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MATING SYSTEM CONSEQUENCES ON RESISTANCE TO HERBIVORY AND LIFE HISTORY TRAITS IN *DATURA STRAMONIUM*¹

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The consequences of selfing and outcrossing in *Datura stramonium*, a colonizing annual species, were experimentally assessed in terms of the fecundity of maternal plants and the performance of their progeny. Selfed and cross-pollinated plants were produced through controlled crosses and were then planted in their native environment. We recorded size at establishment, final height, number of branches, fruits, total biomass, and resistance to herbivores for each plant. Natural selection was measured for plant size and resistance to herbivores in both types of plants. Seed production (fecundity) was higher for fruits developed from outcrossing than for fruits derived from selfing. Among-family differences were detected for seed mass, but these were not related to mating system. No differences in germination, plant size, and fruit number were found between the two mating systems. Damage produced by two herbivores, *Epitrix parvula* and *Sphenarium purpurascens*, ranged from 0 to 95% of total leaf area; however, there were no differences in resistance to both herbivorous insects either among families or between mating systems. Plant survival through the whole season was similar for plants of both types of crossing. The direction and magnitude of phenotypic selection for each trait were similar for both types of progeny. Directional selection to increase plant size and reduce resistance to *E. parvula* was detected, while no selection was detected for resistance to *S. purpurascens*. Curvilinear (stabilizing/disruptive) and correlational selection modes were not detected for any trait. Inbreeding depression coefficients were all statistically indistinguishable from zero, except for seed number per fruit. The inbreeding depression coefficient for seed number was high and close to 40%, suggesting that evolution toward complete selfing may be prevented in this population of *Datura stramonium*.

Key words: *Datura stramonium*; herbivory; life history traits; mating system; outcrossing; selfing; Solanaceae.

Plants possess several mechanisms to promote cross-pollination and/or to avoid selfing. Floral characters like nectar, pollen, and oils (Steiner and Whitehead, 1991) are considered rewards to enhance pollination service by animals (Faegri and van der Pijl, 1979; Baker 1983; Wyatt, 1983). Several mechanisms are known that prevent selfing (though not inbreeding necessarily). The separation of sexual organs in different plants (dioecy) is the most extreme. Species with both sexual functions in the same organism (monoecy) or even in the same flower (hermaphroditic) may possess temporal (dichogamy, see Robertson and Lloyd, 1991) or spatial mechanisms (herkogamy, heterostyly) to avoid selfing. Genetic self-incompatibility may prevent both selfing and outcrossing (Richards, 1986). In spite of these mechanisms, selfing occurs in many plants species, although complete selfing is rare (Jain, 1976).

One reason for the avoidance of self-fertilization in plants may be the deleterious effects of inbreeding, known as inbreeding depression. Alternatively, selfing

may reduce the potential advantages brought about by heterosis, which is thought to be promoted by outcrossing (Charlesworth and Charlesworth, 1987). Thus, one would expect selection to act on the mating system of plants or traits related to it.

There is evidence that inbreeding depression and heterosis occur in plants, and that these phenomena can affect (in a negative and a positive manner, respectively) fitness components such as reproduction or even survival of the organism (see Wright, 1977; Schemske, 1983; Schoen, 1983; Charlesworth and Charlesworth, 1987). However, the effects of inbreeding depression are not equal in all plants and can be expressed at different life stages (Charlesworth and Charlesworth, 1987) and with different magnitudes in different life history traits (Falconer, 1981). Some plants can afford selfing without showing marked negative effects of inbreeding, while others show marked inbreeding depression (Charlesworth and Charlesworth, 1987).

Although the effects of inbreeding have been analyzed on many plant traits, such effects on plant resistance to herbivores have rarely been considered. Herbivory is a common phenomenon in plants, and available evidence shows that it affects plant fitness or several of its components (see Marquis, 1992). Resistance to herbivory refers to genetically controlled characteristics of individual plants of a species that result in it being less damaged by herbivores (Kennedy and Barbour, 1992). The mating system could affect those genetic qualities of individuals and thus could potentially affect resistance to herbivores.

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For instance, Schoen (1983) has shown that the ability of plants to recover after damage by herbivores (i.e., tolerance, a component of resistance to herbivores) was higher for outcrossed than for selfed progenies of the annual *Gilia achilleifolia*, though this difference was just marginally statistically significant. Although there is evidence for cultivated plants (see Kennedy and Barbour, 1992), we are unaware of other published reports for natural plant populations.

Simulations by Lande and Schemske (1985) have shown that assuming a constant genetic cost of outcrossing (reduction in the parent-offspring relatedness), the evolution of the mating system is controlled by inbreeding depression. In their model, a threshold value of 0.5 (i.e., the mean fitness of inbreeders is only 50% that of outcrossers) of inbreeding depression determines if selfing or outcrossing is favored. Only the extremes (complete selfing or obligatory outcrossing) represent evolutionarily stable mating system conditions, and populations that exhibit intermediate levels of outcrossing could represent transient stages during mating system evolution (see Schemske and Lande, 1985; Barrett and Eckert, 1990). Although there is evidence supporting this prediction (Schemske and Lande, 1985; Barrett and Eckert 1990), it has created some contention (see Aide, 1986; Waller, 1986; Schemske and Lande, 1986, 1987). Intermediate levels of outcrossing may represent stable mating systems if other factors, such as biparental inbreeding (Uyenoyama, 1986), pollen discount (Uyenoyama, Holsinger, and Waller, 1993), or covariation between the cost of outcrossing, selfing rate and inbreeding depression (Yahara, 1992) are taken into account.

A first approach to study mating system evolution is to evaluate the occurrence, magnitude, and consequences of inbreeding depression. *Datura stramonium* is a colonizing, annual, hermaphroditic species with a mixed-mating system, i.e., produces seeds through outcrossing (allogamy) and selfing (autogamy), although the rate of outcrossing is rather low (Motten and Antonovics, 1992). A remarkable characteristic is that individual plants of this species are frequently heavily damaged by herbivorous insects, and this damage has been found to affect plant fecundity (Núñez-Farfán and Dirzo, 1994). These two ecological aspects give relevance to the study of the interactions between plant mating system and plant resistance to herbivores. Thus, our goal was to determine the extent of inbreeding depression for traits related to fitness in *D. stramonium*, with particular emphasis on resistance to herbivores and natural selection on phenotypic variation in resistance among progenies derived from selfing and outcrossing.

The plant species—*Datura stramonium* is an annual, hermaphroditic plant with an average rate of outcrossing (t) of 3.9% for four experimental populations in North Carolina, and 1.3% for a natural population in the same region; however individual plants can reach a t of 18.7%, while others have a very low outcrossing rate (Motten and Antonovics, 1992). Some floral characteristics of *D. stramonium* are thought to enhance cross-pollination (Knuth, 1909; Faegri and van der Pijl, 1979). For example, the large, funnel-shaped corollas, with purple or white colors, and the production of floral nectar and large amounts of pollen are considered traits that attract polli-

nators. *D. stramonium*'s flowers open at dusk and pollen is exposed first. Late at night or the following morning, the stigma is receptive (J. Núñez-Farfán, personal observations). These flowers are visited mainly by long-tongued sphingids (Grant and Grant, 1983; Motten and Antonovics, 1992) and in daytime by bees (Sharma, 1972; Grant and Grant, 1983; Motten and Antonovics, 1992). The production of nectar starts at 1900, and ends at 2400. Nectar sugar concentration ranges from 3.9% (Sharma, 1972) to 10–24% (J. Núñez-Farfán, personal observation at Morelos, México).

Selfing has been proposed as advantageous for colonizing plants like *D. stramonium*, which occupy new habitats where there may be a limited number of potential mates or scarcity of pollinators (Baker, 1955, 1967). Floral characteristics could enhance cross-pollination in this species, yet selfing is readily achieved under certain circumstances, especially because (1) anthers dehisce before the corolla opens and (2) due to the proximity of the anthers to the stigma, pollen often falls onto the stigma and seeds may be produced by self-fertilization ("automatic pollination"; Knuth, 1909; see also Motten and Antonovics, 1992). However selfing may be reduced if there is a spatial separation between anthers and stigma (i.e., herkogamy), which indeed happens in this species (Motten and Antonovics, 1992; and J. Núñez-Farfán, personal observations). In this paper we experimentally examined the ecological consequences of selfing or outcrossing in one population of *D. stramonium* in the Valley of Mexico. If the floral characteristics mentioned above have evolved to promote outcrossing and avoid selfing in this plant species, it would be expected that individuals derived from self-pollination would have a decreased fitness (due to inbreeding depression) when compared to plants produced by cross-pollination. Given the prevalence of herbivory found naturally in this species (from 0 to 90% of total leaf area eaten; Núñez-Farfán and Dirzo, 1994), we attempted to assess the effects of mating system on plant resistance to herbivores as a contribution to the study of the consequences of mating systems in plant evolutionary ecology.

MATERIALS AND METHODS

Experimental design—In the fall of 1987, seeds from 450 individuals were collected from an 8-ha area within the Pedregal de San Angel Preserve (Ciudad Universitaria, D.F.) in southern Mexico City. Here, scattered patches of *D. stramonium* can be found during the rainy season (May–October) along roadways, waste places, and where the native vegetation (xerophitic scrubland) has been removed. We sowed seeds from 40 randomly chosen individuals (henceforth families) to carry out controlled self- and cross-pollination on each plant to evaluate seed production and seedling performance. To produce seeds by cross-pollination we removed the anthers of a given flower 2–3 d before opening and we covered the flower with a soft mesh bag, which was re-opened to cross-pollinate the plants when the stigma was receptive. We took pollen from another randomly chosen plant and applied an excess of pollen to the stigma. In order to produce seeds by self-pollination, we rubbed the anthers of a given flower onto the receptive stigma of the same flower and the flower was covered with a soft mesh bag to avoid pollen contamination. To check whether *D. stramonium* produces seeds through agamospermy, an additional set of 32 plants was used. In one flower of each of these plants, the anthers were removed as described above, but the stigmas were not supplied with pollen; these were also bagged as de-

scribed above. The pollen of *D. stramonium* is sticky and hardly moved by wind. This, and the fact that the plants were maintained in a glasshouse, diminished the probability of contamination with alien pollen. Self- and cross-pollinated flowers within a plant were labeled with colored thin wires. When the fruits ripened, we collected and counted the seeds from plants in each pollination type and family. Two random samples of seeds ($N = 30$ each) per type of mating, per family, were individually weighed using an analytical balance prior to their germination ("pregermination" seed mass). Seeds were germinated in a controlled-environment chamber (12 h light, 25°C; 12 h dark, 20°C). Percentage of germination per type of mating was recorded, and the emerged seedlings were placed in jiffy pots and transplanted within 2 wk to an experimental outdoor plot in the Pedregal de San Angel Preserve.

Survival and size (the number of expanded leaves on each plant) were evaluated after 1 mo. By this time, plants were damaged by the tobacco flea beetle (*Epitrix parvula*, Coleoptera: Chrysomelidae), and at reproduction, 2 mo later, by a grasshopper, locally known as "chapulín" (*Sphenarium purpurascens*, Orthoptera: Acrididae), which is an abundant generalist herbivore. The two types of damage are not correlated (Núñez-Farfán and Dirzo, 1994). We measured total number of leaves (TNL) and leaves damaged by *E. parvula* (DL) on 1-mo-old plants, and defined relative resistance per plant as $R = 1 - (DL/TNL)$. For *S. purpurascens* damage, we collected all leaves of each plant 3 mo after planting (i.e., at the end of the study) and measured them with a leaf area meter (Delta-T-Devices, Ltd., Cambridge, England). For each plant, we obtained total leaf area (TLA), and leaf area damaged (LAD). We defined relative resistance as $R = 1 - (LAD/TLA)$ (see also Núñez-Farfán and Dirzo, 1994). Data on relative resistance to herbivores were arcsine-transformed for statistical analyses and retransformed for data presentation. At reproduction, we measured plant height, biomass, number of branching units, and number of initiated fruits. Variables measured as counts were square-root transformed for statistical analysis (Sokal and Rohlf, 1981). Plant survival was recorded every 4 wk until harvest at the beginning of November 1989.

Statistical analysis—Two-way ANOVA mixed models were performed to assess the effects of family (random effect), mating system (fixed effect), and interaction ($F \times M$; random effect) on each progeny variable (see Mitchell-Olds and Waller, 1985). For number of seeds per fruit, germination, and survivorship we did not have replication within families (i.e., we had one fruit per family of each type of mating) and thus we only made the comparison between the two types of mating system (pooling all plants). To determine whether seed mass affects the probability of germination, we measured seed mass prior to germination and recorded which seeds germinated. This analysis allowed us to see if mean seed mass before germination (all seeds of a fruit) differs from the mean mass of those seeds that actually produced a seedling.

Survival of progeny from each mating system was analyzed using a log-linear model, where time was a continuous variable and mating system a categorical variable. This model was fitted using GLIM (Baker and Nelder, 1978), following the equation:

$$N_t = e^{(a - bt + cm + dmt)}$$

where N_t is the number of survivors at time t , a is $\ln(N_0)$, being N_0 the initial number of individuals, b , c , and d are the coefficients for time (t), mating system (m), and interaction (mt), respectively. b is the rate of mortality through time, and c is the effect of cross type, and d indicates if the type of mating system affects the slope of the survivorship curve through time. We used a Poisson error and a logarithmic link function (see Valiente-Banuet and Ezcurra, 1991).

Inbreeding depression—The inbreeding depression coefficient for each character was estimated as suggested by Lande and Schemske (1985) as:

$$\delta = 1 - (\bar{w}_s/\bar{w}_o),$$

where \bar{w}_o is the mean fitness of outcrossed progenies and \bar{w}_s is the mean

fitness of selfed progenies. Mean values were compared by Tukey's HSD test (Sokal and Rohlf, 1981).

Phenotypic selection analysis—The mode, intensity, and direction of natural selection on phenotypic variation for plant resistance to herbivores and plant size were estimated independently for selfed and outcrossed progenies using multiple regression (Lande and Arnold, 1983). In order to estimate directional (β , coefficients) and curvilinear (stabilizing, disruptive, and correlational; γ_{ij} , coefficients) selection, we used partial multiple linear regression and quadratic multivariate regression, respectively, of relative plant fitness against plant size and resistance to herbivores (Lande and Arnold, 1983; Mitchell-Olds and Shaw, 1987; Rausher and Simms, 1989; Núñez-Farfán and Dirzo, 1994). Relative fitness (ω_i) of a given plant was estimated as $\omega_i = W_i/\bar{W}$, where W_i is the number of initiated fruits for plant i , and \bar{W} is the mean number of fruits for each type of progeny; thus, $\bar{\omega} = 1$ (Lande and Arnold, 1983). Our estimator of individual fitness was the total number of fruits per plant, which is significantly correlated with maternal reproductive output (Núñez-Farfán and Dirzo, 1994). This estimate of fitness ignores the contribution of the male function to total reproductive fitness and was considered as a constant for families and progeny types. Other fitness components (e.g., plant survival, growth) were analyzed independently, and we estimated the selection coefficients independently for each type of progeny.

RESULTS

Compatibility and agamospermy—Results of the controlled pollinations revealed that *D. stramonium* is a highly compatible species. Ninety out of 98 flowers (91.8%) supplied with self-pollen produced fruits, a frequency similar ($\chi^2 = 0.008$; $P > 0.50$) to flowers supplied with pollen from another plant (92.1%, i.e., 106 out of 115). Emasculated flowers that were bagged without any pollination failed to produce fruits (0 fruits in all cases, $N = 32$ flowers), indicating that seeds are not produced via agamospermy.

Morphological and life history characters—Analyses of variance of life history traits revealed, with one exception, no differences related to mating system (Tables 1, 2). The only significant difference among progenies derived from selfing and outcrossing was total number of seeds per fruit. "Pregermination" mean seed mass was significantly different among families and the interaction family \times type of mating was significant; the same result was obtained for "postgermination" seed mass (i.e., mass of those seeds that germinated), indicating again that mating system did not affect seed mass (Table 1). Germination percentage did not differ significantly between selfed (79.3%) and outcrossed (67.9%) progeny (Table 1) and, although individual plants differed in their mean seed mass, there was not a consistent pattern of germination related to family or mating type. No differences were detected for morphological or life history related traits, such as plant size at establishment, number of branching units and fruits, and total biomass (Table 1).

Resistance to herbivores—Damage produced by herbivores was high, with average values of relative damage for selfed and outcrossed progenies of 53 and 47%, respectively, for *E. parvula*, and 32 and 34%, respectively, for *S. purpurascens*. The analysis revealed a lack of significant effects of family or mating system on relative resistance to both herbivores (Table 1). This result is reinforced by the distributions of relative resistance dis-

TABLE 1. Results of ANOVA on each character measured on selfed and outcrossed progenies of *Datura stramonium*. Sum of squares (SS) and *F* statistics are given for the family effect (fam), mating system (ms), interaction (f × ms), and error (err). Degrees of freedom for each source of variation are given in parentheses. * *P* < 0.05; *** *P* < 0.001; NS = not significant.

Character	SSfam	SSms	SSfxms	SSerr	Ffam	Fms	Ffxms
Seed number		122.127 (1)		393.344 (64)		18.244***	
Seed mass ^a	40963.491 (15)	1573.376 (1)	13803.141 (15)	30357.367 (928)	83.481***	1.710NS	28.130***
% seed germination		0.259 (1)		2.784 (30)		2.793NS	
Seed mass ^b	4723.712 (9)	95.814 (1)	2586.669 (9)	5200.384 (132)	13.322***	0.333NS	7.295***
Leaf number at 4th wk	0.659 (9)	0.008 (1)	0.657 (9)	5.472 (132)	1.766NS	0.105NS	1.760NS
Stature (cm)	870.232 (9)	26.768 (1)	520.601 (9)	9839.392 (132)	1.297NS	0.463NS	0.776NS
Number of branches	7.225 (9)	0.890 (1)	13.518 (9)	190.096 (132)	0.557NS	0.592NS	1.043NS
Number of flowers	5.616 (9)	1.509 (1)	14.956 (9)	158.210 (132)	0.521NS	0.908NS	1.387NS
Number of fruits	0.310 (9)	0.037 (1)	0.534 (9)	9.114 (132)	0.498NS	0.619NS	0.859NS
Total biomass (g)	0.454 (9)	0.022 (1)	0.819 (9)	9.559 (132)	0.697NS	0.243NS	1.257NS
Resistance to <i>Epitrix parvula</i>	1.266 (9)	0.033 (1)	2.405 (9)	26.447 (132)	0.702NS	0.122NS	1.334NS
Resistance to <i>Sphenarium purpurascens</i>	0.366 (9)	0.011 (1)	0.218 (9)	5.394 (132)	0.995NS	0.457NS	0.592NS

^a "Pregermination" (see Methods.)

^b "Postgermination."

played by each type of progeny (Fig. 1). A Kolmogorov-Smirnov two-sample test comparing the distributions did not reveal differences among them for either *E. parvula* or *S. purpurascens* ($D_{\max} = 0.101$, $P = 0.835$; $D_{\max} = 0.107$, $P = 0.781$, respectively; Fig. 1).

Plant survival—Survivorship curves of plants derived from selfing and outcrossing appeared to be very similar (Fig. 2) and parallel through time. Only the linear coefficient (*b*) from the log-linear model was statistically significant, indicating a constant rate of mortality (Fig. 2). There was no detectable effect of mating system on the rate of mortality nor on the shape of the survivorship curve. The Type II survivorship curve fitted explained

96% of the variance in mortality and the mean survival at reproduction for self- and outcross-derived plants was nearly 70% (Fig. 2).

Inbreeding depression—For "pre-" and "postgermination" seed mass, percentage of germination, size-related traits (height, number of branches and total biomass), and reproductive characters (number of fruits), the progenies from the two mating systems behaved similarly and no significant effects of inbreeding depression were detected (Table 2), except for the number of seeds per fruit, which displayed a strong negative effect of inbreeding (Table 2).

Both progenies showed a strikingly similar level of

TABLE 2. Mean (\bar{X}) and standard deviation (SD) for fitness-related characters in progenies of *Datura stramonium* derived from selfing and outcrossing. δ estimates the magnitude of inbreeding depression. NS indicates that δ is statistically not different from zero, while * means that δ was significantly different from zero ($P < 0.001$) (after a Tukey HSD Test).

Character	Mating system				δ
	Selfing		Outcrossing		
	\bar{X}	SD	\bar{X}	SD	
Seed number	58.32	39.79	95.91	39.10	+0.39*
Seed mass ^a (g)	66.60	10.04	64.20	8.74	-0.03 NS
% germination	79.30	23.05	67.90	29.19	-0.16 NS
Seed mass ^b (g)	67.18	10.20	64.40	9.00	-0.04 NS
Leaf number at 4th wk	6.74	1.10	6.71	1.17	-0.01 NS
Stature (cm)	24.88	8.10	23.76	11.50	-0.04 NS
Total biomass (g)	2.89	2.90	2.66	2.78	-0.08 NS
Number of branches	15.50	10.20	13.90	12.50	-0.11 NS
Number of fruits	10.40	7.88	8.90	8.17	-0.16 NS
Resistance to <i>Epitrix parvula</i>	0.464	0.318	0.530	0.458	+0.08 NS
Resistance to <i>Sphenarium purpurascens</i>	0.683	0.164	0.665	0.198	-0.02 NS

^a "Pregermination."

^b "Postgermination."

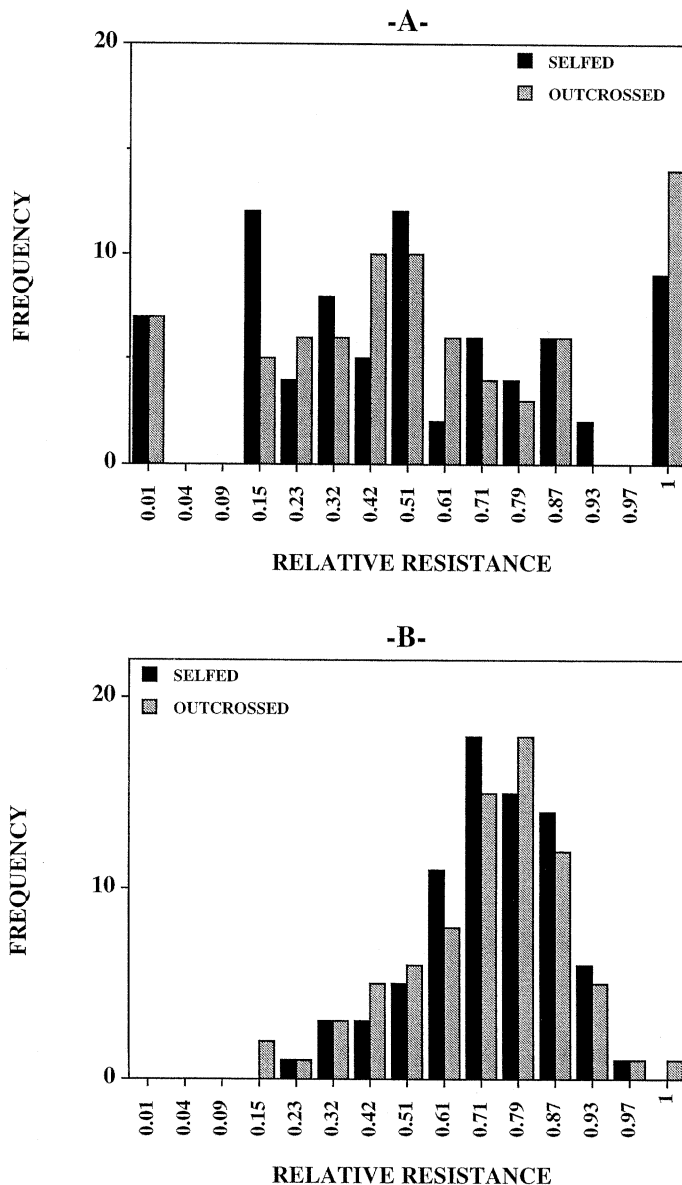


Fig. 1. Frequency distribution of relative resistance to *Epitrix parvula* (A) and *Sphenarium purpurascens* (B) for selfed and outcrossed progenies of *Datura stramonium* (see Results). Sample size = 78, for each type of progeny.

resistance to both herbivores, with no inbreeding depression detected for this trait (Table 2). Besides the similarity of the mean levels of resistance of both progenies, variances and frequency distributions of resistance scores were also similar (Table 2; Fig. 1). Thus, inbreeding appears to have no effect on the system of resistance to herbivores of *D. stramonium*.

Phenotypic selection—While the estimates of inbreeding depression focused on the mean values of both types of progenies, the selection analysis concentrated on the effects of phenotypic variation of plant size and resistance to herbivores on individual fitness. Selection analyses detected a significant effect of plant size and resistance to the flea beetle on fitness, but selection was equal for selfed and outcrossed progenies (Tables 3A, 4A). For plant size

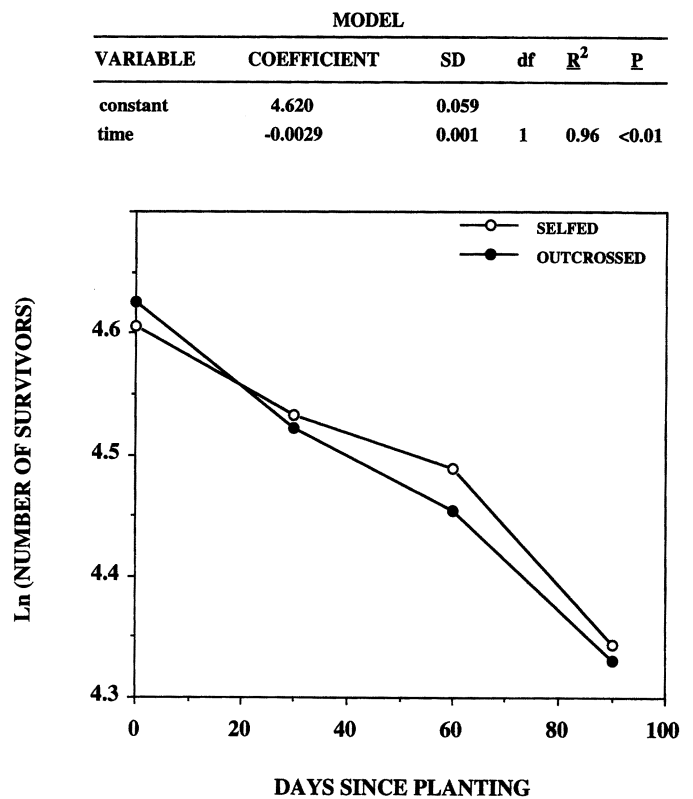


Fig. 2. Survivorship curve for selfed and outcrossed progenies of *Datura stramonium* in the Valley of Mexico. Values of coefficients, variance explained, and probability are given at the figure's top. The only significant coefficient indicates a Type II survivorship curve. Model's terms related to mating system were not significant. Sample sizes were 102 and 101 for selfed and outcrossed progenies, respectively.

the result was similar, even in the magnitude of the selection gradient, for selfed and outcrossed progenies (Tables 3A, 4A). In the same analysis, selection for reduced resistance to *E. parvula* was detected. The directional selection gradient (β_j), which can be expressed in terms of phenotypic standard deviations ($\beta_{j[std]}$ in Tables 3A, 4A), indicates that a change in one standard deviation in plant size would have an increase in fruit production of 23.5% for selfed progenies and 29.5% for outcrossed progenies (Tables 3A, 4A). Also, an increase of one standard deviation in resistance to *E. parvula* would reduce relative fitness by only 7.1% and 6.4% in selfed and outcrossed progenies, respectively (Tables 3A, 4A). Thus, directional selection is "strong" for plant size and "weak" for resistance to *E. parvula*. No selection was detected for resistance to *S. purpurascens*. Both models were statistically significant and explained 46.5% (Table 3B) of the variance in fitness for selfed progenies and 64.7% for outcrossed progenies (Table 4B). None of the quadratic selection gradients were statistically significant, indicating that there is no evidence of curvilinear selection on plant size or resistance to herbivores in either type of progeny.

DISCUSSION

Strong inbreeding depression in *D. stramonium* was found for seed number per fruit. The inbreeding depression coefficient showed nearly a twofold difference be-

TABLE 3. (A) Directional selection gradients (β_i) and standardized selection gradients [$\beta_{i(Std)}$] for three traits in selfed progeny of *Datura stramonium*. Values in parentheses are standard errors of coefficients. (B) Analysis of the variance and adjusted R^2 for the regression model.

A)						
Plant character	β_i	$\beta_{i(Std)}$	t	P		
Resistance to <i>Epitrix parvula</i>	-0.164 (0.071)	-0.071 (0.030)	-2.307	<0.05		
Resistance to <i>Sphenarium purpurascens</i>	0.300 (0.177)	0.054 (0.032)	1.688	NS		
Plant size	0.0293 (0.0040)	0.235 (0.032)	7.320	<0.001		
B)						
Source	Sum of squares	df	Mean square	F	P	R^2
Regression	4.6220	3	1.5407	21.163	<0.001	0.465
Residual	5.3130	73	0.0728			

tween outcrossing (95.9 seeds per fruit, on average) and selfing (58.3). Although no differences were found regarding resistance to herbivores, survival, morphological, and life history traits between selfing and outcrossing, seed number is an important component of fitness for annual plants. Thus, inbreeding depression on this single trait may prevent evolution toward complete selfing in this population. Similar results have been found in three species of the genus *Costus* (Schemske, 1983), where natural and hand cross-pollination produced more seeds than self-pollination, indicating that inbreeding depression can be expressed in reproductive characters (although it could also be expressed in other life stages; see Schemske, 1983; McCall, Waller, and Mitchell-Olds, 1994). The magnitude of inbreeding depression for seed number in *D. stramonium* is similar to values reported for other herbaceous angiosperms (see Table 2 in Charlesworth and Charlesworth, 1987).

The most parsimonious explanation for our results is that inbreeding depression is acting during fertilization or zygote formation, perhaps through the expression of recessive deleterious loci, and that this might explain the differences in seed set between fruits developed from self- and cross-pollination. Other conceivable explanations for the differences in seed number produced by the two types of mating are (1) that there are differences among mother plants in their seed-filling capacity (fecundity) and (2) sexual selection. The first hypothesis can be ruled out, since each mother plant produced both outcrossed and selfed fruits and thus there is no possible reason for differences in fecundity among fruits other

than inbreeding depression. The second possibility is not as easy to discard since pollen donor plants may vary in their ability to fertilize and mother plants may select against particular "fathers," aborting selectively the zygotes or immature seeds (Stephenson and Winsor, 1986). However, the possibility that sexual selection may produce differences in fecundity between the two types of mating is probably low, since a given outcrossed fruit developed from the pollen of only one randomly selected father, so there was not much opportunity for sexual selection to act.

Herbivory—Although inbreeding could be expected to alter the level of genetically based resistance to herbivores (see Kennedy and Barbour, 1992), this character has not been explicitly analyzed in plants from this perspective. There are some studies that tangentially indicate that inbreeding may reduce plant survival under intense levels of herbivory (see Schoen, 1983; McCall, Waller, and Mitchell-Olds, 1994). Moreover, several studies of cultivated plants have shown that, during the process of artificial selection to maximize yield using inbred lines, a reduction in resistance takes place (see Kennedy and Barbour, 1992 and references therein), indicating the presence of trade-offs between fitness components. Production of inbred lines would increase the probability of producing homozygous deleterious loci and inbreeding depression, which could reduce (or change) defenses to herbivores and increase the probability of death due to extensive damage. In our study, however, plants derived from selfing and outcrossing showed similar values of

TABLE 4. (A) Directional selection gradients (β_i) and standardized selection gradients [$\beta_{i(Std)}$] for three traits in outcrossed progeny of *Datura stramonium*. Values in parentheses are standard errors of coefficients. (B) Analysis of the variance and adjusted R^2 for the regression model.

A)						
Plant character	β_i	$\beta_{i(Std)}$	t	P		
Resistance to <i>Epitrix parvula</i>	-0.141 (0.058)	-0.064 (0.0265)	-2.4305	<0.05		
Resistance to <i>Sphenarium purpurascens</i>	0.034 (0.128)	0.0077 (0.0286)	0.2709	NS		
Plant size	0.0325 (0.0031)	0.295 (0.0286)	10.2913	<0.001		
B)						
Source	Sum of squares	df	Mean square	F	P	R^2
Regression	6.982	3	2.232	42.206	<0.001	0.647
Residual	3.8055	72	0.0529			

damage caused by both herbivorous insects, indicating that the level of resistance was comparable in both types of progenies and that there is no detectable effect of inbreeding depression on this trait in *D. stramonium*.

Survival and life history—Survival of selfed and outcrossed progenies was very similar ($\approx 70\%$ by the end of the growing season), reinforcing the conclusion that inbreeding depression acted only during seed formation. For several correlated growth characters such as height, number of branching units, number of fruits, and total biomass (see Núñez-Farfán, 1991), the analyses again showed no significant differences between the progenies, indicating that they were genetically similar. An analogous interpretation has been advanced for the grass *Danthonia spicata*, in which seedlings derived from seeds produced by chasmogamous and cleistogamous flowers showed similar survival rates in the field (Clay, 1983; Clay and Antonovics, 1985). In contrast, it has been found that inbreeding significantly reduces survivorship in *Impatiens capensis* (McCall, Waller, and Mitchell-Olds, 1994).

The influence of inbreeding on the different life stages of a plant may depend on its environment (Dole and Ritland, 1993). For instance, inbreeding depression may be stronger when the environment is more “harsh,” both physically (e.g., reduced soil humidity and shading) and biotically (e.g., competitors, herbivores; Wolfe, 1993), than when it is more “benign.” In a study of the effects of inbreeding in *D. stramonium*, inbred- and outcrossed-derived plants were grown in two types of environment. In an environmental regime with periods of dryness and flooding, the effects of inbreeding depression on plant reproductive output were greater than in a benign, mesic environment (A. Motten, personal communication). The physical conditions of the site in which the plants of the present study were grown were similar for both types of progenies and very close to the average natural conditions. However, the regular spatial arrangement of plants used in this study (1.2 individuals/m²) differs from the usual natural distribution of clumps of higher local density, implying that the density might have been lower than that normally observed at reproduction in natural populations of *D. stramonium* at this locality. Thus, the possibility that inbreeding depression could be stronger in a more drastic environment (e.g., intense competition during establishment) cannot be ruled out. Likewise, the average levels of damage we estimated in the present study were, by far, greater than in previous years (cf. Núñez-Farfán, 1991), yet no detectable effects of inbreeding were found in terms of plant resistance to herbivory.

In relation to life history traits, Kalisz (1989) found significant differences in seed mass, germination, and emergence rates among plants of *Collinsia verna* derived from selfing and outcrossing. Comparable results were found in *Impatiens capensis* when comparing seed mass of progeny derived from random outcrosses and selfing (McCall, Waller, and Mitchell-Olds, 1994). In the present study, average seed mass was similar for both types of mating system (seed size “pregermination” in Table 2) and, although there were differences among families in average seed mass, the seeds showed similar germination

levels. We found no differences in average seed mass based on all seeds and only those that germinated. In contrast to the findings with *C. verna* and *I. capensis*, the present results indicate that inbreeding does not always reduce the size of the seed nor its capacity to germinate.

Natural selection on selfed and outcrossed progenies—Selection on plant size and resistance to herbivores was equivalent (in mode and magnitude) on both types of progeny. Previous results revealed that herbivores affect fitness components in *D. stramonium* and that genetic variation for resistance in this population is low (Núñez-Farfán and Dirzo, 1994). The results obtained here confirm previous results in the sense that plant size is highly related to fruit production, so selection for this trait is directional and positive. As in our previous study, we detected selection for reduced resistance to *E. parvula*, a result that is difficult to explain. It is possible that heavily damaged plants possess high growth rates and can tolerate damage, although we would not expect damaged plants to perform better than undamaged plants. Another possible explanation is that plants are overcompensating. Compensatory growth has been observed in other plant species (see Paige and Whitham, 1987), although the prevalence of this phenomenon in nature is still controversial (Whitham et al., 1991). A similar result has been found for another species of flea beetle feeding upon *Ipomoea purpurea* (Rauscher and Simms, 1989) and the authors suggested that perhaps low to moderate levels of damage could be beneficial to the plant.

Mating system evolution—The level of inbreeding depression for *D. stramonium* found in this study indicates, according to Lande and Schemske's (1985) model, that this population can afford the cost of inbreeding and may evolve toward an endogamous mating system (obligate selfer). However, both empirical and theoretical studies indicate that this prediction is not warranted. First, the inbreeding depression coefficient found for seed number is high and near 50%, a fact that may delay or even prevent evolution toward complete selfing in *D. stramonium*. Also, studies in this species suggest that the mixed-mating system could represent an evolutionarily stable state if we take into account the plant's floral biology through time (see discussion in Motten and Antonovics, 1992), in which outcrossing, although reduced, is likely to occur at some point during the plant's life-span. The spatial variation of the sexual structures within the flower (herkogamy) will lead to an increased probability of outcrossing (Motten and Antonovics, 1992). Theoretically, if inbreeding depression is not the only factor contributing to the evolution of the mating system, mixed-mating systems could be evolutionarily stable if (1) there is pollen discounting (Uyenoyama, Holsinger, and Waller, 1993), (2) there are biases in the cost of outcrossing (parent-offspring relatedness) from 0.5 (biparental inbreeding; Uyenoyama, 1986), or (3) there is covariation among selfing rate, inbreeding depression, and the cost of outcrossing (Yahara, 1992).

The magnitude of inbreeding depression is expected to evolve jointly with the mating system (Lande and Schemske, 1985; Uyenoyama, 1986; Charlesworth and Charlesworth, 1987; Uyenoyama, Holsinger, and Waller, 1993). In this context, it would be interesting to test predictions

of the models regarding the factors leading to the maintenance of mixed-mating systems, particularly in species where the outcrossing rates vary. Populational variation in outcrossing rate has been reported for several plant species (e.g., Grant, 1975; Kahler, Clegg, and Allard, 1975; Rick, Fobes, and Holle, 1977; Wyatt, 1986; Cugen et al., 1989) and we are currently exploring its occurrence in natural populations of *D. stramonium*.

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