

EFFECTS OF INBREEDING ON TRAITS THAT INFLUENCE DISPERSAL AND PROGENY DENSITY IN *CAKILE* *EDENTULA* VAR. *LACUSTRIS* (BRASSICACEAE)¹

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Inbreeding may influence the intensity of sibling competition by altering the number of offspring produced or by changing plant morphology in ways that influence seed dispersion patterns. To test this possibility, effects of inbreeding on seed production and on traits that influence progeny density were measured using experimental pollinations of flowers of *Cakile edentula* var. *lacustris*. Different flowers on a plant were either hand pollinated with self pollen (with and without emasculation) or foreign pollen, or they were allowed to be pollinated naturally. Selfed flowers matured significantly fewer viable seeds than outcrossed flowers (10.3% less seed maturation with inbreeding depression of 19.2%), due in large part to a greater percentage of proximal seed abortions and lower germination success. Plants grown from selfed seeds tended to have lower seed production (37 fewer seeds on average, with inbreeding depression of 16.2%), caused in part by an increase in the percentage of fruits with proximal seed abortions, although this effect was not significant. Inbreeding depression in total fitness was 29.0%, which corresponds to a difference of 46 seeds per pollinated ovule. Selfing rate estimates were usually intermediate to high, indicating that inbreeding effects observed in this study would be present in naturally pollinated progeny. Although the influence of inbreeding directly on dispersal was negligible, the predicted reduction in sibling competition caused by reduced seed production resulted in an estimate of inbreeding depression of 17.5%, which is 11.5% lower than that measured under uniform conditions. Consequently, inbreeding depression estimated under natural dispersion patterns may be lower than that estimated under uniform conditions since seeds from self- and cross-pollination may not experience the same competitive environment in the field. Inbreeding in the maternal generation, therefore, could influence progeny fitness not only by determining the genetic composition of progeny, but also by influencing the competitive environment in which progeny grow.

Key words: Brassicaceae; *Cakile edentula*; density; dispersal; inbreeding; inbreeding depression; maternal effects; maternal environmental effects.

Plants resulting from inbreeding often have lower biomass and lower fitness compared to plants resulting from outcrossing (e.g., Schemske, 1983; Schoen, 1983; Waller, 1984; Levin, 1989, 1991; Dudash, 1990; Holtsford and Ellstrand, 1990). Many traits that are associated with fitness and biomass also influence seed or fruit dispersal. Such traits may be height, branchiness, number of seed abortions, or seed size (e.g., Cremer, 1977; Rabinowitz and Rapp, 1981; Augspurger and Hogan, 1983; McCanny and Cavers, 1989; Donohue, 1993). Abundant seed crops are often associated with higher progeny density after dispersal (Platt, 1976; Rabinowitz and Rapp, 1981; McCanny and Cavers, 1989; Donohue 1993). Traits that influence dispersal and progeny density are therefore expected to show some effects of inbreeding.

Effects of inbreeding on dispersal and progeny density

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could influence the relative fitness of selfed and outcrossed progeny since dispersal itself often is not selectively neutral, and since progeny density often influences progeny fitness (Gadgil, 1971; Janzen, 1971, 1972; Liew and Wong, 1973; Burdon and Chilvers, 1975; Platt, 1976; Bullock, 1981; Howe and Smallwood, 1982; Augspurger, 1983a, b; Horvitz and Schemske, 1986; Howe, 1989). If inbred and outcrossed progeny are dispersed differently or grow at different densities, and if dispersal or density influences fitness, then the realized fitness difference between inbred and outcrossed progeny would differ from estimates of inbreeding depression that omitted the fitness effects of dispersal and density (Lloyd, 1980; Holsinger, 1986).

Inbreeding can potentially influence dispersal or abundance either of the inbred seeds themselves, by altering morphology, mass, or seed maturation within the propagule, or it could influence dispersal or density of the propagules of plants grown from inbred seeds by altering plant morphology or the morphology, mass, or abundance of fruits on these plants. Thus, dispersal and density of two generations of progeny could be influenced by inbreeding in one generation, depending on the life stage in which inbreeding depression is expressed. Altering the dispersal or density of grandprogeny could lead to time lags in fitness differences associated with inbreeding. If inbreeding does influence dispersal or progeny density, then inbreeding could have fitness consequences to progeny and grandprogeny through an ecological mechanism

TABLE 1. Inbreeding depression estimates for fitness and dispersal. ndf = numerator degrees of freedom, ddf = denominator degrees of freedom. $N = 555$ for the last three characters.

Character	df	Statistic	<i>P</i>	δ
Total lifetime fitness	17	$t = 2.18$ (outcross–self)	0.044*	0.290
First-generation viable seed maturation/ovule	17	$t = 2.65$ (outcross–self)	0.024*	0.192
Second-generation fruit segments/germinant	ndf = 1 ddf = 14	$F = 2.49$	0.137	0.162
Dispersal factor 1 = size	ndf = 1 ddf = 14	$F = 2.43$	0.143	–1.447
Dispersal factor 2 = compactness	ndf = 1 ddf = 14	$F = 0.07$	0.790	0.975

* Significant using sequential Bonferroni criterion (see text for justification of Bonferroni criteria).

as well as the genetic mechanism of increased homozygosity, which is usually considered to be the sole cause of inbreeding effects on fitness (Lloyd, 1980; Holsinger, 1986; Charlesworth and Charlesworth, 1987).

Below, I report measures of seed production and traits that influence dispersal in *Cakile edentula* var. *lacustris* plants produced from self pollination, cross pollination, or natural pollination of flowers in the field. This study had the following goals: (1) to estimate the total magnitude of inbreeding depression on fitness, and to identify the stage at which inbreeding depression is expressed; (2) to estimate the magnitude and direction of inbreeding effects on traits that influence dispersal and progeny density; (3) to determine whether inbreeding depression acts independently on each trait; (4) to estimate the frequency of self pollination in the field in order to determine how common the observed effects of inbreeding may be in natural situations; and (5) to estimate the effects of inbreeding on progeny density and on fitness differences between selfed and outcrossed progeny after dispersal, based on previous studies of dispersal and selection on seed dispersion patterns in this system (Donohue, 1993, 1997).

MATERIALS AND METHODS

The study organism—*Cakile edentula* (Bigel.) Hook var. *lacustris* (Brassicaceae) (*C. edentula* hereafter), or the Great Lakes sea rocket, is an annual plant that grows along the shores of the Great Lakes. *C. edentula* has segmented fruits, with a proximal segment and a deciduous distal segment. Distal fruit segments detach more readily and are dispersed farther and to lower densities than are proximal segments, which often stay attached to the dead maternal plant through germination (Donohue, 1993). Seed abortions are common and result in a fruit with either only a proximal segment (usually <5% of all fruits), or more commonly, a fruit with only a distal segment (~40% of all fruits). Therefore, seed abortion could influence the proportion of fruit segments on a plant that is dispersed. In *C. edentula*, taller, more sparsely branched plants with a greater percentage of fruits on their primary stem had impeded dispersal, and plants with more fruit segments had denser seed shadows (Donohue, 1993). Traits of size, shape, and seed maturation, therefore, influence dispersal in *C. edentula*. The species is highly self compatible, with high rates of autogamy in the greenhouse.

Experimental methods—Eighteen plants of *C. edentula* growing at the Indiana Dunes National Lakeshore were arbitrarily selected for the study. Plants that had at least four flowering branches were chosen. The plants were located, on average, ~15 m apart, and the entire sampling area covered a strand of beach ~0.5 km in length.

Four pollination treatments were performed. One, two, or three branches were chosen for each treatment, depending on the number of available branches. In all treatments, flowers and fruits already produced were removed so that the pollination of all flowers on that branch could be controlled. To minimize this mutilation, younger branches with many buds were chosen for all manipulations.

In the first pollination treatment, referred to as the selfed-emasculated treatment, the branch was bagged, and buds nearing maturity were emasculated well before anther dehiscence. On the first day that the flowers opened, the stigmas were brushed with mature pollen from flowers elsewhere on the plant (on nonexperimental branches). Pollination was repeated on the following two days in order to ensure that pollination occurred when the stigma was receptive. The branch remained bagged until fruit maturation. In the outcrossed treatment, the branch was also bagged, and the buds were emasculated. When the flowers opened, they were brushed with mature anthers from five plants located within a 10-m radius of the plant. This distance is within the radius in which cross pollination is likely to occur in this system, based on casual observations of insect visitors to the plants. Cross pollination was repeated for the next two days. The branch remained bagged until fruit maturity. In the open-pollinated treatment, the branches were not initially bagged or emasculated. This treatment represented natural pollination, so insect visitation was not inhibited. Soon after the flowers showed signs of being pollinated, as evidenced by swelling of the receptacle, the spent flower was emasculated to control for the effects of emasculation among treatments. After all flowers on the branch had bloomed, the branch was bagged to control for the effects of bagging among treatments. To test for the effects of emasculation, a fourth pollination treatment was performed. In the selfed treatment (different from the selfed-emasculated treatment), the buds were not emasculated until after the flowers had been pollinated and showed signs of fertilization. This treatment, resembling the open-pollinated treatment, could then be compared to the selfed-emasculated treatment to determine whether there was any effect of emasculation.

The following information was recorded for each branch: number of flowers pollinated, number of flowers that produced mature fruits, number of distal seed abortions, and number of proximal seed abortions.

After the fruits had ripened, up to 15 seeds from each treatment on each plant were randomly selected to grow in the greenhouse. To induce germination, the seed coats were slit, and the seeds were allowed to soak in water for 12 h. Then the seed coats were dissected off and the embryos planted in individual plug trays in a mixture of Pro-Mix (Premier Brands, an artificial soil) and Terra-green (Oil-Dry, Inc., baked clay). Germination success was recorded. After ~2 wk, when the first true leaves appeared, the seedlings were transplanted into clay pots (10 cm [4 inch]) filled with Terra-green and randomly arranged on a greenhouse bench. Plants were watered as needed and fertilized once a week.

The following traits that influence dispersal and seed maturation were recorded on adult plants: height, number of secondary branches per

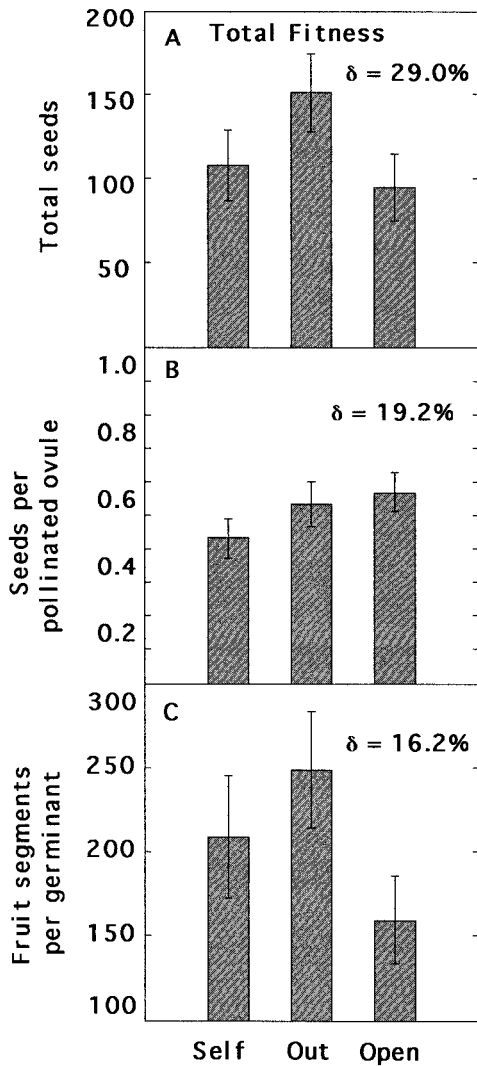


Fig.1. Means and standard errors of total fitness, viable seed maturation from pollinated ovules, and fruit segment production of plants grown from germinated seeds for selfed, outcrossed, and open-pollination treatments.

centimeter of primary stem, number of tertiary and higher level branches, percentage of fruits that were located on the primary stem, the number of fruits produced, the number of distal seed abortions, and the number of proximal seed abortions. Each fruit segment typically has only one seed, with <2% of all fruit segments containing more than one seed. The number of fruit segments, therefore, closely approximates the number of seeds produced. Total fitness was estimated as the total number of fruit segments produced by each pollinated ovule, assuming two viable ovules per flower, as is typically observed (Rodman, 1974; Donohue, 1993). Because this plant is highly selfing in the greenhouse, and because fruits are usually single-seeded, the total number of fruit segments produced closely approximates total reproductive success. It should be noted that two sets of measurements of fruit production, distal seed abortions, and proximal seed abortions exist in this study. The first set pertains to the flowers that were pollinated, and thus the measurements represent the number of pollinated flowers that successfully matured fruits, the number of flowers that matured fruits with distal seed abortions, and the number of flowers that matured seeds with proximal seed abortions, respectively. The second set pertains to plants grown from successfully matured seeds of the experimentally pollinated flow-

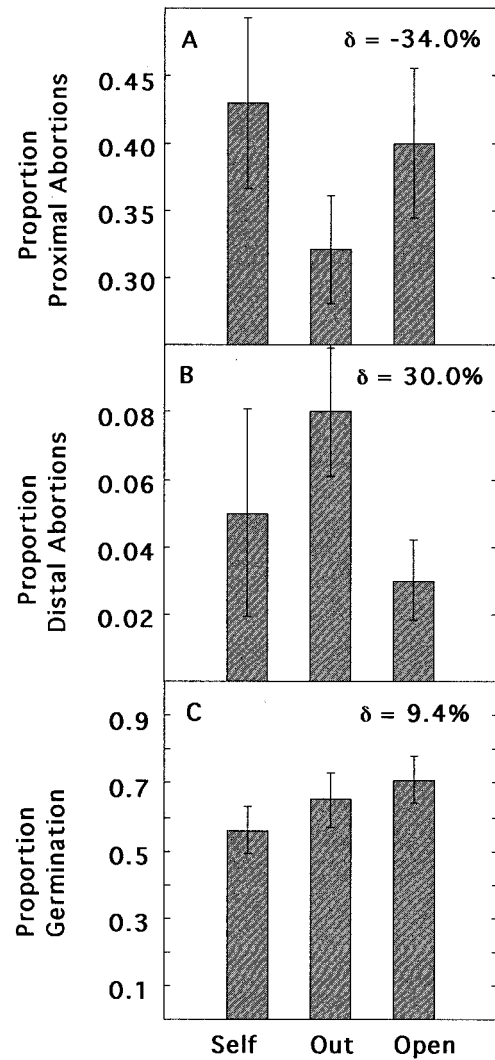


Fig. 2. Means and standard errors of traits of seeds from flowers that were experimentally selfed, outcrossed, or open pollinated .

ers, and the measurements represent the number of fruits produced by the plant, the number of fruits with distal seed abortions on that plant, and the number of fruits with proximal seed abortions on that plant, respectively. Therefore, in contrast to previous measures of inbreeding depression, the measure of inbreeding on “total fitness” includes two generations of inbreeding effects—the first being viable seed maturation from ovules, and the second being seed maturation by a mature plant grown from those viable seeds.

Data analysis—Family mean phenotypic values were calculated for siblings sharing a pollination treatment to avoid pseudoreplication within a family. The effects of inbreeding on viable seed maturation of pollinated flowers and total fitness were tested by comparing family mean values of the self-emasculated and the outcrossed treatments with paired *t* tests. Principal components analysis was performed on the sample of adult plants to identify the two major axes of variation in morphological traits that influence dispersal. The first two principal components and seed production by adult plants were compared between self-pollinated (emasculated) and outcrossed treatments using a mixed-model analysis of variance, with maternal sibship as the random factor, and pollination treatment as the fixed factor. Bonferroni criteria were applied based on the observation that first-generation seed maturation

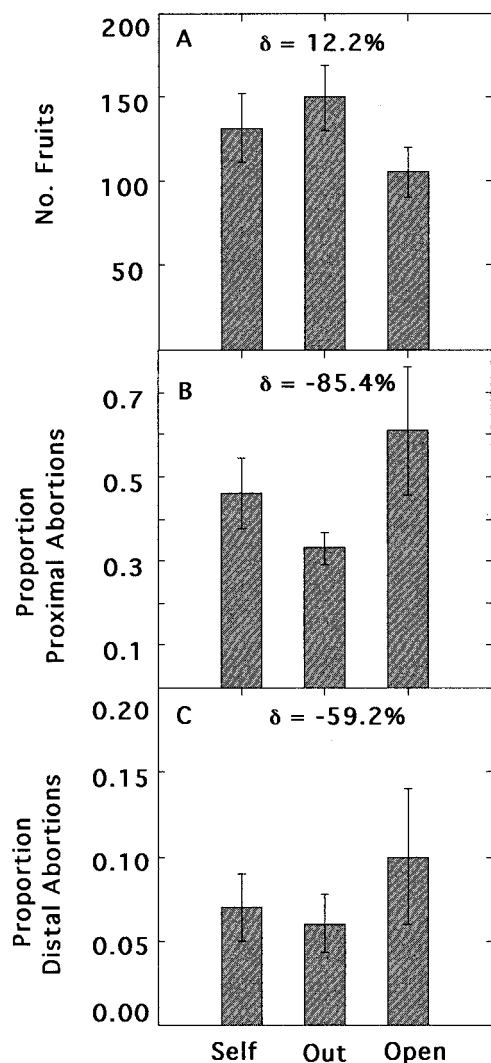


Fig. 3. Means and standard errors of fitness components of plants grown from seeds that matured from flowers that were experimentally selfed, outcrossed, or open pollinated.

and total fitness were calculated based on the sample of all pollinated ovules and used family mean values, and can therefore be considered to be the same sample. However, second-generation seed maturation and dispersal traits were calculated from a random sample of those seeds that germinated, and individual plant values rather than family mean values were used in the analyses. The latter measures were not included in Bonferroni corrections for seed maturation and total fitness, since they used a different random sample and a different analysis. Uncorrected probabilities are provided, however.

The magnitude of inbreeding effects on each trait was defined as $\delta = (p_{out} - p_{self})/p_{out}$, where p_{out} is the family mean phenotypic value of traits on plants grown from cross-pollinated seeds, p_{open} is the family mean phenotypic value of the trait on plants grown from open-pollinated seeds, and p_{self} is the family mean phenotypic value of the trait on plants grown from self-pollinated seeds.

Pearson correlations were calculated for the magnitude of difference in traits of selfed and outcrossed progeny. Mean values of maternal sibships were calculated for fitness and dispersal traits for the selfed and outcrossed pollination treatments, and the difference in the values was used to calculate the correlation coefficients among traits. If inbreeding influences each trait independently, as was shown to be the

TABLE 2. Principal components analysis of dispersal traits.

Trait	PC1	PC2	PC3	PC4
Height	0.40	0.77	0.28	-0.43
No. secondary branches/cm	0.43	-0.62	0.59	-0.29
% Primary fruits	-0.52	0.20	0.75	0.36
Total no. branches	0.62	0.09	0.03	0.78
% Variance explained	50.2	24.2	16.8	8.8

case for some traits in *Salvia pratensis* (Ouborg and Van Treuren, 1994), then correlations would not be significantly different from zero.

Estimates of selfing rates were calculated using the method of Charlesworth (1988). To test for an effect of emasculation, family mean values of the selfed and selfed-emasculated treatments were compared using paired t tests. Only traits that showed no effect of emasculation were used for further analysis. Moreover, traits were excluded from this analysis if open-pollinated samples had phenotypic values that were not intermediate between selfed and outcrossed samples within a range permitted by random error. Selfing rate is defined as: $S = (p_{out} - p_{open}) / (p_{out} - p_{self})$, where p_{out} , p_{open} , and p_{self} are defined as before. The sampling variance of the selfing rate was calculated using Bulmer's (1985) delta method of determining the variance of functions with three variables. In addition to these estimates of selfing rate based on the population sample, traits that showed significant inbreeding effects within individual plants (as indicated by t tests comparing mean values of self-emasculated and outcrossed treatments within a family) were used to estimate the selfing rate of individual plants.

RESULTS

Inbreeding significantly reduced total fitness by an average of 46 seeds per pollinated ovule, with 29.0% inbreeding depression in total fitness (Table 1, Fig. 1). This reduction in fitness of selfed ovules was due to a significant decrease in the proportion of self-pollinated flowers that matured seeds that were viable through germination (10.3% fewer, $\delta = 19.2\%$) and a nonsignificant reduction in fruit segment maturation in plants grown from selfed seeds (37 fewer seeds, $\delta = 16.2\%$). The difference in viable seed production from ovules can be attributed to an increase (12.0%, $\delta = -34.0\%$) in the percentage of flowers that aborted their proximal seeds and a slight decrease in the percentage of selfed seeds that germinated (6.2%, $\delta = 9.4\%$; Fig. 2). The trend for decreased fruit segment production in adult plants growing from selfed seeds was due to a decrease in fruit production (15 fruits, $\delta = 12.2\%$) and an increase (13.0%, $\delta = -85.4\%$) in proximal seed abortions within those fruits (Fig. 3). Therefore, inbreeding appears to influence fitness most strongly at early life stages in this system, particularly at the stage of ovule maturation.

Principal components analysis of traits that influence dispersal provide two axes that explain a total of 74.4% of the variance in dispersal traits (Table 2). The first axis can be interpreted as size, and the second reflects branch placement, or the compactness of the plant. No significant effects of inbreeding were detected for the morphological traits that influence dispersal (Table 1, Fig. 4).

Significant correlations of magnitudes of inbreeding effects between some traits indicate that inbreeding does not always influence traits independently (Table 3). A large effect of inbreeding on the number of branches corresponds to a large effect of inbreeding on the number

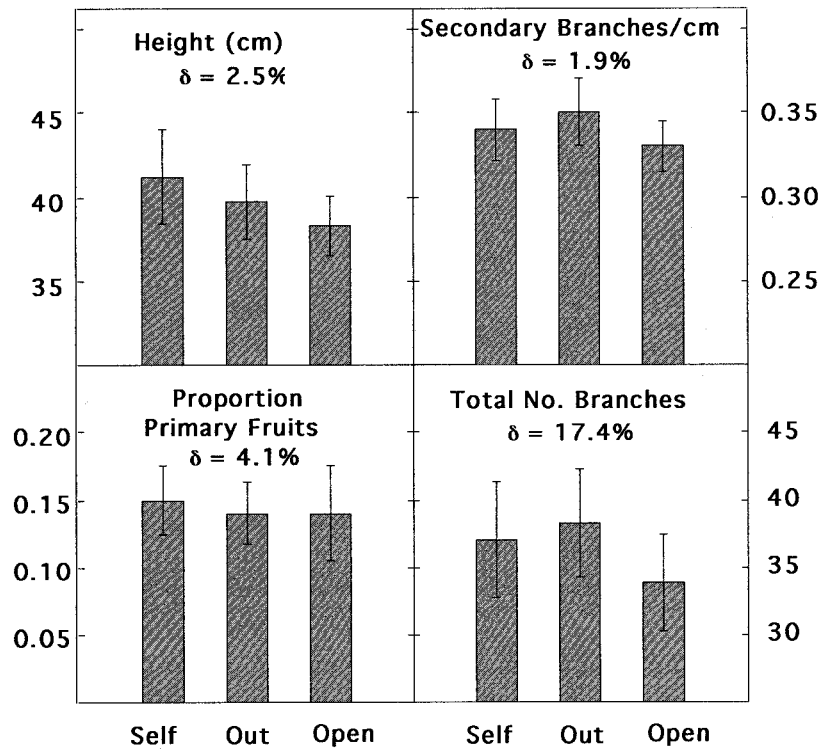


Fig. 4. Means and standard errors of dispersal traits of plants grown from seeds that matured from flowers that were experimentally selfed, outcrossed, or open pollinated.

of seeds produced by plants grown from successful germinants. This correlation in inbreeding effects can be explained in part by the magnitude of the correlation between the traits themselves (Pearson's $r = 0.89$ in selfed [$P < 0.001$], and 0.92 in outcrossed [$P < 0.001$]). This result suggests that inbreeding can influence some traits concurrently if the traits are correlated.

Emasculation significantly influenced seed mass (mean difference = -0.001 , $t = -3.250$, $P < 0.05$) and percentage germination (mean difference = -0.244 , $t = -2.666$, $P < 0.05$), but had no significant effect on other traits. Therefore, neither seed mass nor percentage germination were used to estimate selfing rates. Estimates of selfing rates were $>50\%$ in all cases except one (Table 4), with most of the estimates significantly greater than zero, and half of them significantly greater than 0.5.

To calculate the average number of seeds produced by selfed and outcrossed seeds while taking into account fitness effects of dispersal and density, results from previ-

ous studies are used (Table 5). Given the results of this study, a plant growing from a selfed seed would have 63% distal fruit segments, whereas a plant growing from an outcrossed seed would have 58% distal segments. These values were obtained using the values of second-generation abortion frequencies in Fig. 3. If a plant growing from a selfed seed were in turn to self pollinate, then of the 47% of the fruits that have no abortions, 43% of them would suffer abortions of the proximal fruit segment, and 5% of them would suffer abortions of the distal segment. This would lead to a final value of 27% proximal segments in selfing lineages. Likewise, outcrossing lineages would have 35% proximal segments.

Distal segments are dispersed to lower densities than are proximal segments (Table 5; Donohue, 1993). A previous experimental manipulation of density in the field (Donohue, 1997) demonstrated a significant regression relationship for total fruit segment production as a function of density (Table 5; $t = -2.22$, $P = 0.027$, $N = 261$,

TABLE 3. Correlations of magnitudes of inbreeding effects between pairs of fitness and dispersal traits. Pearson's correlations are shown on the lower diagonal (boldface) and uncorrected probabilities are shown on the upper diagonal. Family mean values were used to calculate the difference in values of each trait between selfed and outcrossed treatments. N ranges from 14 (adult traits) to 18 (seeds/ovule).

Trait	Seeds/ovule	Height	2° br./cm	% 1° fruits	No. branches	No. segments
No. seeds/ovule	1.00	0.245	0.091	0.552	0.443	0.253
Height	0.33	1.00	0.584	0.449	0.285	0.870
2° br./cm	-0.47	0.16	1.00	0.174	0.659	0.377
% 1° fruits	-0.17	-0.22	0.11	1.00	0.868	0.380
No. branches	0.21	-0.31	-0.13	-0.05	1.00	0.003*
No. segments	0.32	-0.05	-0.26	0.25	0.69	1.00

* Significant using Bonferroni correction for multiple tests.

TABLE 4. Selfing rates of the population sample and individual selfing rates of four plants. Selfing rates were estimated using plant traits that exhibited detectable inbreeding effects. The estimates of population selfing rate were calculated using family mean values of the 18 families. Standard errors are shown in parentheses.

Sample used for estimate	Trait used for estimate	Selfing rate (SE)
Population sample	% Matured from ovules	-0.12 (1.10)
	No. fruit segments/germinant	2.22 (3.01)
	Total fitness	1.29* (0.99)
Family	1 Proportion proximal abortions ^a	0.561** (0.505)
	2 Proportion distal abortions ^a	0.723* (0.227)
	2 Proportion proximal abortions ^b	0.425* (0.406)
	3 No. branches	1.375** (0.375)
	3 Proportion proximal abortions ^b	0.888** (0.133)
	4 No. fruit segments/germinant	0.892** (0.268)
	4 Proportion distal abortions ^a	0.788* (0.367)

* Estimate is significantly greater than 0. ** Estimate is significantly greater than 0.5.

^a First-generation abortions; proportion of flowers pollinated that produced fruits with proximal abortions.

^b Second-generation abortions; proportion of proximal abortions produced by plants grown from seeds of a pollination treatment.

using family mean values; $t = -2.96$, $P = 0.003$, $N = 1044$ using individual values; probabilities are based on natural log-transformed data. The regression equation is the same for both analyses). When considering the fitness consequences of density experienced by proximal and distal fruit segments, the adjusted inbreeding depression is estimated to be 26.5% rather than 29% (Table 5).

Total seed production of self- and cross-pollination can influence progeny density as well. As an approximation, we can let seed crop size be proportional to seed density after dispersal (Table 5). Note, however, that seed crop size could influence dispersal distance or evenness and thereby not reflect seed density directly (Sinha and Davidar, 1992). With this approximation, total inbreeding depression is estimated to be 17.5% or 11.5% lower than the estimate that omits fitness consequences of density.

DISCUSSION

Inbreeding significantly reduced total seed production and thereby is expected to reduce competitive conditions experienced by progeny under natural conditions. The actual fitness difference between progeny of parents that selfed vs. parents that outcrossed, therefore, could be substantially less than that estimated while omitting fitness effects of sibling competition. In this system, realized inbreeding depression after dispersal could be up to 11.5% less than that estimated under uniform conditions. Field experiments that measure dispersal, density, and fitness of selfed and outcrossed progeny in situ would be able to determine their fitness differences under ecologically realistic conditions.

The observation that self pollination by the maternal parent can influence not only the genetic composition of the progeny, but also the competitive environment in which the progeny grow, can be considered to be a sort of maternal effect. Just as germination behavior, which is maternally determined, can influence the environment in which progeny grow and thereby influence progeny fit-

TABLE 5. Estimates of realized inbreeding depression as a function of progeny density. Due to proximal seed abortions, selfed and outcrossed plants have different proportions of proximal seeds (line 1). As known from previous studies (Donohue, 1993), proximal seeds are dispersed to higher densities than distal seeds (208 seeds/m² vs. 115 seeds/m²) (line 2). A regression line (line 3) predicts the number of seeds produced by proximal and distal seeds, based on differences in their density (Donohue, 1997) (line 4). The number of seeds produced by selfed and outcrossed seeds is the sum of the products of the elements in lines 1 and 4. Selfed progeny also experience inbreeding depression (29% as estimated in this study), leading to the total number of seeds produced by selfed and outcrossed progeny. In the lower table, the density of selfed seeds is approximated as 81% that of outcrossed seeds, since selfed flowers produce only 81% the number of viable seeds. The other calculations are as before.

Variable	Selfed	Outcrossed
Proportion proximal seeds	0.27	0.35
Proportion distal seeds	0.73	0.65
Density of proximal seeds	208 seeds/m ²	208 seeds/m ²
Density of distal seeds	115 seeds/m ²	115 seeds/m ²
Regression line:	Total seeds = 18 - 0.5 (no. seeds/m ²)	
No. seeds produced by proximals	7.6	7.6
No. seeds produced by distals	12.3	12.3
Total seeds produced	11 - (11 × δ) = 7.8	10.6
Total inbreeding depression		26.5%
Density of proximal seeds	169 seeds/m ²	208 seeds/m ²
Density of distal seeds	93 seeds/m ²	115 seeds/m ²
No. seeds produced by proximals	9.6	7.6
No. seeds produced by distals	13.4	12.3
Total seeds produced	12.3 - (12.3 × δ) = 8.75	10.6
Total inbreeding depression		17.5%

ness (Roach and Wulff, 1987; Donohue and Schmitt 1997), so can the maternal selfing rate influence the progeny competitive environment and, through it, progeny fitness. A maternal effect occurs when the maternal parent influences progeny fitness or phenotype not only through the Mendelian transmission of its genes to its progeny, but also through indirect mechanisms such as altering the environment that the progeny experience (Kirkpatrick and Lande, 1989). Therefore, if selfing rate is genetically determined, if it influences the progeny environment by decreasing competition, and if that environment influences progeny fitness, then inbreeding can be said to elicit maternal effects. This intergenerational, environmentally mediated fitness consequence of inbreeding has not explicitly been considered. However, it may have important implications for estimates of inbreeding depression under ecologically realistic conditions. Moreover, it may inform studies of the evolutionary dynamics of the evolution of selfing rates, since characters with maternal effects have unique evolutionary responses to selection (Kirkpatrick and Lande, 1989).

The density-mediated fitness consequence of inbreeding observed in this study could be common in many systems, particularly when high sibling density significantly reduces offspring fitness. This study also suggests

that it could occur in both selfing and outcrossing systems. Substantial inbreeding depression (29.0%) was detected in this plant, which appears to have an intermediate to high selfing rate. High inbreeding depression in selfing plants has been observed before (Holtsford and Ellstrand, 1990; Agren and Schemske, 1993; Rathcke and Real, 1993) and accords with theoretical work that indicates that appreciable inbreeding depression could be expected even in populations with high levels of selfing (Charlesworth and Charlesworth, 1987, 1990; Charlesworth, Morgan, and Charlesworth, 1990). More important to this study, however, is the suggestion that selfing could be common in this system, and therefore effects of inbreeding on seed production observed in this study are likely to be expressed in the field as well. In outcrossing systems, inbreeding depression is likely to be even more severe than that observed in this system, though less frequent.

Effects of inbreeding were apparent only in one generation in this study; inbreeding significantly reduced maturation of ovules into viable seeds, but it did not reduce the seed production of plants grown from those viable seeds. Inbreeding depression has been shown to influence seed maturation at this early stage in other systems as well (e.g., Griffin and Lindgren, 1985; Levin, 1989; Woods and Heaman, 1989). However, an even greater number of studies have shown effects of inbreeding at later stages than were observed in this study, particularly with respect to seed production by plants grown from inbred seeds (e.g., Dudash, 1990; Levin, 1991; Willis, 1993). In such a system, inbreeding in one generation may influence seed production not only by the plant that inbred but also by the progeny of that plant. That is, inbreeding in one generation may influence seed maturation in two generations. In such systems, the density-dependent fitness consequences may also persist for two generations.

An effect of inbreeding on dispersal in outcrossing species could be especially interesting if dispersal, in turn, influences the probability of inbreeding. Dispersal determines the spatial relationships among plants, which could, in turn, influence pollination probability and relatedness of pollen donors (Pitelka, 1942; Baker, 1955; Grant and Grant, 1968; Levin and Kerster, 1968, 1969, 1971; Wolfe, 1989; Schlesling and Turpin, 1971; Ehrlich and Gilbert, 1973; Linhart, 1973; Price and Waser, 1979; Augspurger, 1980). The expression of inbreeding effects in traits that influence dispersal and density could affect the probability of future inbreeding, therefore, by altering the spatial relationship among potential pollen donors. Fluctuations between generations in the selfing rate could result from fluctuations in dispersal and density. The fact that dispersal often is not selectively neutral could influence equilibrium probabilities of dispersal and inbreeding as well. More theoretical modeling is required to investigate the dynamic consequences of interacting fitness effects of dispersal and inbreeding on the evolution of plant mating systems.

In conclusion, self pollination reduced the expected progeny density, and this reduction in sibling competition lowered estimates of inbreeding depression by up to 11.5%. Therefore, the fitness difference between selfed and outcrossed progeny may be smaller in the field under

natural dispersion patterns than that typically calculated under controlled conditions, since selfed progeny may occur at lower densities than outcrossed progeny in the field. Empirical studies of the dispersal of progeny of selfed and outcrossed plants would provide important information on how inbreeding and dispersal interact to determine the realized fitness difference between selfed and outcrossed progeny. Because dispersal and density often have significant effects on fitness, considering the interaction of dispersal and inbreeding could alter estimates of the effects of inbreeding on fitness in ecologically realistic contexts. Doing so could foster different predictions concerning magnitudes of inbreeding depression and selection for inbreeding avoidance.

LITERATURE CITED

- AGREN, J., AND D. W. SCHEMSKE. 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. *Evolution* 47: 125–135.
- AUGSPURGER, C. K. 1980. Mass-flowering of a tropical shrub (*Hybanthus prunifolius*): influence on pollinator attraction and movement. *Evolution* 34: 475–488.
- . 1983a. Offspring recruitment around tropical trees: changes in cohort distance with time. *Oikos* 20: 189–196.
- . 1983b. Seed dispersal of the tropical tree, *Platypodium elegans*, and the escape of its seedlings from fungal pathogens. *Journal of Ecology* 71: 759–771.
- , AND K. P. HOGAN. 1983. Wind dispersal of fruits with variable seed number in a tropical tree (*Lanchoarpus pentaphyllus*: Leguminosae). *American Journal of Botany* 70: 1031–1037.
- BAKER, H. G. 1955. Self-compatibility and establishment after “long-distance” dispersal. *Evolution* 9: 347–349.
- BULLOCK, S. H. 1981. Aggregation of *Prunus illicifolia* (Rosaceae) during dispersal and its effects on survival and growth. *Madrono* 28: 94–95.
- BULMER, M. G. 1985. The mathematical theory of quantitative genetics. Clarendon Press, Oxford.
- BURDON, J. J., AND G. A. CHILVERS. 1975. Epidemiology of damping-off disease (*Pythium irregulare*) in relation to density of *Lepidium sativum* seedlings. *Annals of Applied Biology* 81: 135–143.
- CHARLESWORTH, D. 1988. A method for estimating outcrossing rates in natural populations of plants. *Heredity* 61: 469–471.
- , AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.
- , AND ———. 1990. Inbreeding depression with heterozygote advantage and its effects on selection on modifiers changing outcrossing rate. *Evolution* 44: 870–887.
- , M. T. MORGAN, AND B. CHARLESWORTH. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* 44: 1469–1489.
- CREMER, K. W. 1977. Distance of dispersal in eucalypts estimated from seed weight. *Australian Forest Research* 7: 225–228.
- DONOHUE, K. 1993. The evolution of seed dispersal in *Cakile edentula* var. *lacustris*. Ph.D. dissertation, University of Chicago, Chicago, IL.
- . 1997. Seed dispersal in *Cakile edentula* var. *lacustris*: Decoupling the fitness effects of density and distance from the home site. *Oecologia* 110: 520–527.
- , AND J. SCHMITT. In press. Maternal environmental effects: Adaptive plasticity? In A. T. Mousseau and C. W. Fox [eds.], Maternal effects as adaptations, Oxford University Press, Oxford.
- DUDASH, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* 44: 1129–1139.
- EHRLICH, P. R., AND L. E. GILBERT. 1973. Population structure and dynamics of the tropical butterfly *Heliconius ethilla*. *Biotropica* 5: 69–82.

- GADGIL, M. 1971. Dispersal: population consequences and evolution. *Ecology* 52: 253–260.
- GRANT, K. A., AND V. GRANT. 1968. Hummingbirds and their flowers. Columbia University Press, New York, NY.
- GRIFFIN, A. R., AND D. LINDGREN. 1985. Effect of inbreeding on production of filled seeds in *Pinus radiata*—experimental results and a model of gene action. *Theoretical and Applied Genetics* 71: 334–343.
- HOLTSFORD, T. P., AND N. C. ELLSTRAND. 1990. Inbreeding effects in *Clarkia tembloriensis* (Onagraceae) populations with different natural outcrossing rates. *Evolution* 44: 2031–2046.
- HOLSINGER, K. E. 1986. Dispersal and plant mating systems: The evolution of self fertilization in subdivided populations. *Evolution* 40: 405–413.
- HORVITZ, C. C., AND D. W. SCHEMSKE. 1986. Seed dispersal and environmental heterogeneity in a neotropical herb: a model of population and patch dynamics. In A. Estrada and T. H. Flemming [eds.], *Frugivores and seed dispersal*. Dr. W. Junk, Dordrecht.
- HOWE, H. F. 1989. Scatter- and clump-dispersal and seedling demography: hypothesis and implications. *Oecologia* 79: 417–426.
- , AND J. SMALLWOOD. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 7: 469–495.
- JANZEN, D. H. 1971. Escape of *Cassia grandis* L. beans from predators in time and space. *Ecology* 52: 964–979.
- . 1972. Escape in space by *Sterculia apetala* from the bug *Dysdercus fasciatus* in a Cost Rican deciduous forest. *Ecology* 53: 350–361.
- KIRKPATRICK, M., AND R. LANDE. 1989. The evolution of maternal characters. *Evolution* 43: 485–503.
- LEVIN, D. A. 1989. Inbreeding depression in partially self-fertilizing *Phlox*. *Evolution* 43: 1417–1423.
- . 1991. The effect of inbreeding on seed survivorship in *Phlox*. *Evolution* 45: 1047–1049.
- LEVIN, D. A., AND H. W. KERSTER. 1968. Local gene dispersal in *Phlox pilosa*. *Evolution* 22: 130–139.
- , AND ———. 1969. The dependence of bee-mediated pollen dispersal on plant density. *Evolution* 23: 560–571.
- , AND ———. 1971. Neighborhood structure in plants under diverse reproductive methods. *American Naturalist* 105: 345–354.
- LIEW, T. C., AND F. O. WONG. 1973. Density, recruitment, mortality, and growth of Dipterocarp seedlings in virgin and logged forests in Sabah. *Malayan Forester* 36: 3–15.
- LINHART, Y. B. 1973. Ecological and behavioral determinants of pollen dispersal in hummingbird-pollinated *Heliconia*. *American Naturalist* 107: 511–523.
- LLOYD, D. G. 1980. Demographic factors and mating patterns in angiosperms. In O. T. Solbrig [ed.], *Demography and evolution in plant populations*, 67–88. Blackwell, Oxford.
- MCCANNY, S. J., AND P. B. CAVERS. 1989. Parental effects on spatial patterns of plants: a contingency table approach. *Ecology* 70: 368–378.
- OUBORG, N. J., AND R. VAN TREUREN. 1994. The significance of genetic erosion in the process of extinction. IV. Inbreeding load and heterosis in relation to population size in the mint *Salvia pratensis*. *Evolution* 48: 996–1008.
- PITELKA, F. A. 1942. Territoriality and related problems in North American hummingbirds. *Condor* 44: 189–204.
- PLATT, W. J. 1976. The natural history of a fugitive prairie plant (*Mirabilis hirsuta* (Pursh)). *Oecologia* 22: 399–409.
- PRICE, M. V., AND N. M. WASER. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsonii*. *Nature* 277: 294–297.
- RABINOWITZ, D., AND J. K. RAPP. 1981. Dispersal abilities of seven sparse and common grasses from a Missouri prairie. *American Journal of Botany* 68: 616–624.
- RATHCKE, B., AND L. REAL. 1993. Autogamy and inbreeding depression in mountain laurel, *Kalmia latifolia* (Ericaceae). *American Journal of Botany* 80: 143–146.
- ROACH, D. A., AND R. D. WULFF. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18:209–235.
- RODMAN, J. E. 1974. Systematics and evolution of the genus *Cakile* (Cruciferae). *Contributions of the Gray Herbarium, Harvard University* 205: 3–146.
- SCHEMSKE, D. W. 1983. Breeding system and habitat effects on fitness in three neotropical *Costus* (Zingiberaceae). *Evolution* 38: 817–832.
- SCHLESING, R. A., AND R. A. TURPIN. 1971. Hummingbird dispersal of *Delphinium cardinale* pollen treated with radioactive iodine. *American Journal of Botany* 58: 401–406.
- SCHOEN, D. J. 1983. Relative fitness of selfed and outcrossed progeny in *Gilia achilleifolia* (Polemoniaceae). *Evolution* 37: 292–301.
- SINHA, A., AND P. DAVIDAR. 1992. Seed dispersal ecology of a wind dispersed rain forest tree in the Western Ghats, India. *Biotropica* 24: 519–526.
- WALLER, D. M. 1984. Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. *Evolution* 38: 427–440.
- WILLIS, J. H. 1993. Effects of different levels of inbreeding on fitness components in *Mimulus guttatus*. *Evolution* 47:864–876.
- WOLFE, L. M. 1989. Consequences of selfing and seed size variation on offspring performances in *Hydrophyllum appendiculatum*. *American Journal of Botany* (Supplement) 76: 131.
- WOODS, J. H., AND J. C. HEAMAN. 1989. Effect of different inbreeding levels on filled seed production in Douglas-fir. *Canadian Journal of Forest Research* 19: 54–59.