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RELATIVE FITNESS OF SELFED AND OUTCROSSED PROGENY IN A SELF-COMPATIBLE, PROTANDROUS SPECIES, *SABATIA ANGULARIS* L. (GENTIANACEAE): A COMPARISON IN THREE ENVIRONMENTS

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Abstract.—The consequences of selfing were examined for a population of self-compatible, protandrous, *Sabatia angularis* L. (Gentianaceae). Field-collected plants were hand-pollinated in the greenhouse to produce selfed progeny and outcrossed progeny from parents separated by a maximum of 5 m (near-outcross) and 85 m (far-outcross) in the field. Self, near-outcross, and far-outcross half sib progeny were grown in the greenhouse, a garden plot, and their native habitat. Progeny were compared by multiplicative fitness functions based on seed production per hand-pollination, seed germination, rosette formation, survival to reproduction, and reproduction in each environment. Variation in reproduction among progeny groups was influenced by the environment in which they were grown. Significant inbreeding depression was detected between the self and far-outcross progeny in each environment. Only the natural environment demonstrated a greater than 50% reduction in relative fitness of self compared to near-outcross progeny. This is of biological relevance since near-outcross hand-pollinations occurred within the range of pollen and seed dispersal suggesting that inbreeding depression in *S. angularis* is strong enough to maintain outcrossing in the study population. In the field, the far-outcross progeny outperformed the near-outcross progeny suggesting local population substructure. The magnitude of the inbreeding depression expressed among the self progeny was the greatest in the field, intermediate in the garden plot, and the least in the greenhouse.

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Inbreeding depression is thought to be the only selective agent in outcrossing plant populations strong enough to prevent the evolution of self fertilization (Charlesworth and Charlesworth, 1979). A self fertile individual can contribute genes through pollen and ovules by selfing, in addition to contributing genes by pollen from cross fertilization. In contrast, an outcrossing individual contributes on average only one-half of its genes to its progeny. The heterosis experienced from outcrossing must overcome the two-fold advantage of selfing if outcrossing is to persist; with inbreeding depression greater than or equal to 50% a population will evolve toward or maintain an outcrossed mating system, respectively. Conversely, if inbreeding depression is less than 50%, a population may evolve higher selfing rates in the absence of pollen discounting, i.e., the contribution that a plant makes to cross-fertilization through its pollen is unaffected by the pollen used in self-fertilization and vice versa (Fisher, 1941; Nagylaki, 1976; Lloyd, 1979; Wells, 1979; Lande and Schemske, 1985). However, recent theoretical work (Holsinger, 1988; Charlesworth et al., unpubl.) has demonstrated that the role of inbreeding depression in the evolution of selfing may depend upon the intensity of selection for selfing and the genetic basis of the inbreeding depression.

There are few empirical data on natural plant populations that show a two-fold advantage from outcrossing (Schemske, 1983;

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Schoen, 1983). The empirical relationship expected in natural plant populations after imposed selfing is dependent on the genetic mechanisms causing inbreeding depression for the species in question. Inbreeding depression following selfing may result from 1) the breakdown of heterozygote advantage at a single locus (Wright, 1977), or many loci (Lande and Schemske, 1985), and 2) the expression of recessive alleles with deleterious effects (Wright, 1977; Falconer, 1981; Charlesworth and Charlesworth, 1987). An empirical estimate of the magnitude of inbreeding depression may be influenced by the environment in which self and outcross progeny are compared and the life history stages examined (Schemske, 1983).

The major goal of this study was to determine if progeny derived from outcrossing are at least twice as fit as progeny derived from selfing (Maynard Smith, 1971; Williams, 1971) in self-compatible, protandrous *Sabatia angularis* L. (Gentianaceae). Protandry has long been considered a classic adaptation to promote outcrossing in plants (Darwin, 1876). Progeny resulting from hand-selfing and outcrossing at two interparent distances were compared in their native habitat, a garden plot, and greenhouse throughout their entire life cycle. The progeny were grown in three environments to determine if the magnitude of inbreeding depression expressed by the progeny varied in relation to the environment in which they were grown. The progeny were compared by multiplicative fitness functions within each environment and the environments were compared as well.

MATERIALS AND METHODS

Study Plant and Study Site

Sabatia angularis L. (Gentianaceae), commonly known as Rose Pink, is an obligate biennial in the northern portion of its range (pers. obs.). In northern Indiana, seeds disperse in the fall and germinate late the following spring. Rosettes form by mid July, overwinter and bolt in their second spring. Flowering occurs from late July into early September, peaking in August. Fruits mature from early September through October. The reproductive biology of *S. angularis* is further detailed in Dudash (1987).

Sabatia angularis is abundant at Miller Dunes in Gary, Indiana, a protected site which is part of the Indiana Dunes National Lakeshore, approximately 35 miles southeast of Chicago. Adults range from 3–80 cm in height with 1–200 flowers per individual and are found in densities ranging from 1–200 individuals per square meter, primarily around the margins of interdunal ponds.

Fitness Experiment

Production of Progeny.—A greenhouse crossing program was conducted during the summer of 1983 to compare the consequences of one generation of selfing and outcrossing on progeny fitness. Twenty-five randomly chosen bolting rosettes were collected and field locations mapped from two five-square-meter plots separated by 85 meters in two study areas. The two study areas were separated by a large dune complex. Plants were grown under pollinator-free conditions at the University of Illinois at Chicago greenhouse, where all crosses were performed. Each individual was selfed (self), outcrossed to an individual from the same five-square-meter plot (near-outcross), and outcrossed to an individual from a plot separated by 85 meters (far-outcross). All pollinations on a given plant were completed within a day of one another and each pollination occurred by day three in the lifespan of the flower. These criteria resulted in approximately 10 pollen donors for each category of outcrossed pollination in the two study areas. A maternal family was defined by each of the 25 plants used as females that were chosen from all four plots and the progeny that were generated from the three types of hand-pollination. Progeny resulting from the self, near-outcross, and far-outcross hand-pollinations are hereafter referred to as self, near-outcross, and far-outcross progeny.

Seeds were counted from each of three fruits representing the three types of hand-pollination on each female with a dissecting microscope and weighed en masse to measure total seed mass. Seed mass was divided by seed number to estimate mean seed mass per fruit, and pericarp mass was also determined for these same fruits. The square root of seed number and pericarp mass, and the reciprocally transformed mean seed mass data were compared using a two-way analysis of variance (ANOVA) without replication with pollination type fixed and maternal parent random (Brownlee, 1965).

In December 1983, seed from each pollination treatment for each maternal plant were sowed in separate trays and randomly placed in a protected cold frame for stratification. In April 1984, the seed trays were returned to the greenhouse and germination occurred in about one month. Germination and rosette formation were monitored on a stratified random subset of the 25 families in the greenhouse and the transformed data (angular) were analyzed using a two-way ANOVA without replication with the same main effects as the above model.

Full sibs from each pollination treatment within each maternal family were equally represented and grown in three environments: (1) a field site at Miller Dunes, and (2 and 3) a garden plot and greenhouse at the University of Illinois at Chicago. The progeny were monitored for survival and reproduction in each environment. The proportionate survival data for each environment were analyzed with a Friedman Rank test with maternal parent as the replicated block. An a posteriori multiple comparisons test on Friedman Rank sums was used to test for significant differences among the three pollination treatments (Hollander and Wolfe, 1973).

Field Habitat.—Rosettes were transplanted during September, 1984 into areas inhabited by adult *S. angularis* in previous years. The plants were not returned to their maternal environment because only 25 plants were removed and 2,700 plants were returned to Miller Dunes. The plants were stratified randomly into 27 one-square-meter quadrats, such that all crosses from all the females were represented equally in each quadrat. Each quadrat contained 100 plants in a hexagonal array. The transplanting density was well within the natural densities of plants in the field. The 2,700 plants represented 36 each of self, near-outcross, and far-outcross progeny from each of 25 females. Survival was monitored after transplanting into the field (September, 1984), after the winter (March, 1985), spring (June, 1985), and end of summer (September, 1985).

Total seed production per individual progeny was estimated from total fruit mass per individual, because it was impractical to count all of the seed produced by a single individual. A regression of seed number per fruit (square root transformed values) was performed on total fruit mass for a subsample of the fruits collected for all maternal parents across all quadrats with surviving individuals (30 self, 30 near-outcross, and 30 far-outcross fruits). Regressions of seed number per fruit on total fruit mass were performed separately for the self, near-outcross, and far-outcross progeny. A homogeneity of slopes test revealed no significant differences among pollination treatments ($F = 1.24$, $df = 2,84$, $P = 0.30$), so the data were pooled into a single regression of seed number per fruit on total fruit mass ($r = 0.84$, $F = 207.91$, $df = 1,88$, $P < 0.0001$). Based on this regression, total fruit mass provided a good estimate of total seed production. Total fruit mass per individual (natural log transformed) for the three categories of progeny was analyzed using a three-way ANOVA with pollination type fixed and maternal parent and transplantation site random. The mean square denominator to test for the fixed main effect was obtained from Brownlee (1965, p. 512, Table 15.3) and the degrees of freedom were calculated following Winer (1971).

Garden Plot.—The garden plot was composed of a six-inch top layer of sand to simulate the natural field environment, with garden soil underneath. The garden plot contained 912 rosettes representing 12 replicates per cross × 3 crosses × 25 females. There were 12 rosettes transplanted per square meter quadrat stratified randomly in a hexagonal array. A two-way ANOVA was performed on natural log transformed values to determine if the maternal parent (random effect) or pollination type (fixed treatment) affected total fruit production per individual. The density of plants was much lower in the experimental garden plot than in the natural habitat quadrats because mortality was expected to be lower in the garden plot.

Greenhouse.—Approximately 55 rosettes per cross for each of 25 maternal parents were transplanted into standard greenhouse soil and stratified in cold frames. The 4,155 plants were located randomly by maternal family to eliminate position effects within a family. The plants completed their life cycle in the greenhouse, where they were also located randomly by maternal family. Total fruit production per individual by the progeny was analyzed using a two-way ANOVA with maternal parent random and pollination type fixed.

The coefficient of variation and its standard error (Simpson et al., 1960) were calculated for reproduction on untransformed data of the self, near-outcross, and far-outcross progeny in each environment.

Total Relative Progeny Fitness.—The progeny after rosette formation were monitored throughout their life cycle in each environment. A multiplicative fitness function was calculated to compare relative fitness for progeny resulting from the three categories of crosses. The function was calculated by multiplying seed production per hand-pollination, the probability of germination, the probability of rosette formation, the probability of survival, and reproduction of those individuals that survived to reproduce. Relative fitness was standardized within each family such that the pollination type with the largest value was assigned the value of one, and the other two were a fraction of one. A Friedman Rank test was performed on the relative multiplicative fitness functions using maternal parent as the replicated block. An a posteriori multiple comparison test on the Friedman Rank sums was used to test for significant differences among pollination treatments (Hollander and Wolfe, 1973). This allowed the comparison of the total relative progeny fitness of the self, near-outcross, and far-outcross progeny within environments. The magnitude of differences among environments was also compared.

Family Variation in Inbreeding Depression.—To determine if a relationship existed between the interparent distances used to generate the near-outcross progeny and their resultant fitnesses, quadratic and linear regression analyses were performed (Statview 512+, 1986). The interparent distances ranged from 10 to 500 cm and the dependence of fitness on interparent distance was compared in the field.

Second Generation of Selfing and Outcrossing.—A second greenhouse crossing program was conducted during the summer of 1985 to examine the consequences of a second generation of selfing and outcrossing on progeny fitness. Twenty-five self progeny were randomly chosen from the greenhouse portion of the fitness experiment that represented the four original field sites. The same crossing program and methods were performed as in 1983. The second generation of progeny was analyzed for seed production per fruit, mean seed mass per fruit, and pericarp mass with a two-way ANOVA without replication using pollination type fixed and maternal parent random.

All statistical analyses unless noted were conducted with the SAS statistical package (SAS, 1985) on the University of Illinois at Chicago mainframe. Analysis of variance was performed using the GLM (general linear model) procedure and all assumptions of ANOVA were met by the appropriate transformations when necessary.

RESULTS

Fitness Experiment

Greenhouse Breeding Experiment, Germination and Rosette Formation.—Seed number per fruit and pericarp mass were significantly lower for self-pollinations compared to far-outcross pollinations (Table 1). Mean seed mass did not significantly differ among the three types of hand-pollinations. There was a significant effect of maternal parent (female) in each of the analyses ($P < 0.0001$). Percentage of germination (ca. 73%) and rosette formation (ca. 48%) did not differ significantly among the categories of progeny (germination: $F = 0.74, P = 0.69$; rosette formation: $F = 0.29, P = 0.77$). There was no significant maternal effect in either of these analyses.

Progeny Survival.—A total of 1,348 individuals survived to reproduce in the field (ca. 50%). In the field significantly fewer self progeny survived to flower than either of the outcross progeny (Fig. 1A, $P < 0.05$). Despite the overall higher survival in the garden (ca. 89%), significantly fewer self progeny survived to reproduce than either of the outcross progeny (Fig. 1B, $P < 0.05$). There were no significant differences in survival to reproduction among the three types of progeny in the greenhouse (Fig. 1C).

TABLE 1. Mean seed number, seed mass, and pericarp mass (standard deviation) of *S. angularis,* following one generation of hand-self and cross pollinations in the greenhouse. Sample sizes (*N*) are the number of maternal parents. Superscripts indicate differences among the categories of progeny ($P < 0.05$). The data are presented untransformed. The *F* and *P* values are from the hand-pollination treatment in the respective ANOVA.

Hand pollination	*N*	Seed number per fruit	Mean seed mass/fruit (mg)	Pericarp mass (mg)
Self	25	730^{a} (273)	0.022^{a} (0.007)	24.56^{a} (8.91)
Near-out-cross	25	$755^{a,b}$ (264)	0.023^{a} (0.007)	$24.99^{a,b}$ (9.34)
Far-out-cross	25	834^{b} (268)	0.023^{a} (0.007)	26.74^{b} (9.46)
F		4.53	0.25	3.78
P		0.02	0.78	0.03

However, only 14 of the original 25 families survived (ca. 27% of individuals) because of overwintering conditions in the cold-frame and were therefore used in the analysis.

Progeny Reproduction.—In the field, all fruit produced from 1,143 of the surviving 1,348 individuals was collected in six areas. Two of the areas could not be used in the analysis owing to extensive mortality and only those individuals where all fruit produced were collected were used in the analysis. The ANOVA revealed significant effects of maternal parent, transplantation site, and pollination type on total fruit mass per individual (Table 2). The only significant interaction was maternal parent × pollination type (Table 2, $P < 0.05$). Tukey's a posteriori comparison of means test indicated that self progeny produced significantly less fruit mass per plant than near-outcross progeny, which in turn produced significantly less fruit mass per individual than far-outcross progeny (Fig. 2A, $P < 0.05$).

In the garden plot individual fruit production was significantly less for self progeny then near-outcross progeny, which was significantly less than far-outcross progeny (Fig. 2B, $P < 0.05$). The ANOVA revealed significant effects of maternal parent, pollination type, and their interaction (Table 3).

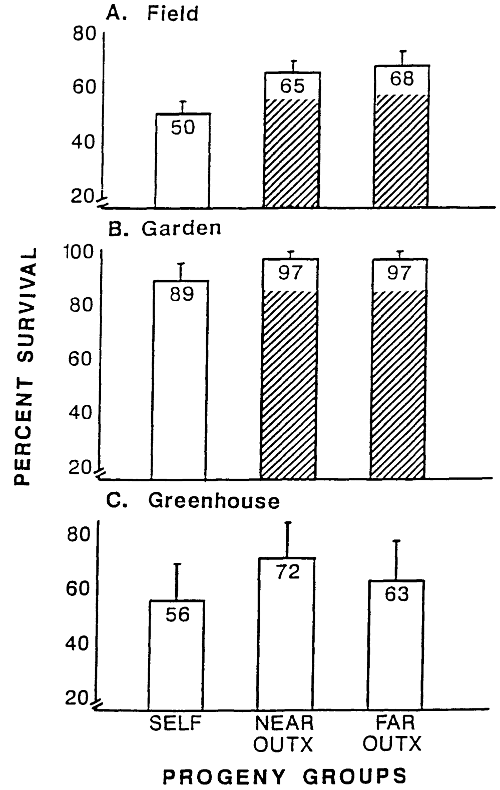

FIG. 1. Survival of self, near-outcross, and far-outcross progeny in the (A) field, (B) garden, and (C) greenhouse. The means of the progeny groups are shown within the bar graphs with 95% confidence intervals in one direction. Different shading patterns among the progeny bar graphs indicate significant differences ($P < 0.05$).

In the greenhouse Tukey's contrast revealed no significant difference between the self and near-outcross progeny in individual fruit production. However, the far-outcross progeny produced significantly more fruit per individual than either the self or near-outcross progeny (Fig. 2C, $P < 0.05$). The greenhouse ANOVA of fruit production results was the same as the garden plot (data not shown).

Total Relative Progeny Fitness.—In the field the multiplicative fitness function demonstrated a greater than 50% decrease in progeny fitness following self-pollination compared to progeny derived from either category of outcrossing (Fig. 3A). The relative fitness of self progeny was significantly less than that of near-outcross progeny, which was significantly less fit than far-out-

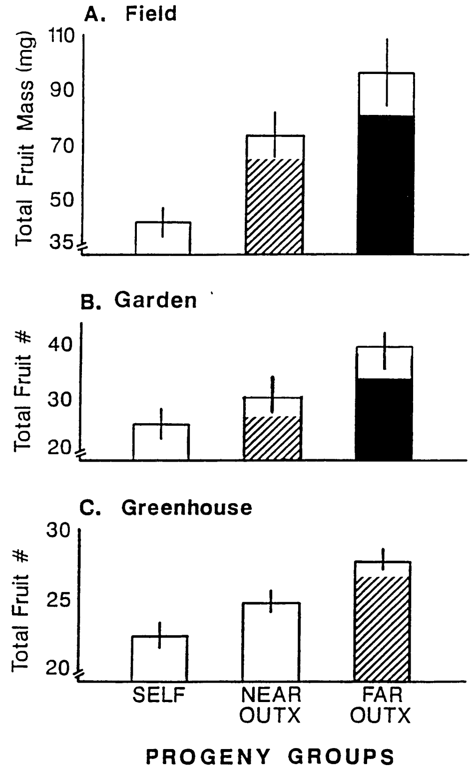

FIG. 2. Reproduction of the self, near-outcross, and far-outcross progeny grown in the (A) field, (B) garden, and (C) greenhouse. The measure of reproduction in the field was total fruit mass per individual. Total fruit production per individual was the measure of reproduction in the garden and greenhouse. Different shading patterns among the progeny bar graphs indicate significant differences at the 0.05 level. Vertical lines indicate 95% confidence intervals around the means.

TABLE 2. Analysis of variance of total fruit mass of field individuals ($N = 1{,}143$) grown from seed produced by hand-self, near-outcross, and far-outcross pollinations.

Dependent variable: ln(total fruit mass (mg))

Source	*df*	MS	*F*	*P*
Female (Fem)	24	2.22	1.91	<0.025
Site	5	45.04	38.90	<0.0001
Pollination (Poll)	2	47.27	26.25	<0.0001
Fem*Site	120	1.16	1.03	0.32
Fem*Poll	48	1.57	1.45	<0.05
Site*Poll	10	1.31	1.21	0.30
Fem*Site*Poll	219	1.08	0.96	0.63
error	714	1.13		

cross progeny. The relative fitness advantage of outcrossing over selfing in the field was 2.3 for near-outcross progeny and 4.0 for far-outcross progeny.

The multiplicative fitness functions for progeny grown in the garden plot showed no significant differences between the total relative fitness of the self and near-outcross progeny. Far-outcross progeny were significantly more fit than the self and near-outcross progeny (Fig. 3B, $P < 0.05$). The relative fitness advantage for outcrossing was 1.7 for near-outcross progeny and 2.4 for far-outcross progeny.

The Friedman Rank analysis of the multiplicative fitness functions of plants grown in the greenhouse revealed the self progeny to be significantly less fit than either category of outcross progeny (Fig. 3C, $P < 0.05$). There was no difference, however, in total relative fitness among the near- and far-outcross progeny. The relative fitness advantage for outcrossing was 1.9 for near-outcross progeny and 2.1 for far-outcross progeny.

Family Variation in Inbreeding Depression.—There was significant variation in the magnitude of inbreeding depression expressed among the 25 maternal families grown in the field (Tables 2 and 4). These results were corroborated by the significant interaction between maternal parent and pollination type in both the garden plot (Fem*Poll, Table 3) and greenhouse (data not shown). Neither linear ($r = 0.21$, $df = 1{,}23$, $P = 0.33$) or quadratic ($r = 0.24$, $df = 2{,}22$, $P = 0.51$) regression analysis detected any significant relationship between interparent distance and relative fitness of the near-outcross progeny of the maternal families grown in the field.

Coefficient of Variation.—Coefficients of variation for progeny reproduction were greatest in the field, intermediate in the gar-

TABLE 3. Analysis of variance of total fruit production of garden plot individuals ($N = 816$) grown from seed produced by hand-self, near-outcross, and far-outcross pollinations. See text for details.

Dependent variable: ln(total fruit production)

Source	*df*	MS	*F*	*P*
Female	24	3.29	3.64	<0.0001
Pollination	2	21.43	17.04	<0.0001
Fem*Poll	48	1.26	1.39	<0.045
error	741	0.91		

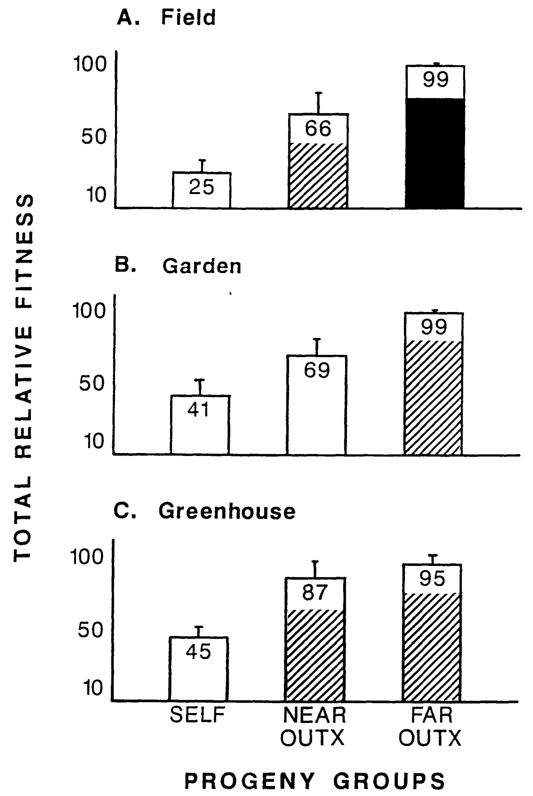

FIG. 3. Relative fitness of the self, near-outcross, and far-outcross progeny in the (A) field, (B) garden, and (C) greenhouse. The means of the relative fitness of the progeny are shown within the bar graphs with 95% confidence intervals in one direction. Different shading patterns among the progeny indicate significant differences ($P < 0.05$).

TABLE 4. Total relative fitness of field grown progeny for each of the 25 maternal parents used in the crossing program. The maternal parents are listed in order of increasing relative fitness of the self progeny.

Maternal parent	Total relative progeny fitness		
	Self	Near-outcross	Far-outcross
1	0.02	0.24	1.00
2	0.06	0.87	1.00
3	0.12	0.22	1.00
4	0.12	0.61	1.00
5	0.12	1.00	0.96
6	0.13	0.29	1.00
7	0.17	0.81	1.00
8	0.18	0.44	1.00
9	0.18	0.63	1.00
10	0.19	0.45	1.00
11	0.22	0.29	1.00
12	0.22	0.60	1.00
13	0.22	1.00	0.48
14	0.23	0.55	1.00
15	0.24	0.36	1.00
16	0.25	0.33	1.00
17	0.25	0.82	1.00
18	0.28	1.00	0.94
19	0.37	1.00	0.94
20	0.40	0.41	1.00
21	0.40	0.50	1.00
22	0.41	0.97	1.00
23	0.51	0.31	1.00
24	0.52	0.34	1.00
25	0.77	1.00	0.85

den plot, and smallest in the greenhouse (Table 5). A significant difference between the coefficient of variation of reproduction values was considered when two values were separated by more than two standard errors (0.05 level). In the greenhouse the self progeny had a significantly greater coefficient of variation than the near-outcross progeny, which was significantly greater than the far-outcross progeny. In the garden plot the self and near-outcross progeny coefficients of variation were significantly greater than the far-outcross progeny (no significant difference between self and near-outcross). In the field, the opposite was true: the far-outcross progeny had significantly greater coefficients of variation than the self and near-outcross progeny, which again were not significantly different from one another (Table 5).

Second Generation of Selfing and Outcrossing.—There were no significant differences among the second generation hand-self, near-outcross, and far-outcross pollinations in terms of seed number per fruit and pericarp mass. The mean seed mass resulting from the near-outcross pollinations was significantly greater ($P < 0.05$) than from the self pollinations, although no other comparison was significant. Significant maternal effects ($P < 0.0001$) were also detected in each of the analyses.

DISCUSSION

The magnitude of inbreeding depression in *S. angularis* was greatest in the natural field habitat and the pattern of inbreeding depression was consistent among the three categories of progeny in each environment. The significant two-fold difference between the self and the near-outcross progeny in the field is the most biologically relevant result

TABLE 5. Coefficient of variation (CV = SD/MEAN × 100) of reproduction of self, near-outcross, and far-outcross individuals grown in the greenhouse, garden, and field. In the field, reproduction was measured as total fruit mass per individual, and total fruit number per plant was the measure of reproduction in the garden and greenhouse. The standard error for coefficient of variation is given in parentheses. Means and sample sizes are given in Figure 2 and the text. Superscripts indicate differences among the progeny groups ($P < 0.05$, see text for details).

Environment	Coefficient of variation of reproduction of the progeny: Self	Near-outcross	Far-outcross
Greenhouse	39.54^{a} (1.49)	34.02^{b} (1.21)	29.46^{c} (1.08)
Garden	109.20^{a} (4.84)	108.42^{a} (4.58)	94.04^{b} (3.96)
Field	118.66^{a} (4.65)	117.80^{a} (4.11)	133.63^{b} (4.68)

since the near-outcross hand-pollinations (within a five-square-meter quadrat) were performed between plants that occurred within the range of pollen and seed dispersal in the field (Dudash, unpubl.). However, the relative fitness of the self progeny may be inflated, because the early stages of growth of all plants occurred in the greenhouse and cold frame, and the greenhouse environment resulted in the lowest expression of inbreeding depression.

Statistically significant inbreeding depression stages (i.e., survival and reproduction) and non-significant stages (i.e., germination and rosette formation) in the life cycle culminated in a significant effect on total relative fitness of the progeny. Others (Schemske, 1983; Schoen, 1983; Clay and Antonovics, 1985) have also used a product of survival and a measure of reproduction or at least a character expected to be strongly correlated with reproductive fitness (i.e., growth, adult size, and flower production) to estimate fitness. However, unless individuals are monitored throughout their entire life cycle, relative fitness comparisons among individuals will often be underestimated because inbreeding depression has a cumulative effect on fitness and varies with the life history stage monitored.

Previous field studies comparing relative fitness of hand-generated progeny of angiosperm populations were done by Schemske (1983) and Schoen (1983). Schemske (1983) examined *Costus* species in the greenhouse and field and detected no significant differences in germination and survival between the self and outcross progeny in the field. In contrast, Schemske (1983) found a 1.4–2.3 fold advantage to outcrossing after 12 months of growth in the greenhouse. Schemske was unable to monitor progeny reproduction because *Costus* is a perennial. Schoen (1983) estimated a 1.79 and 1.75 advantage of open pollinated progeny of *Gilia achilleifolia* compared to progeny generated from one and two generations of selfing when grown in the field. In *S. angularis* total fruit mass by the progeny in the field demonstrated the most dramatic inbreeding depression in the life history stages monitored, perhaps because it occurs at the end of the life cycle when the combined effects of inbreeding depression are manifested most. In contrast, Schoen observed that differences in survival contributed the most to reduced fitness in *G. achilleifolia.* Inbreeding depression probably varies from year to year within the same population and as to which life history stages are more susceptible to inbreeding depression within a population and across taxa.

In *S. angularis* no reduction in progeny performance was detected in the far-outcross progeny compared to the near-outcross progeny. Within the near-outcross hand-pollinations there was also no evidence for any relationship between interparent distance and relative progeny fitness on a local scale. Studies on *Delphinium nelsonii* and *Ipomopsis aggregata* have shown evidence for an optimal outcrossing distance on a local scale (Price and Waser, 1979; Waser and Price, 1983, 1989). The lack of evidence for optimal outcrossing in *S. angularis* suggests that selection is operating on mechanisms to maintain and/or promote pollen and seed dispersal because long distance mating events produce superior genotypes. Local populations of *S. angularis* may be separated by less than 85 m in the field. However, the significant difference in relative progeny fitness between the near- and far-outcross progeny may not have been detected with an interparent distance less than 85 m.

Limited pollen and seed dispersal have been found to contribute to population subdivision (Ehrlich and Raven, 1969; Levin, 1981). *Sabatia angularis* is restricted to areas close to the water table surrounding the margins of interdunal ponds separated by uninhabitable xeric areas. The superior fitness of far-outcross progeny compared to near-outcross progeny indicates appreciable spatial population subdivision. Fruit production of greenhouse grown progeny from naturally pollinated field plants was found to be similar to only the near-outcross greenhouse grown progeny values (Dudash, 1987). These results suggest that the naturally pollinated progeny of the field plants were primarily a result of outcrossed pollinations between individuals within the same local population in the field.

The level of inbreeding depression following selfing of the maternal families was found to differ among the four original field sites at the 0.1 level ($F = 2.39$, $df = 3,21$). This trend suggests that there is variation in the degree of inbreeding in the local populations owing to the frequency and magnitude of bottlenecks (Wright, 1977) and/or variation in the size structure within the local populations (Heywood, 1986).

As a consequence of population subdivision of *S. angularis* at Miller Dunes, the near-outcross hand-pollinations were probably often between related individuals. If frequent biparental inbreeding was practiced in the past it does not appear to have reduced the genetic load, since strong inbreeding depression is expressed following selfing (uniparental inbreeding) (Uyenoyama, 1986). The inheritance of inbreeding depression also will affect the magnitude of inbreeding depression expressed following selfing in local populations practicing biparental inbreeding (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). However, biparental inbreeding can occur between full sibs, half sibs, cousins, and any other degree of relatedness, and the variation detected among maternal families in relative progeny fitness of the self and outcross progeny supports this idea. The survival data from the field suggest a hierarchy of probability of survival dependent on the degree of relatedness, so reproductive individuals in a local population may be only distantly related even though seed dispersal has been found to be quite limited (Dudash, unpubl.). *Sabatia angularis* produces hundreds of seeds per fruit and seed set can be pollinator limited (Dudash, unpubl.), suggesting that more than a single pollinator visit is required for production of a full seed set. Therefore, the progeny from one female may often consist of both half and full sibs, which would reduce the potential genetic relatedness among reproductive individuals by a factor up to one-half.

The ability to detect inbreeding depression at different stages in the life cycle varies with environmental conditions. In the fitness experiment the harshest environment in terms of plant mortality was the field. Decreasing soil moisture levels during the flowering season and lower absolute water levels late in the flowering season resulted in harsher field conditions compared to the garden plot. The latter received supplemental watering and exhibited very little plant mortality (Dudash, 1987). The greenhouse was the least harsh environment since progeny were watered and fertilized regularly and there was little mortality associated with the greenhouse following stratification. In *Costus,* Schemske (1983) also observed differences in progeny performance between greenhouse and field experiments and within environments grown at different stages in the life cycle. It has been argued that heterosis increases under adverse conditions and the data presented here on the magnitude of inbreeding depression expressed in the three environments support this general assumption (Lerner, 1954; Allard, 1965; Levin, 1970; Lloyd, 1980).

Selfing increases homozygosity and more homozygous genotypes may be susceptible to environmental harshness. In contrast, outcrossing usually increases or maintains heterozygosity that tends to produce genotypes better able to buffer themselves against environmental variability and harshness (Lerner, 1954; Falconer, 1981). The expected empirical evidence suggesting this line of reasoning was found in the greenhouse and garden and is in agreement with other empirical studies conducted under controlled conditions that demonstrated higher coefficients of variation for selfed individuals than outcrossed individuals (Griffing and Langridge, 1963; Jain, 1978; Wilken, 1982; Schemske, 1983). However, in the field the relationship was reversed, which suggests there was a genotype × genotype interaction or genetic complementarity between the parent plants used in the crossing program independent of heterosis and inbreeding depression, which has been referred to as combining ability by Falconer (1981). The field environment enhanced the detection of differences in combining abilities among the parent plants because the progeny were successfully grown in six sites at Miller Dunes (progeny genotype × environment interaction) compared to the more benign and less variable greenhouse and garden plot. Individuals have been found to combine as parents producing progeny that perform well in one environmental background (site) but not in another (Pederson, 1968).

The protandry exhibited by *S. angularis* has been shown to promote outcrossing in other plant species (Breese, 1959; Moore and Lewis, 1965; Vasek, 1965; Arroyo, 1973; Ganders et al., 1977; Schoen, 1982; Wyatt, 1984). Since an individual plant may have flowers in both male and female stages on any given day, the protandry is incomplete, allowing for geitonogamy, or transfer of pollen between two flowers on the same individual (Lloyd and Webb, 1986). Therefore, total flower production by an individual may also influence the potential for geitonogamy (Dudash, unpubl.). Garnock-Jones and Molloy (1982) have suggested that varying levels of geitonogamous pollinations of protandrous *Hebe amplexicaulis* may explain why self progeny from one maternal parent exhibited striking inbreeding depression and the other did not, and this may be partly the case for *S. angularis.* A selfing rate of 7% was estimated from reproductive adults of *S. angularis* (Dudash, 1987) grown from open pollinated seed in a greenhouse environment using the method described by Charlesworth and Charlesworth (1987) and suggests that protandry is an effective outcrossing mechanism which is responsible for maintaining a high genetic load and/or naturally produced self progeny in the field do not survive to reproduce. Adult representation of the gene pool has been argued to be a more accurate estimate than zygote estimates for genetic contributions to subsequent generations (Eldridge, 1976; Lloyd, 1979). Regardless of the life history stage influenced, inbreeding depression appears to be a major selective force maintaining outcrossing in the study populations of *S. angularis.*

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