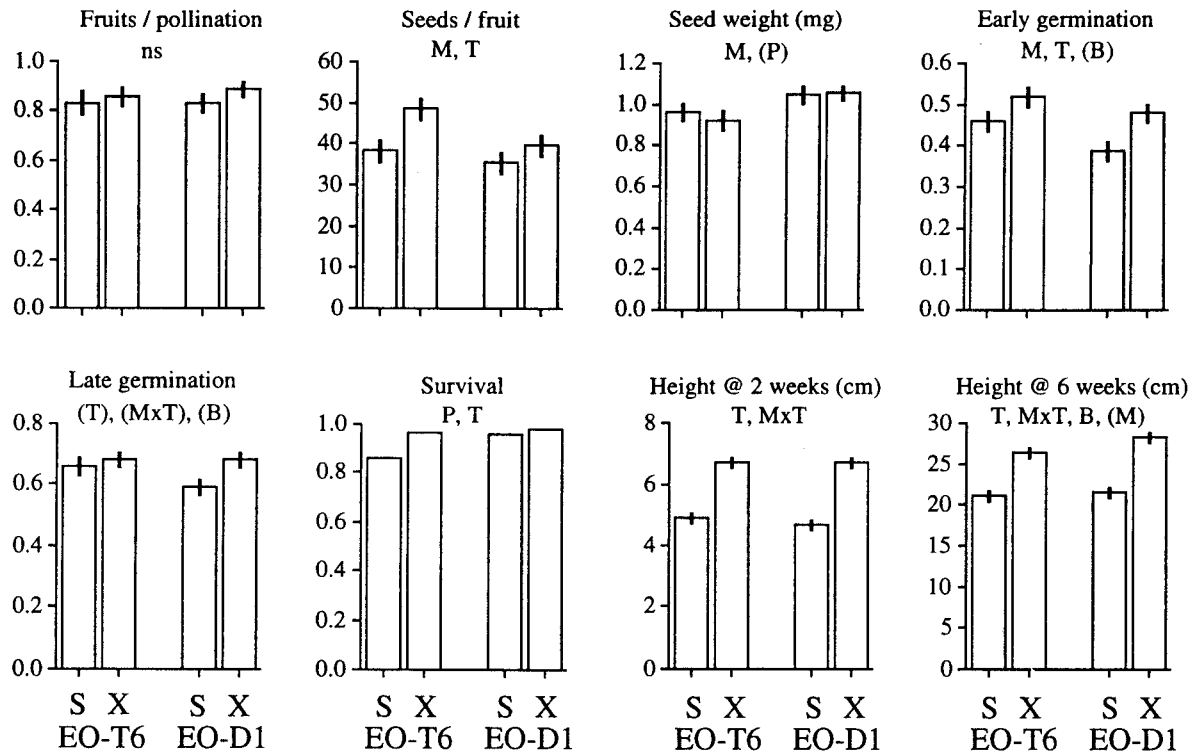
FIG. 1. The fitness consequences of self- (S) and cross-fertilization (X) in two populations of *Decodon verticillatus* measured under uniform glasshouse conditions. Means (± SE, $N \approx 430$) are presented for eight components of fitness. Binomial SEs for survival were all < 0.04. Significant effects in ANOVAs (tables 2 to 4) are shown above bars for each variable (NS, not significant; P, population; M, maternal family, T, pollination treatment; and B, block). Effects in parentheses are marginally significant.

Experimental Comparison of Fitness Components

Fruit and Seed Set. —The proportion of flowers producing fruit after experimental pollination was high (0.85 ± 0.13 SE) and did not differ between treatments or populations (fig. 1, $F = 0.5$; df = 3,68; $P = 0.696$). The number of seeds per fruit varied significantly among maternal plants and was 23 ± 4% ($N = 36$ families) higher for cross-pollinated than self-pollinated fruits. The effect of pollination treatment varies significantly among maternal plants; however, seed set was greater after self- than cross-pollination for only 3 of 36 maternal plants. Average seed weight varied significantly among maternal families but not between treatments.

Germination and Early Growth. —On average, 46% and 65% of seeds had germinated after 2 and 5 wk, respectively (fig. 1, table 2). Levels of both early and late germination were higher for

outcrossed than selfed seed (29 ± 8% higher for early, 13 ± 5% higher for late). Early germination success also varied significantly among families; however, this effect disappeared by late germi-

TABLE 3. Analysis of variation in growth of seedlings from experimental self- and cross-fertilization of *Decodon verticillatus*. Values are *F*-ratios calculated as in table 2. Significance is indicated as in table 2. MS_{Error} df was 1222 for week 2 and 1167 for week 6. Means are in figure 1.

Source of variation (df)	Height at 2 wk‖ ($r^2 = 0.34$)	Height at 6 wk ($r^2 = 0.61$)
Population (1)	0.2^{NS}	3.4^{NS}
Maternal family(P) (34)	1.0^{NS}	1.8*
Treatment (1)	81.9****	122.5****
T × P (1)	0.3^{NS}	1.6^{NS}
T × M(P) (34)	3.4**	1.9**
Block (2)	2.7^{NS}	14.3***

‖ Square-root transformed.

Proportion of progeny flowering: P, T, D

Number of flowering leaf axils

Dry weight (g): T, MxT, D, (DxP)

S   X   S   X   Density
EO-T3   EO-D1   □ High
                   ▣ Medium
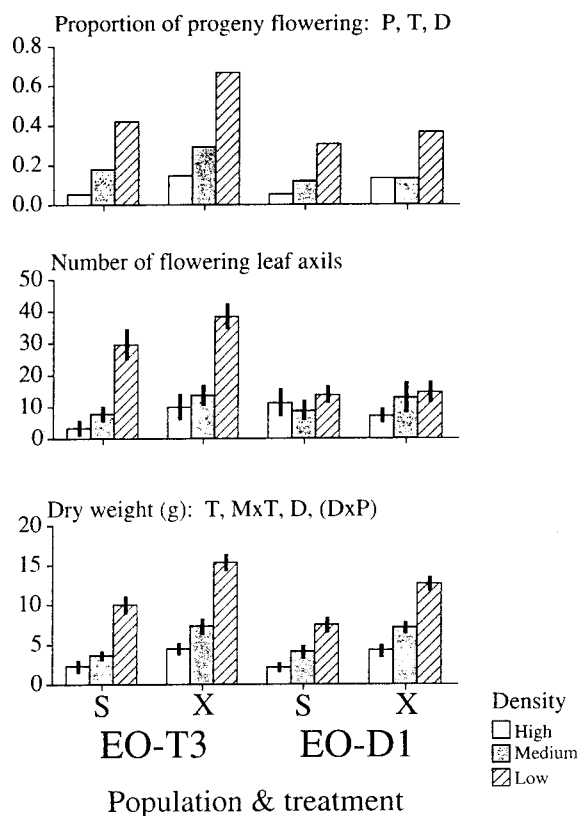Population & treatment   ▨ Low

FIG. 2.   Comparison of growth and flowering between progeny from experimental self- (S) and cross-fertilization (X) of *Decodon verticillatus* grown under three density regimes in the glasshouse. Bars are means ± 1 SE (binomial SEs for the probability of flowering were all < 0.002). Significant effects in ANOVAs for probability of flowering and dry weight (table 4) are shown above the bars. The effects are the same as in figure 1, except that Block has been replaced by Density (D). See text for analysis of flowering leaf axils.

TABLE 4. Analysis of variation in above-soil dry weight of progeny from experimental self- and cross-fertilization of *Decodon verticillatus*. The analysis uses ranked data. The df for MS_{Error} was 1002. Values are F-ratios. Significance is indicated as in table 2. Means are in figure 2.

Source of variation	df	F#
$r^2 = 0.56$		
Population	1	0.5^{NS}
Maternal family(P)	34	1.3^{NS}
Treatment	1	155.8^{****}
T × P	1	0.3^{NS}
T × M(P)	34	2.1^{**}
Density	2	406.6^{****}
D × P	2	3.3^{*}
D × M(P)	68	1.0^{NS}
D × T	2	1.5^{NS}
D × T × P	2	1.0^{NS}
D × T × M(P)	68	0.8^{NS}

\# The F-test for P used $MS_{M(P)}$ as the denominator; that for M(P) used a synthetic denominator consisting of $(MS_{T \times M(P)} + MS_{D \times M(P)} - MS_{D \times T \times M(P)})$; those for T and T × P used $MS_{T \times M(P)}$; those for D and D × P used $MS_{D \times M(P)}$; D × T × M(P) used MS_{Error}; and the remaining effects used $MS_{D \times T \times M(P)}$.

nation. For late germination success, the effect of treatment was only marginally significant, and varied among families, with 11 of 36 showing greater germination success for selfed than outcrossed seed. Growth after transplanting showed stronger inbreeding depression. Plant height at both 2 and 6 wk was significantly higher for outcrossed seedlings (week 2, outcrossed 40 ± 6% larger; week 6, outcrossed 30 ± 3% larger; fig. 1, table 3). The effects of pollination treatment varied among families at both times. However, selfed seedlings were larger than outcrossed seedlings in only one family.

Growth and Flowering at Three Densities.— Survival until harvest was high (94%; fig. 1). Categorical analysis with population, treatment, and density as main effects revealed significant differences between populations (91% of 648 plants from EO-T6 and 97% of 648 from EO-D1; $LR = 20.9$, $P < 0.001$) and between selfed (91% of 648) and outcrossed (97% of 648) offspring ($LR = 25.7$, $P < 0.001$) but not among densities. Of the 1219 surviving plants, 24% flowered by harvest (fig. 2). The probability of flowering differed among densities (10% of 407 in high, 18% of 406 in medium, and 44% of 406 in low density; $LR = 148.5$, $P < 0.001$), populations (30% of 591 from EO-T6 and 19% of 628 from EO-D1; $LR = 29.6$, $P < 0.001$), and pollination treatments (19% of 589 selfed and 29% of 630 outcrossed offspring; $LR = 19.0$, $P < 0.001$). The binomial SE of the means reported above were all less than 0.07%.

The analysis of flower production (indexed by the number of flowering leaf axils) was complicated by large differences among densities in the proportion of plants flowering. Only 40 of 432 and 73 of 432 plants reached flowering under high- and medium-density conditions, respectively. Pooling across populations and maternal plants to increase sample sizes, the data suggest higher flower production by outcrossed progeny at both high (outcrossed = 8.8 ± 0.5 flowering leaf axils, $N = 30$; selfed = 7.4 ± 0.5, $N = 10$) and medium density (outcrossed = 13.5 ± 0.8, $N = 44$; selfed = 8.2 ± 0.4, $N = 29$), but neither difference neared significance (high: $F = 0.3$, df = 1,38; $P = 0.609$; medium: $F = 3.6$; df = 1,71;

$P = 0.061$). Although more plants flowered under low-density conditions, there was no difference in flower production between selfed (22.1 ± 1.4, $N = 71$) and outcrossed (23.4 ± 1.5, $N = 108$) offspring (two-way ANOVA with population and pollination treatment as main effects—population: $F = 22.0$; df = 1,175; $P < 0.001$; treatment: $F = 0.0$; df = 1,175; $P = 0.992$).

Differences between selfed and outcrossed progeny were most pronounced for above-soil dry weight (fig. 2). On average, outcrossed plants were 130 ± 20%, 112 ± 16%, and 79 ± 12% heavier than their selfed counterparts under high, medium, and low densities, respectively. Although the magnitude of this difference was largest under the highest density, there was no significant treatment by density interaction (table 4). Again, the variation in treatment effects among families neared significance, but the average dry weight of selfed offspring exceeded that for outcrossed offspring in only 2 of 36 families. Above-soil dry weight was correlated with both flowering variables. Analyzing separately each density by treatment combination, progeny that flowered were 1.5- to 2-fold heavier than those that did not (all contrasts significant at $P < 0.001$). Among flowering offspring, heavier plants tended to produce more flowering leaf axils, however, log-log regressions were significant only for low-density plants (selfed: $r^2 = 17.0\%$; $F = 14.2$; df = 1,69; $P = 0.0003$; outcrossed: $r^2 = 21.6\%$; $F = 29.2$; df = 1,106; $P < 0.00001$).

Survival Outdoors.—Survival of plants in medium- and high-density garden arrays was high (94%, table 5). Categorical analysis with density, population, and pollination treatment as main effects detected significant differences between treatments (selfed = 90%, outcrossed = 97%; $LR = 21.1$, $P < 0.0001$) and populations (EO-T6 = 92%, EO-D1 = 95%, $LR = 4.8$, $P = 0.029$) but not densities (all SE's for % survival < 0.02%). Survival of plants transplanted into natural habitats was substantially lower (67%, table 6) and varied significantly between sites (inhabited = 74%, uninhabited = 59%; $LR = 9.9$, $P = 0.002$) and treatments (selfed = 56%, outcrossed = 76%; $LR = 17.8$, $P < 0.0001$). The effect of population was marginally significant (EO-T6 = 62%, EO-D1 = 71%; $LR = 3.7$, $P = 0.053$; all SEs < 0.2%).

Measures of Inbreeding Depression.—Inbreeding depression estimated from multiplicative survival in the glasshouse was moderate ($\delta = 0.27$ ± 0.05, fig. 3). After two summers in garden arrays the relative survival of selfed offspring was
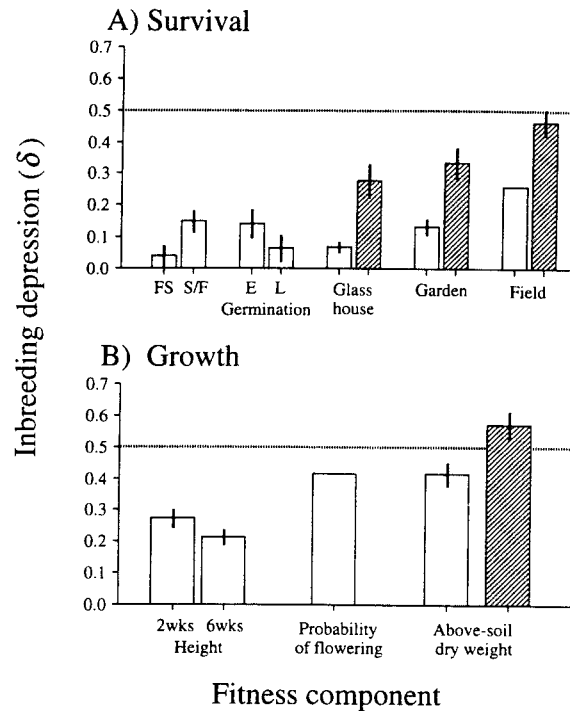

A) Survival

B) Growth

Fitness component

FIG. 3. Experimental estimates of inbreeding depression (δ) in *Decodon verticillatus* under glasshouse, garden, and field conditions. In (A), mean δ (± SE, $N = 36$ families) is presented for six components of survival: fruits set (FS), seeds per fruit (S/F), early (E) and late (L) germination, survival in the glasshouse, garden arrays, and field sites. Multiplicative survival (hatched bars) is also presented for glasshouse plants at harvest and for garden and field plants two summers later. In (B), estimates of δ are presented for four components of offspring vigor measured in the glasshouse: height at 2 and 6 wk, probability of flowering in the first year and final above-soil dry weight. A composite measure of δ (hatched bar) was derived by combining multiplicative glasshouse survival and above soil dry weight. The hatched line at $\delta = 0.5$ indicates the threshold level of inbreeding depression below which increased selfing should evolve.

further reduced ($\hat{\delta}$, medium density = 0.31 ± 0.05; high density = 0.35 ± 0.05). Stronger inbreeding depression was expressed under field conditions. Multiplying the mean multiplicative survival of each family by relative survival at the two field sites produced estimates of $\hat{\delta} = 0.39$ ± 0.04 for the inhabited site and $\hat{\delta} = 0.54$ ± 0.03 for the uninhabited site. Under glasshouse conditions, inbreeding effects were stronger for growth variables than survival and tended to increase throughout the course of the experiment (fig. 3). A composite measure of δ was calculated for each family by multiplying multiplicative survival by their respective family means for the final measure of offspring vigor (above-soil dry
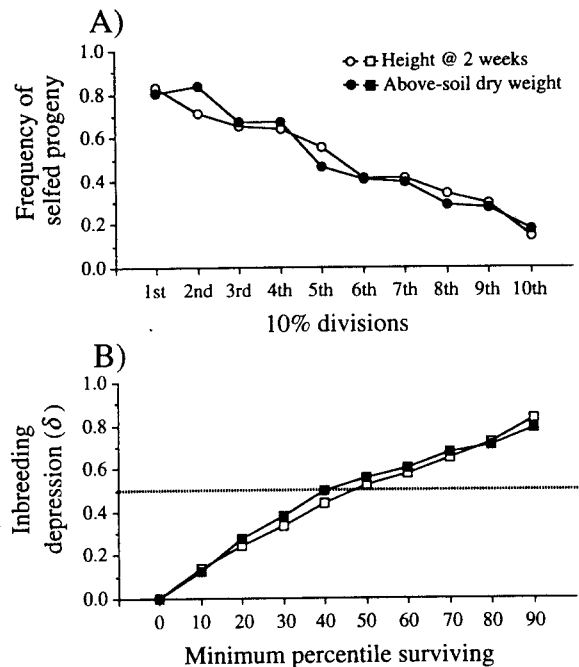
A)

B)

FIG. 4. Levels of inbreeding depression arising from truncation selection on offspring vigor: (A) the representation of selfed progeny across the overall distribution of seedling height and dry weight. The first 10% division includes the lowest trait values; the tenth includes the highest. (B) the value of δ arising from truncation selection at the various percentiles. The hatched line indicates $\delta = 0.50$. The total distribution involves 1296 plants ($\approx$ 130 per 10% division). The values for each trait are averages over density regimes because there was no difference in the proportional distribution of selfed progeny across percentiles among densities.

weight). This measure represents the relative amount of surviving biomass from each self-versus outcross pollination, and indicates that substantial inbreeding depression ($\delta > 0.5$) was expressed in the first year of growth. Estimates of δ based on family means could not be calculated for other measures of vigor such as the probability of flowering and number of flowering leaf axils, because in some families, no progeny flow-

ered (i.e., $\omega_s = \omega_x = 0$). Estimates calculated using the overall proportion of selfed and outcrossed offspring flowering were 0.64, 0.30, and 0.30 for the high-, medium-, and low-density regimes, respectively. Estimates of δ for flower production involving only those progeny that flowered were 0.16, 0.39, and 0.06.

The fitness consequences of reduction in vigor because of inbreeding were also assessed by examining the relative frequency of selfed offspring in the upper percentiles of the distributions for two growth variables. In figure 4, we have plotted both the frequency of selfed offspring in each 10% division of the distribution of seedling height and above-soil dry weight along with the values of δ arising from different intensities of truncation selection. For both variables, the proportion of selfed offspring declines steadily towards the upper percentiles of the distribution. However, the proportional representation of selfed offspring in each division is roughly the same for both variables, even though the proportional difference between the means is much higher for dry weight ($\hat{\delta} = 0.41$, averaged across densities) than seedling height ($\hat{\delta} = 0.27$). In addition, the frequency of selfed offspring across the percentiles of dry weight did not differ among density treatments. Calculating δ for truncation selection in which only the top 10% survive, $\hat{\delta}_{10\%} = 0.83$ for seedling height and $\hat{\delta}_{10\%} = 0.78$ for dry weight. Even relatively relaxed selection in which the top 50% survive results in $\hat{\delta} > 0.50$ (fig. 4).

DISCUSSION

Populations of Decodon verticillatus exhibit moderate levels of self-fertilization, yet severe reductions in the fitness of selfed compared to outcrossed progeny were indicated by both population-genetic and experimental measures of inbreeding depression. Below we discuss these results and the assumptions underlying the population-genetic and experimental approaches

TABLE 5. Survival of progeny from experimental self- and cross-fertilization of Decodon verticillatus after two consecutive growing seasons in natural habitats and an outdoor garden. Values are the proportion of plants surviving, with sample sizes in parentheses.

Population	Treatment	Field site or garden density			
		Inhabited	Uninhabited	Medium	High
		Natural habitats		Outdoor garden	
EO-T6	Selfed	0.60 (40)	0.46 (50)	0.89 (94)	0.86 (94)
	Crossed	0.79 (53)	0.62 (50)	0.95 (103)	0.96 (104)
EO-D1	Selfed	0.73 (53)	0.46 (50)	0.95 (103)	0.88 (102)
	Crossed	0.81 (53)	0.82 (50)	0.99 (106)	0.99 (106)

used in this study. In addition, we consider the implications of these results for mating-system evolution in *D. verticillatus*.

Population Genetic Estimates of Inbreeding Depression

The high estimates of inbreeding depression obtained using Ritland's (1990) equilibrium approach arise because of an association between moderate levels of self-fertilization, on the one hand, and estimated population genetic structures among parental plants conforming to Hardy-Weinberg genotype frequencies (i.e., $\hat{F} \approx 0$), on the other. Similar findings are known for other species (*Limnanthes douglasii* var. *rosea*: $\hat{s} = 0.18$, $\hat{F} = -0.07$ and *L. douglasii* var. *nivea*: $\hat{s} = 0.18$, $\hat{F} = 0.01$ — Kesseli and Jain 1985; *Echium plantagineum*: $\hat{s} = 0.27$, $\hat{F} = 0.06$ — Burdon and Brown 1986; *Larix laricina*: $\hat{s} = 0.27$, $\hat{F} = -0.07$ — Knowles et al. 1985; and *Plantago coronopus*: $\hat{s} = 0.23$, $\hat{F} = 0.03$ — Wolff et al. 1988). In these cases, the products of self-fertilization contribute little to the genetic structure of the adult population; hence, the relative fitness of selfed offspring is essentially zero.

The main drawback of this inferential approach is that individual population estimates of δ often possess large standard errors. However, when progeny array data are available for a large sample of populations, one can obtain a fairly robust estimate of δ that reflects selective processes occurring in nature. Estimates for *D. verticillatus* agree with the experimental results in showing strong inbreeding depression but further suggest that the experimental approach may have underestimated the magnitude of δ. Evidence for stronger inbreeding depression under field than glasshouse conditions comes from several studies (reviewed in Barrett and Kohn 1991). In this study, population genetic estimates of δ potentially include differential survival during severe episodes of selection that could not be simulated in the glasshouse, as well as the expression of genetic load during later life-history stages (Johnston 1992). However, because the average relative fitness of selfed offspring was zero, most of the selection against selfed offspring is probably occurring before reproductive maturity (Dole and Ritland 1993).

Estimates of δ from population genetic data are based on three major assumptions: (1) the population is at inbreeding equilibrium; (2) the inbreeding coefficient is affected mainly by consanguineous matings; and (3) the selfing rate and inbreeding coefficient do not vary greatly between years. Population genetic studies of morph-frequency variation in *D. verticillatus* (Eckert and Barrett 1992, 1995) suggest that some populations may deviate from equilibrium genetic structure because of founder effects and genetic drift. However, the biases introduced by departures from inbreeding equilibrium depend not so much on whether a given population is at equilibrium but on whether the inbreeding coefficient is currently increasing or decreasing. If F is increasing, then $\hat{\delta}$ is biased upwards; if F is decreasing, then $\hat{\delta}$ is biased downwards. It is unclear which of these situations would be more or less common in populations of *D. verticillatus*. However, estimating δ from many populations reduces the importance of the departures of individual populations from equilibrium. Violation of the second assumption will occur if genetic drift is making a significant contribution to the level of inbreeding within populations, which should bias $\hat{\delta}$ downwards. Fluctuations in s and F among years will have similar effects. Following Ritland (1990), we assessed the effect of these fluctuations by assuming that variation in s and F among years within a population is no greater than that observed among populations. The maximum bias calculated from the parametric variance in $\hat{s}$ and $\hat{F}$ among populations was small (-0.016); hence, the actual bias is probably negligible.

Experimental Analysis of Inbreeding Depression

Experimental results showed that genetic load in *D. verticillatus* was expressed throughout the first year of growth in the glasshouse. Seed set and germination were 10%–15% lower after self- compared with cross-pollination. Outcrossed seeds appeared to germinate earlier than their selfed counterparts, which may provide an advantage during seedling competition in nature (Waller 1985; Schmitt and Ehrhardt 1990). After germination, growth differences between selfed and outcrossed seedlings became more exaggerated with time, culminating in a close to two-fold difference in above-soil dry weight. Inbreeding also led to a small but significant decrease in survival and a large decrease in the probability of flowering. Differences in the production of flowering leaf axils between selfed and outcrossed offspring were less pronounced.

Survival of year-old plants over two summers in natural habitats and garden arrays indicated

that inbreeding depression is expressed beyond the early life-history stages. As in other studies (Schemske 1983; Dudash 1990), harsher conditions in the field led to greater reductions in the relative survival of selfed offspring ($\hat{\delta}$, field = 0.26, garden = 0.08). Moreover, there existed some indication that inbreeding was expressed more strongly in the uninhabited ($\hat{\delta}$ = 0.36) compared with the inhabited site ($\hat{\delta}$ = 0.16), but the contrast between sites was not significant (see Schemske 1983).

Our experimental data strongly suggest that selfed offspring are less than half as fit as their outcrossed counterparts; however, the populations genetic analysis indicates much stronger inbreeding depression and that selfed offspring rarely survive to reproduce. Comparison of experimental and population-genetic estimates of δ in *Mimulus* show a similar pattern (Ritland 1990; Dole and Ritland 1993; Latta and Ritland 1993). The discrepancy between experimental and population-genetic results is important because if $\delta \approx 0.5$ there is little or no selection on the selfing rate, whereas if $\delta \approx 1.0$ there is strong selection against self-fertilization. Glasshouse studies may often underestimate the component of δ expressed during severe episodes of selection that are likely to occur during seedling establishment and overwintering; especially in populations of long-lived perennials (Harper 1977). The few studies that have examined early life-history stages in the field suggest that levels of mortality may be extremely high under natural conditions; thus large sample sizes are required to obtain a statistically robust estimate of δ (Schemske 1983; Kohn 1988; Ashman 1992). We addressed this problem by formulating an alternative measure of δ based on truncation selection rather than a linear relationship between offspring vigor and fitness. Application of this percentile-based measure to growth data from our glasshouse experiment shows that even relatively relaxed truncation selection may substantially decrease the relative fitness of selfed offspring. Hence, the mode of selection may greatly influence the ultimate fitness consequences of differences in vigor between selfed and outcrossed progeny.

Implications for Mating-System Evolution

Theoretical models of coevolution between self-fertilization and inbreeding depression have shown that the evolutionary fate of alleles affecting the selfing rate will depend on the magnitude and nature of their effects as well as on the strength and genetic basis of inbreeding depression (Lande and Schemske 1985; Uyenoyama 1986; Holsinger 1988; Charlesworth and Charlesworth 1990; Charlesworth et al. 1990, 1991, 1992; Uyenoyama and Waller 1991a, 1991b, 1991c). If inbreeding depression is caused primarily by the expression of recessive mutations (rather than heterozygote advantage), levels of δ greater than 0.5 are considered sufficient to discourage the evolution of self-fertilization. The data presented here clearly indicate that inbreeding depression in populations of *D. verticillatus* exceeds this threshold value, despite significant levels of self-fertilization. Strong inbreeding depression in partially selfing populations has generally been interpreted as evidence that genetic load involves mildly deleterious alleles at many loci rather than relatively few recessive lethals or strongly deleterious mutations (e.g., Kohn 1988; Barrett and Charlesworth 1991; Johnston 1992; Ågren and Schemske 1993; Wolfe 1993; Latta and Ritland 1993).

Although this study primarily concerns the maintenance of inbreeding depression in partially selfing populations, our results also raise questions concerning the adaptive significance of self-fertilization in *D. verticillatus*. Why are 30% of all offspring produced by self-fertilization if their fitness is more than halved by strong inbreeding depression? Recent theory suggests that the evolution of self-fertilization may depend on the proximate ecological, morphological, and physiological mechanisms that determine how and when selfing occurs, in addition to the balance between the cost of meiosis and inbreeding depression (Lloyd 1979, 1980, 1992; Lloyd and Schoen 1992). For example, selfing may be favored, despite strong inbreeding depression, if it occurs after opportunities for outcross pollination have passed. Although *D. verticillatus* does not possess obvious floral mechanisms for delaying self-pollination, outcross pollen enjoys a substantial siring advantage over self pollen (Eckert and Barrett 1994b), which may effectively restrict self-fertilization to occasions when there is insufficient outcross pollen for full seed set. Partial selfing may also be a nonadaptive consequence of geitonogamous pollen transfer, which may commonly occur in mass-flowering species like *D. verticillatus* with extensive clonal spread (Handel 1985; Schemske and Lande 1985; Aide 1986; Lloyd 1992). Experimental studies of the proximate mechanisms controlling the amount and timing of self-fertilization are required to evaluate these hypotheses (e.g., Dudash and Ritland 1991; Schoen and Lloyd 1992; Bar-

rett et al. 1994). In the long-term, it is likely that only integrated ecological and genetic approaches will provide the information necessary to understand the complex forces maintaining partial selfing in *D. verticillatus* and other species with strong inbreeding depression.

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