CORRELATED EVOLUTION OF SELF-FERTILIZATION AND INBREEDING DEPRESSION: AN EXPERIMENTAL STUDY OF NINE POPULATIONS OF *AMSINCKIA* (BORAGINACEAE)

MARK O. JOHNSTON[1] AND DANIEL J. SCHOEN
Department of Biology, McGill University, 1205 Docteur Penfield Avenue, Montreal, Quebec H3A 1B1, Canada

Abstract.—The relation between inbreeding depression and rate of self-fertilization was studied in nine natural populations of the annual genus *Amsinckia*. The study included two clades (phylogenetic lineages) in which small-flowered, homostylous populations or species are believed to have evolved from large-flowered, heterostylous, self-compatible ones. In one lineage the small-flowered species is tetraploid with disomic inheritance. Rates of self-fertilization were 25% to 55% in the four large-flowered, heterostylous populations; 72% in a large-flowered but homostylous population; and greater than 99.5% in the four small-flowered, homostylous populations, which produce seed autonomously. When present, inbreeding depression occurred in the fertility but not the survival components of fitness. Using a cumulative fitness measure incorporating both survival and fertility (flower number), we found inbreeding depression to be lower in the four very highly self-fertilizing populations than in the five intermediate ones. The Spearman rank correlation between inbreeding depression and selfing rate for the nine populations was −0.50, but was not statistically significant (*P* = 0.12). Inbreeding depression was greater in the two tetraploid populations than in the very highly self-fertilizing, diploid ones. Phenotypic stability of progeny from self-fertilization tended to be higher in populations with lower inbreeding depression. We conclude that levels of self-fertilization and inbreeding depression in *Amsinckia* are determined more by other factors than by each other. Estimates of mutation rates and dominance coefficients of deleterious alleles, obtained from a companion study of the four highly self-fertilizing populations, suggest that a strong relationship may not be expected. We discuss the relationship of the present results to current theory of the coevolution of self-fertilization and inbreeding depression.

Key words.—Autogamy, dominance, flower size, heterosis, heterostyly, homeostasis, mating system, mutation rate, mutation-selection balance, overdominance, pollination, polyploidy.

Increased rates of self-fertilization have evolved many times in independent phylogenetic lineages of the flowering plants (Stebbins 1950, p. 176, 1957, 1974, ch. 4). Several ecological and genetic theories have been proposed to explain the evolution of higher selfing rates. These include reproductive assurance when pollinators or pollen are scarce (Hagerup 1951; Baker 1955; Stebbins 1957); the automatic transmission advantage of a gene causing complete self-fertilization while allowing pollen dispersal (Fisher 1941; Jain 1976; Nagylaki 1976); the maintenance of adaptive combinations of alleles at different loci (Stebbins 1957; Grant 1975, ch. 24; Solbrig 1976); and the lower cost of producing selfed offspring (Schemske 1978; Waller 1979; Schoen and Lloyd 1984). The relative importance of each of these possibilities remains unresolved.

The persistence of highly outcrossing species suggests either that self-pollinating taxa are evolutionary dead ends (Stebbins 1957) or that contemporary forces continue to inhibit the evolution of increased selfing rates, or both. Of the likely inhibitory forces, inbreeding depression, the reduced fitness of selfed relative to outcrossed progeny, is one of the most commonly documented phenomena in plants. It is found in species with diverse life histories and mating systems (reviewed by Charlesworth and Charlesworth 1987; Johnston 1992; Uyenoyama et al. 1993), but tends to occur at later life stages in primarily selfing taxa than in outcrossing taxa (Husband and Schemske 1996). This fitness reduction of selfed progeny is usually accompanied by greater phenotypic vari-

ability, often interpreted as a general decrease in buffering ability in the face of environmental fluctuations (Wright 1977, ch. 4). Inbreeding depression is hypothesized to have two primary genetic origins. Both depend on the fact that selfing increases offspring homozygosity relative to outcrossing. The overdominance hypothesis suggests that selfed offspring are less fit because they have reduced heterozygosity at loci with heterozygote advantage. The partial dominance hypothesis suggests that there is ongoing mutation to wholly or partially recessive deleterious alleles at many loci. The available data suggest that partial dominance accounts for most observed inbreeding depression (Wright 1977, ch. 2; Charlesworth and Charlesworth 1987; Johnston and Schoen 1995; but see Fu and Ritland 1994).

Many phenotypic and genetic models of the evolution of selfing conclude that complete outcrossing should evolve whenever selfed offspring are less than one-half as fit as outcrossed (inbreeding depression greater than one-half), and otherwise complete selfing should evolve (e.g., Kimura 1959; Lloyd 1979). The degree to which inbreeding depression determines the evolution of selfing rates, however, has been questioned from several angles. First, whenever selfing reduces paternal success from pollen dispersal, Fisher's (1941) automatic transmission advantage decreases, causing outcrossing to be favored at levels of inbreeding depression below one-half (Nagylaki 1976; Lloyd 1979; Wells 1979; Charlesworth 1980; Holsinger et al. 1984). Second, it may be important to include the effects of inbreeding among relatives, as this reduces the automatic selection advantage (Uyenoyama 1986). Third, mixed cleistogamous selfing and chasmogamous outcrossing can evolve when the resulting

[1] Present address: Department of Biology, Dalhousie University, Life Sciences Centre, Halifax, Nova Scotia B3H 4J1, Canada. E-mail: Mark.Johnston@Dal.Ca

two seed types are dispersed to microhabitats with different selective pressures (Schoen and Lloyd 1984). Fourth, genes causing large increases in the selfing rate can increase in frequency even in the presence of high levels of inbreeding depression (Lande and Schemske 1985; Holsinger 1988). Fifth, partial linkage between a selected locus (Uyenoyama and Waller 1991) or many selected loci (Charlesworth et al. 1992) and a locus determining the selfing rate can reduce the chance that selfing will evolve. Partial linkage between selected loci themselves also decreases inbreeding depression slightly (Charlesworth et al. 1992).

Finally, predictions based on a constant level of inbreeding depression may be unrealistic, because inbreeding depression should evolve in concert with the selfing rate (Lande and Schemske 1985; Campbell 1986; Holsinger 1988). As higher selfing rates cause greater efficiency of selection against deleterious alleles that are recessive or partially recessive, the level of inbreeding depression should be lowest in more highly selfing populations. Several explicit genetic models, however, find that such coevolution does not change conclusions about the role of inbreeding depression when it is caused by deleterious mutations at few or many unlinked loci, and when selfing evolves by small increments (Lande and Schemske 1985; Charlesworth et al. 1990; Charlesworth et al. 1991). With inbreeding depression caused by mutations to partially recessive alleles at unlinked loci, Charlesworth et al. (1990, 1991) studied the effects of four parameters on the equilibrium level of inbreeding depression. Of these, s, the average selective disadvantage of a mutant homozygote, has little effect. In contrast, equilibrium inbreeding depression increased with U, the mutation rate per sporophyte genome, and decreased with both h, the dominance level of mutations, and S, the population selfing rate. The last two results are intuitive in that higher dominance levels cause greater phenotypic effects in heterozygotes, exposing mutations to greater selection, as do higher selfing rates through their effect on homozygous frequencies. Furthermore, higher (more nearly additive) dominance levels flatten the relationship between equilibrium inbreeding depression and the selfing rate. Uyenoyama and Waller (1991) investigated a model in which inbreeding depression was caused by mutation at a single locus (in diploids). They distinguished their approach from that of Charlesworth et al. (1990, 1991) as involving true coevolutionary dynamics rather than serial, stepwise changes in selfing and inbreeding depression. In agreement with Holsinger (1988), they concluded that levels of inbreeding depression and rates of self-fertilization are determined not strictly by each other, but also by associations between loci determining the two parameters.

In addition to the several factors discussed above, the level of inbreeding depression may also be influenced by the level of ploidy (Bennett 1976; Lande and Schemske 1985). Polyploidy is associated with high levels of self-fertilization, especially among annual plants. This association probably results from the fact that a newly arisen polyploid in a diploid population can form successful zygotes only by mating with itself (Stebbins 1971, p. 130; Grant 1981, p. 317; see also Barrett 1989). It is also possible, however, that polyploidy promotes the evolution of self-fertilization by directly decreasing inbreeding depression. It is therefore of interest to know whether, for a given level of self-fertilization, polyploid populations experience less inbreeding depression than diploid ones.

The role of inbreeding depression as a factor influencing the evolution of the mating system remains unclear, and additional experimental data could be of value in distinguishing among the various hypotheses. If levels of inbreeding depression and self-fertilization are closely associated in natural populations, then it is reasonable to conclude that those models predicting a close relationship are sufficient to explain a large part of selfing-rate evolution. If, on the other hand, there is little or no correspondence between inbreeding depression and the selfing rate, then one or several conclusions are possible. First, the relationship is expected to be flat when deleterious mutations are more nearly additive than recessive (Charlesworth et al. 1990; Johnston and Schoen 1995). Second, the selfing rate may evolve too fast for an association to develop. Third, ecological factors may cause the selfing rate to vary among seasons, not only rendering any one season unrepresentative, but also slowing attainment of equilibrium. Fourth, ecological factors (pollination assurance, pollen and seed discounting, changes in population size, mode of self-pollination [Lloyd and Schoen 1992; Lloyd 1992]) may overwhelm the role of inbreeding depression in the evolution of the selfing rate. Finally, the dynamics of the coevolution may be driven by associations between loci causing inbreeding depression and those changing the level of selfing in such a way that no correlation exists between them (see Uyenoyama et al. 1993).

Here we present estimates of selfing rates and levels of inbreeding depression in nine populations of *Amsinckia*, a genus with a considerable range of selfing rates among populations. The presence of polyploidy in one species allowed us to investigate whether polyploid populations have reduced inbreeding depression. In addition, we asked whether inbreeding depression was concentrated in particular life stages and whether selfed progeny were more phenotypically variable than outcrossed.

MATERIALS AND METHODS

The Study System

Amsinckia ("fiddleneck," Boraginaceae) is a genus of yellow- to orange-flowered annuals centered in western North America (Ray and Chisaki 1957a). Heterostylous populations consist of two floral types. Pins present the stigma near the opening of the corolla throat, thrums near the nectary at the base. In each case the five anthers emerge from the corolla at the reciprocal position. Other populations are homostylous, the anthers and stigma occurring at nearly equal heights. "Mixed" populations, consisting of pins, thrums, and homostyles, are also found. Heterostyly in *Amsinckia* is unusual in not being accompanied by self- and within-morph incompatibility (Ray and Chisaki 1957a), though in *A. grandiflora* and *A. douglasiana* both self- and within-morph pollen success is reduced when in competition with intermorph pollen ("cryptic incompatibility," Weller and Ornduff 1977; Casper et al. 1988). Heterostyly is inherited as a single-locus trait, the pin form being a recessive homozygote (Ray and Chisaki 1957a; Ganders 1979; Lewis and Jones 1992). Using mor-

TABLE 1. Characteristics of the nine populations used in this study.

Phylogenetic lineage	Species	Haploid chromosome number	Population	Floral morphology	
				Flower size	Distyly
douglasiana/gloriosa	A. douglasiana	6	Paloma Creek Canyon	Large	Heterostylous
			Lockwood	Large	Heterostylous
	A. gloriosa	12	Paloma Creek Canyon	Small	Homostylous
			New Idria	Small	Homostylous
spectabilis	A. spectabilis	5	Nipomo	Large	Heterostylous
			Lompoc	Large	Heterostylous
			Lompoc 17	Large	Homostylous
			Alisal Slough	Small	Homostylous
			Zmudowski State Beach	Small	Homostylous

phological and karyological information, Ray and Chisaki (1957b) proposed four separate evolutionary transitions from large-flowered heterostyly to smaller-flowered homostyly. Their hypothesis is consistent with restriction-site variation in the chloroplast genome (Schoen et al., unpubl.). Our study consists of nine California populations from two of these putative phylogenetic lineages (Table 1).

In the first lineage, heterostylous A. douglasiana ($1n = 6$) is thought to have given rise to homostylous, tetraploid A. gloriosa ($1n = 12$). Segregation analyses of allozymes indicate that A. gloriosa exhibits disomic inheritance. Amsinckia douglasiana flowers are approximately 15–22 mm long and 10–16 mm wide, while those of A. gloriosa are 12–16 mm long and 6–10 mm wide (pers. obs.; Ganders 1993). Amsinckia douglasiana is relatively rare and occurs primarily on slopes of loose shale in two central California counties. Amsinckia gloriosa is common, often covering thousands of square meters, primarily in disturbed sites such as grazed fields and roadsides. Our study includes two populations of A. douglasiana, Paloma Creek Canyon and Lockwood, and two of A. gloriosa, Paloma Creek Canyon and New Idria (Table 1). The Lockwood population was studied by Casper et al. (1988).

The second lineage consists of populations exhibiting a range of floral forms within the species A. spectabilis (Ganders 1975a). Heterostylous A. spectabilis var. microcarpa is an erect plant inhabiting stabilized sandy areas within 20 km of the Pacific coast. The corollas are 12–19 mm long and 8–14 mm wide. We studied one population in Nipomo and one in Lompoc (respectively the "Nipomo" and "Lompoc" populations of Ganders 1975b and Ganders et al. 1985). A second form of this species, A. spectabilis var. spectabilis, produces slightly larger seeds and has smaller flowers 7–15 mm long and 5–12 mm wide (pers. obs.; Ganders 1993). These populations consist wholly or primarily of homostyles, though some populations having a wide range of stigma-anther separation are sometimes considered a mixture of homostyles and heterostyles (Ray and Chisaki 1957a; Ganders et al. 1985). Two of our populations, Alisal Slough and Zmudowski State Beach, are typical of coastal dune populations in having very small flowers and decumbent habit. These populations are entirely homostylous. A third population of var. spectabilis, Lompoc 17, bears flowers as large as those of heterostyles, but displays a continous range of stigma-anther positions, from pinlike to thrumlike, with most individuals having flowers with anthers and stigma in close proximity. We

consider Lompoc 17 to be a large-flowered homostylous population showing quantitative variation in anther-stigma separation.

We collected seeds from 150 plants in each of eight populations between April and June 1991. Seeds from a smaller number of plants of Lompoc 17 were collected in 1988. Each naturally occurring seed parent defined a family and is referred to below as a "grandparent." The grandparental floral morph (pin or thrum) was recorded in the heterostylous populations at the time of seed collection. The seeds were brought to McGill University where, following a short period of after-ripening at room temperature, they were maintained at approximately 4°C with a desiccant. The procedures described below were conducted between December 1990 and July 1993 as space and time permitted.

Selfing-Rate Estimation

We performed starch gel electrophoresis on seeds to search for allozyme variation to estimate the rate of self-fertilization in each population. One biallelic locus was used for each population, except homostylous A. spectabilis from Zmudowski State Beach, where no polymorphisms could be found. In the two tetraploid populations (A. gloriosa), electrophoretic results from test crosses indicated a duplicated locus, one of which was fixed, the second showing a disomic pattern of inheritance. The three segregating genotypes in these two populations could be distinguished by band intensity. Gel and electrode buffers were Histidine (A. H. D. Brown, unpubl.) for Pgi and Pgm (stain recipe from Brown, unpubl.); Tris-citrate (Werth 1985) for Idh-2 (Werth 1985); Tris-Edta-borate (system 10 of Soltis et al. 1983) for Aat-1 (modification of Werth 1985); and Histidine (A. H. D. Brown, unpubl.) for Adh (Werth 1985).

Selfing rates, S, and parental inbreeding coefficients, f, of plants in the field were estimated using the mixed-mating model with the family as the unit of observation (Ritland and Jain 1981; Ritland 1990). The model assumes that a proportion, S, of offspring is the product of self-fertilization and the remaining proportion is from random outcrossing. Ninety-five percent confidence intervals were obtained as the region spanning the 26th to 975th ordered result from 1000 bootstraps. Resampling was conducted between families in the large-flowered populations and within families in the small-flowered ones.

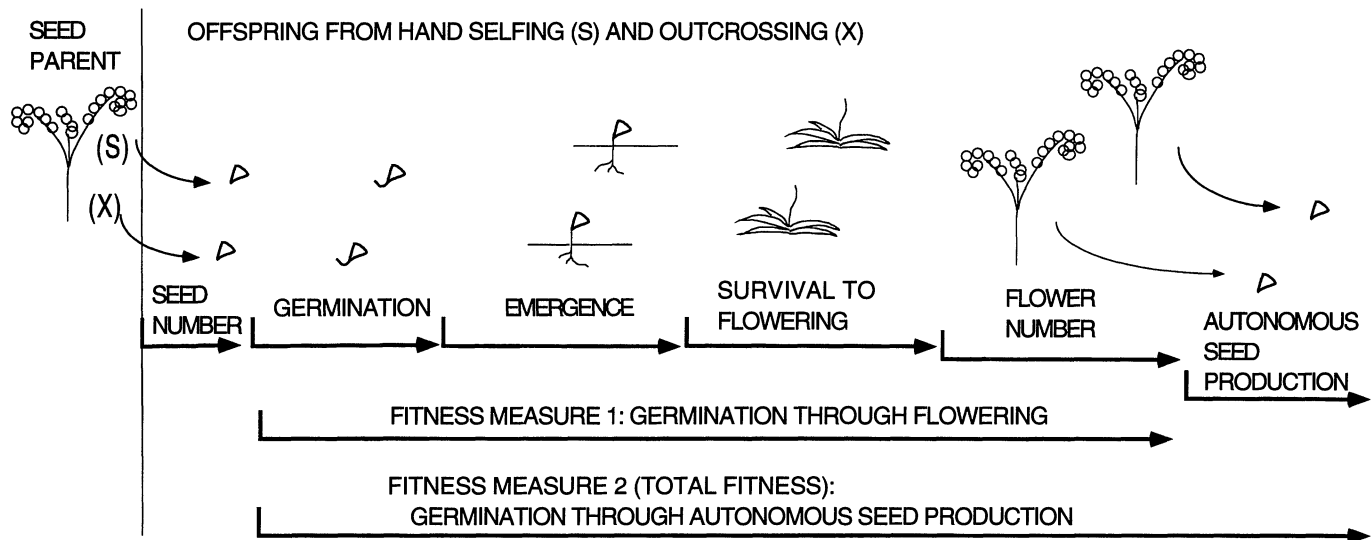Because the Zmudowski State Beach population of A. spec-

FIG. 1. Life stages and fitness measures used in measuring inbreeding depression. Fitness measures were intervals spanning four or five life stages. Fitness measure 1 was used for all populations and is most-comprehensive fitness measure available for the five large-flowered populations. Fitness measure 2 spans the complete life cycle and thus estimates total fitness for the four homostylous populations, which produce seeds autonomously.

tabilis was monomorphic for allozyme expression, we estimated the selfing rate by the inbreeding-depression method (Sorensen et al. 1976). This method compares the mean fitness of offspring produced naturally (w_n) to those from selfing (w_s) and outcrossing (w_x): $S = (w_x - w_n)/(w_x - w_s)$. Natural seeds were sibs of the parents of the self- and outcross progeny, and so were one generation older. They also matured in the field rather than the phytotron. For these two reasons, selfing rates obtained by this method should be considered approximate. Ninety-five percent confidence intervals were determined from standard errors by the method of Charlesworth (1988, with with the sign before the penultimate covariance term corrected to "+" in the variance formula).

Measurement of Inbreeding Depression

For each population one seed from each of 112 grandparents (55 in the case of Lompoc 17) was placed on wet filter paper for germination. These plants were then grown in individual 13-cm plastic pots containing a 2:1:1 mixture of sand, black earth, and vermiculite. Pots were maintained in greenhouse or growth rooms in the McGill University Phytotron at 14°C night and 22°C day. Day length was 12 h for approximately four weeks and 14 h thereafter. Five to eight weeks after sowing, several (one to 30) flowers on each plant were hand pollinated with either self-pollen or outcross pollen from one other haphazardly chosen parent in the population (each pollen parent was used once). To prevent unintentional self-pollination, both homostylous flowers and thrums were emasculated before anther dehiscence and immediately prior to pollen application; this precaution was unnecessary in the case of pin flowers, which were therefore pollinated intact. The resulting seeds from each pollinated flower were counted and collected at maturity, approximately 14 d after pollination in all populations. Following an after-ripening period at room temperature, the seeds were stored dry at 4°C. From each family one (occasionally two) self-

and one (occasionally two) outcross progeny were then grown under the conditions described above to measure fitness at several life-history stages (Fig. 1).

Fitness Components Measured.—We recorded fitness at six sequential life-cycle stages that included both survival and fertility: survival from ovule to seed (referred to as seed maturation or seed number per hand-pollinated flower), germination on moist filter paper in petri dishes, emergence from the soil, survival to flowering, flower number, and autonomous seed production (Fig. 1). An individual's autonomous seed production was the average number of mature seeds in 10–15 haphazardly chosen flowers. It could range from zero to four (flowers in the Boraginaceae have four ovules). In the heterostylous populations, the first and last life-cycle stages were not used in comparing selfed and outcrossed fitnesses because we could not distinguish inbreeding depression from possible pollen-stigma interactions such as cryptic incompatibility (Weller and Ornduff 1977), and heterostylous populations produced no seed autonomously.

These life-cycle components were combined into two cumulative fitness measures (Fig. 1). First, fitness measure 1 incorporated the period from germination through flowering (inclusive). For populations that do not produce all (or nearly all) seeds autonomously, it was the best available estimate of total fitness for progeny growing in the absence of pollinators. Fitness measure 1 was the number of flowers produced by a seed. It was zero if death occurred before flowering; otherwise it equaled the flower number. Second, in the four small-flowered, homostylous populations, fitness measure 2 encompassed germination, emergence, flowering, and autonomous seed production. Fitness measure 2 was the number of seeds autonomously produced by a seed, and thus measured total fitness. It was estimated as the number of flowers produced by a seed multiplied by that individual's average number of seeds set autonomously per flower.

For both the life-cycle stages and the cumulative fitness

TABLE 2. Selfing rate, S, and parental inbreeding coefficients, f, estimated for eight of the nine populations used in this study.

Species and population	Selfing rate		Inbreeding coefficient		Enzyme locus	# Seed parents	# offspring
	S	95% CI	f	95% CI			
A. douglasiana							
Paloma Creek Canyon	0.25	0.029, 0.49	0.047	−0.39, 0.51	*Pgm*	79	512
Lockwood	0.42	0.22, 0.60	0.032	−0.33, 0.40	*Pgm*	50	300
A. gloriosa							
Paloma Creek Canyon	0.999	0.999, 0.999	0.73	0.73, 0.96	*Aat*-1	39	256
New Idria	0.997	0.994, 0.999	0.74	0.68, 0.85	*Idh*-2	64	396
A. spectabilis							
Nipomo	0.55	0.35, 0.70	0.17	−0.10, 0.52	*Pgi*	60	360
Lompoc	0.33	0.19, 0.45	0.35	0.034, 0.66	*Pgi*	60	360
Lompoc 17	0.73	0.54, 0.84	0.036	−0.26, 0.34	*Pgi*	45	398
Alisal Slough	0.998	0.781, 0.999	1.00	1.00, 1.00	*Adh*	49	291

measures described above, we calculated population inbreeding depression as $\delta_p = 1 - w_s/w_x$, where w_s and w_x are the population means of the self- and outcross progeny, respectively. (We use the term "inbreeding depression" to include heterosis, which is the increase in fitness resulting from a cross between highly inbred individuals [Shull 1952], such as in very highly selfing populations.) In the few cases where more than one offspring per parent per cross was available, the mean per parent was used as an observation. Inbreeding depression was determined for each stage using only those families having both a self- and outcross progeny alive at the end of the previous stage. This made the number of self and outcross observations equal and permitted pairwise comparisons. We did not calculate family inbreeding depression, δ_f, which is the mean of per-family values, because it is not used in theoretical models and its estimator is biased downward at small sample sizes per family (Johnston and Schoen 1994). To test for significant differences between self- and outcross progeny, i.e., inbreeding depression, we calculated Wilcoxon's T (signed rank test for paired data, Hollander and Wolfe 1973, ch. 3; significance levels from Zar [1984, table B.11]) for continous characters and G (Sokal and Rohlf 1981, ch. 17) for the two binary characters germination and emergence (P-values calculated from the chi-square distribution with one df).

Effect of Selfing Rate and of Polyploidy on Inbreeding Depression

We used ANOVAs on *ln*-transformed fitnesses to ask whether populations having certain characteristics differed in level of inbreeding depression. With *ln*-transformed data, a significant interaction indicates that the ratio of self:outcross in untransformed units, and therefore the level of inbreeding depression, differed among populations or between the two kinds of population (Johnston and Schoen 1994). We used the following models to test for the effects of three population characteristics. First, we asked whether populations differed in inbreeding depression using the model *ln*(Fitness + 1) = Constant + Population + Cross + Population × Cross + Error. In separate ANOVAs we analyzed flower number and cumulative fitness measure 1. Second, we asked whether the four small-flowered, homostylous populations differed from the five others in level of inbreeding depression. Here the

model was *ln*(Fitness + 1) = Constant + Cross + Mating system + Population (Mating system) + Cross × Mating system + Error. The effect of population on the fitness measure was thus incorporated by nesting it within mating system. Two separate ANOVAs were conducted as above. Third, we asked whether inbreeding depression in the two tetraploid populations of *A. gloriosa* differed from that in the two small-flowered, homostylous populations of *A. spectabilis*. The model was *ln*(Fitness + 1) = Constant + Cross + Ploidy + Population (Ploidy) + Cross × Ploidy + Error. In addition to those stated above, fitness measures included autonomous seed production alone as well as total fitness (measure 2). All ANOVAs were conducted with the General Linear Model procedure of SYSTAT 5.2.1 (1992).

The second ANOVA above tested whether inbreeding depression differed between small-flowered, homostylous populations and the larger-flowered populations. We also asked whether there was a significant Spearman rank correlation between S and δ (probability values from Zar 1984). The power of this test was limited by the sample size (nine). All analyses were conducted with SYSTAT 5.2.1 (1992).

RESULTS

Selfing Rates

In the four large-flowered, heterostylous populations, selfing rates were 25% and 42% in *A. douglasiana* and 33% and 55% in *A. spectabilis* (Table 2). Lompoc 17, the large-flowered but homostylous population of *A. spectabilis*, had a selfing rate of 73%. Each of the four small-flowered, homostylous populations exhibited selfing rates greater than 99.5%. Selfing rates for three of these populations were obtained by electrophoresis (Table 2). For *A. spectabilis* of Zmudowski State Beach, however, the inbreeding-depresssion method estimated the selfing rate at 160%, with confidence interval −120% to 450%. The estimate exceeds 100% because the mean fitnesses of selfed and outcrossed each exceeded that of the naturally produced progeny.

Inbreeding Depression

Survival.—In all nine populations the crossing treatment had no effect on the three stages of offspring survival between seed maturation and flowering: seed germination, seedling
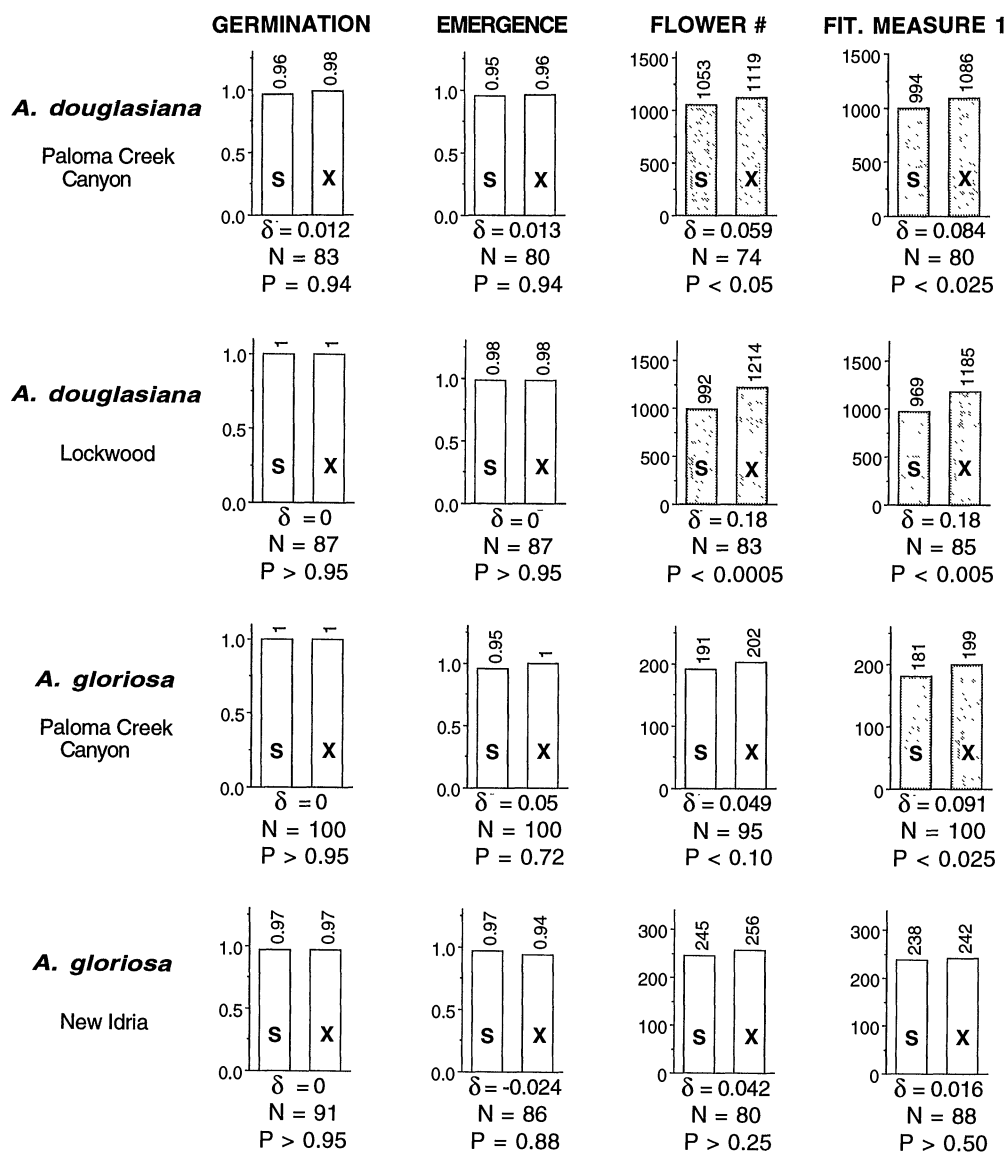
FIG. 2. Inbreeding depression in the *Amsinckia douglasiana-A. gloriosa* phylogenetic lineage. Shaded bars indicate traits showing a significant difference ($P < 0.05$) between selfed (S) and outcrossed (X) progeny. Sample sizes are two times the number of families (N). Fitness measure 1 encompasses germination, emergence from the soil and flower number.

emergence from soil, and survival from emergence to flowering (Figs. 2, 3). For both self and outcross seeds these fitness components exceeded 94% in eight of the nine populations. None of the four small-flowered, homostylous populations exhibited heterosis in seed number per flower, and in the Zmudowski State Beach population of *A. spectabilis* selfed seeds were 8% more likely to reach maturity than outcrossed (two-tailed $P = 0.052$, Table 3).

Fertility.—There was significant inbreeding depression in flower number in all five large-flowered populations, including the large-flowered but homostylous *A. spectabilis* (Figs. 2, 3). These values were all less than 20%. In all small-flowered, homostylous populations, inbreeding depression was less than 5%, being significant in one of the two *A. spectabilis* populations. Significant inbreeding depression in autonomous seed production was found in both *A. gloriosa*

populations (11% and 13%) but not in *A. spectabilis* (3.8% and 1.8%; Fig. 4).

Cumulative Fitness Measures 1 and 2.—Because they were nonautonomous and raised in the phytotron, the most-complete fitness measure for the four heterostylous populations was the number of flowers produced by a seed, or fitness measure 1. In heterostylous populations of both phylogenetic lineages, selfed offspring experienced significantly reduced fitness: 8.4% and 18% in *A. douglasiana* populations and 7.9% and 17% in *A. spectabilis* (Figs. 2, 3). Inbreeding depression in the large-flowered, homostylous *A. spectabilis* population was 25%. Among the four small-flowered, homostylous populations, only *A. gloriosa* of Paloma Creek Canyon experienced significant inbreeding depression in fitness measure 1 (9.1%; Figs. 2, 3).

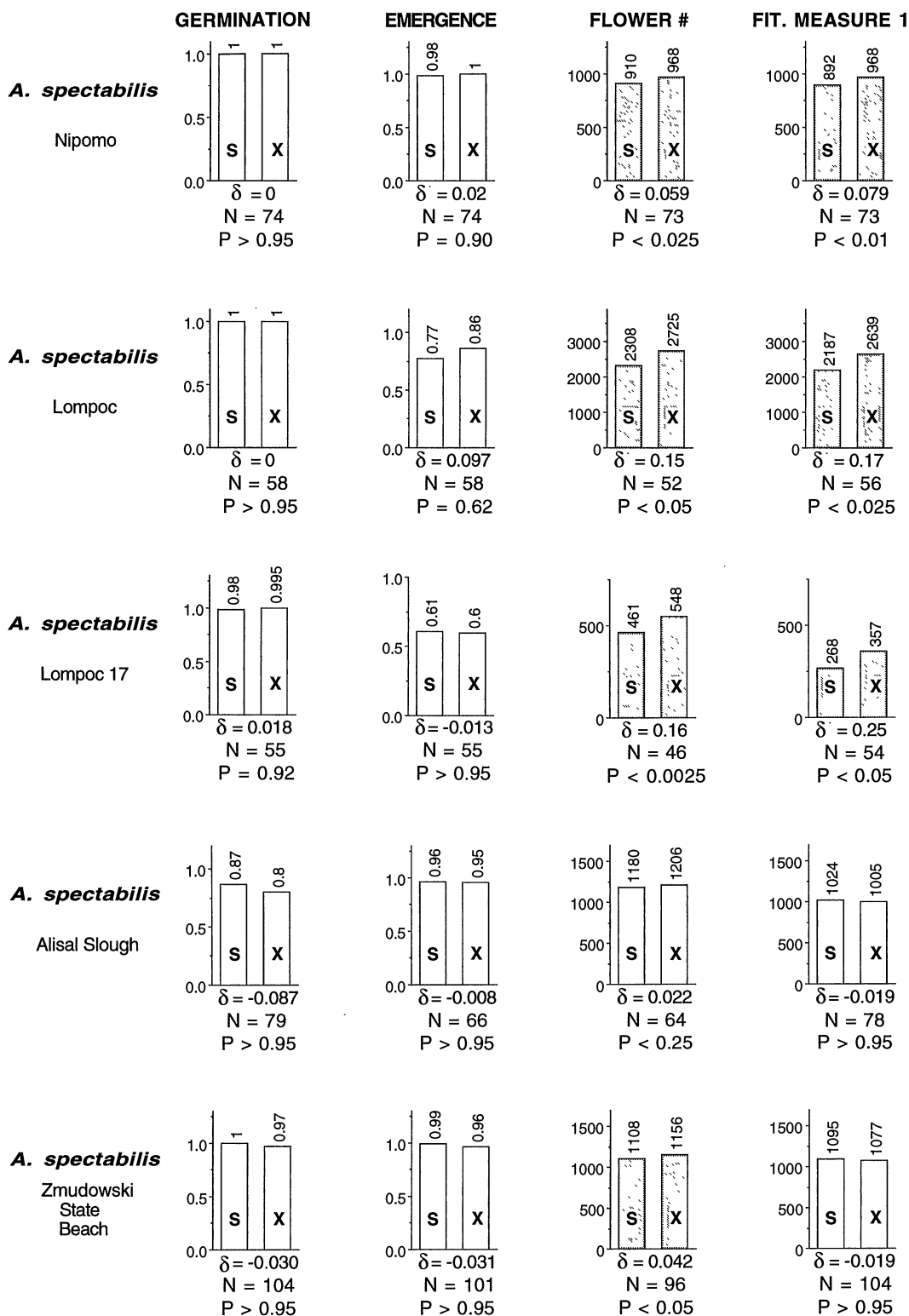Cumulative fitness measure 2, which is total fitness or the

FIG. 3. Inbreeding depression in the *Amsinckia spectabilis* phylogenetic lineage. See Figure 2 for further information.

number of seeds produced by a seed, was available for the four small-flowered populations. Heterosis in this measure was statistically significant in three of the four populations. The values in *A. gloriosa* were 22% at Paloma Creek Canyon ($P < 0.0005$) and 11% at New Idria ($P = 0.025$; Fig. 4).

Values in *A. spectabilis* were 11% at Alisal Slough ($P < 0.025$) and 6.1% at Zmudowski State Beach ($P = 0.14$; Fig. 4).

Relative Variability.—Differences between CVs (selfed minus outcrossed) tended to be lower in the highly selfing pop-

TABLE 3. Population inbreeding depression in seed maturation in the four small-flowered, homostylous populations. The number of seeds produced per flower ranges from 0 to 4. Two-tailed significance levels for the difference between selfed and outcrossed progeny were determined by Wilcoxon's signed rank test for paired values.

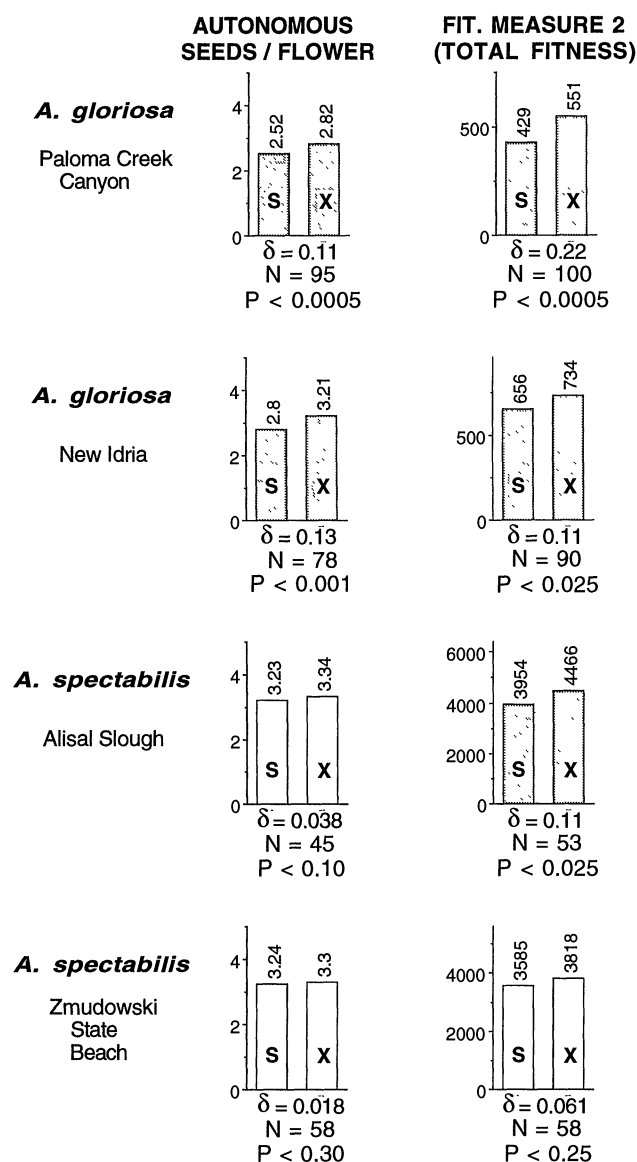| | Fitness component: seed number per flower | | | | | | |
| | Outcrossed | | Selfed | | Inbreeding depression | | # families |
Species and population	Mean	CV (%)	Mean	CV (%)	δ_p	P	
A. gloriosa							
Paloma Creek Canyon	2.47	39	2.32	41	0.06	0.11	100
New Idria	2.42	47	2.29	48	0.05	0.36	91
A. spectabilis							
Alisal Slough	3.11	29	3.18	26	−0.02	0.80	77
Zmudowski State Beach	2.78	36	2.99	37	−0.08	0.052	104

FIG. 4. Inbreeding depression in autonomous seed production and fitness measure 2 in the four small-flowered, homostylous populations. Fitness measure 2 is a measure of total fitnes, encompassing germination, emergence, flower number and autonomous seed production. See Figure 2 for further information.

ulations, and were sometimes negative: within the *A. douglasiana-A. gloriosa* lineage, the average difference between CV's in flower number was 11.5% in large-flowered, heterostylous populations and 4.5% in small-flowered homostylous (Table 4). Mean differences in fitness measure 1 (from germination through flowering) were 12.5% in heterostylous and 7.5% in homostylous (Table 4). Within the *A. spectabilis* lineage, however, outcrossed progeny of the two small-flowered, homostylous populations showed greater variability than selfed progeny in both characters. The mean differences in CV of flower number were 15.5% in heterostylous populations and −3.5% in homostylous. For cumulative fitness measure 1, these mean differences were 16% for heterostylous and −11.5% for homostylous populations.

Effects of Population Characteristics on Inbreeding Depression

Populations differed in level of inbreeding depression in fitness measure 1, as shown by a significant interaction between cross type and population in an ANOVA of *ln*-transformed data (Table 5). There was not a significant interaction ($P < 0.13$) in the case of flower number (Table 5).

That the four highly selfing populations had lower inbreeding depression than the five moderate selfers was indicated by a significant interaction between cross type and mating system (high versus intermediate selfing) for both flower number ($P < 0.04$) and fitness measure 1 ($P < 0.02$; Table 5). As expected in populations differing in average fitness values, the added variance component due to population ($P < 0.000001$ for both traits) was large enough to obscure the effect of mating system by itself ($P < 0.30$; $P < 0.50$ for flower number and fitness through flowering, respectively; interaction term used as error). The effect of cross type in these kinds of analyses, therefore, does not indicate anything about the presence of inbreeding depression, which is a within-in-population phenomenon. Thus, only the interaction terms are presented in Table 5.

The Spearman rank correlation between selfing rate and inbreeding depression was -0.67 ($P = 0.10$, $n = 9$) for the case of flower number and -0.50 ($P = 0.12$) for fitness measure 1. (In all analyses the selfing rate of the Zmudowski State Beach population was assumed to be one.)

For the four very highly selfing populations, it was also possible to ask whether heterosis differed between the tet-

TABLE 4. Coefficients of variation (%) of stage-specific fitness components and of cumulative fitness measures in selfed (S) and outcrossed (X) progeny.

Species and population	Flower number		Cumulative fitness measure 1		Autonomous seed number		Cumulative fitness measure 2	
	S	X	S	X	S	X	S	X
A. douglasiana								
Paloma Creek Canyon	39	34	47	38				
Lockwood	43	25	46	30				
A. gloriosa								
Paloma Creek Canyon	33	26	41	26	35	26	44	30
New Idria	38	36	44	44	24	19	52	47
A. spectabilis								
Nipomo	26	20	27	20				
Lompoc	74	49	79	54				
Lompoc 17	62	59	75	87				
Alisal Slough	58	66	73	86	21	24	62	69
Zmudowski State Beach	22	21	24	34	26	22	32	32

raploid and diploid populations. While no evidence for this was found in flower number ($P < 0.95$), the two tetraploid populations had significantly higher heterosis in fitness measure 1 ($P < 0.04$), and nearly significantly higher heterosis in fitness measure 2 ($P < 0.07$, Table 5).

DISCUSSION

Relation of Data to Theory Based on Mutation-Selection Balance

The evolution of inbreeding depression as a function of the selfing rate can be studied under controlled conditions or in natural populations. Controlled conditions have the advantage of reducing effects of confounding variables. Barrett and Charlesworth (1991) examined the effect of several generations of imposed self-fertilization on both a historically inbreeding and outbreeding population of the annual *Eichhornia paniculata* under greenhouse conditions. They found that five generations of selfing increased the average fitness (flower number) of outcrossed progeny only in the outbreeding population and further concluded that the pattern gave

evidence for inbreeding depression being caused by mutation to partially recessive deleterious genes. The present study, in contrast, examined the relation between natural rates of self-fertilization and inbreeding depression. Such studies are best suited to answer questions about the relation between these two variables when all natural confounding variables are included. A strong relationship would then imply that selfing rates and inbreeding depression are intimately connected, while a weak one means only that other forces are at least as important in determining each variable. Two previous studies have used this approach. In a study of three populations of *Clarkia tembloriensis* with selfing rates ranging from 42% to 97%, Holtsford and Ellstrand (1990) found some evidence for a negative relationship in some reproductive characters. Latta and Ritland (1994) investigated the relationship between inbreeding depression and the inbreeding coefficient (rather than the selfing rate) in *Mimulus* taxa. The relationship was negative for all five traits related to fitness, but significantly so for only one trait.

The present study found that highly selfing populations of *Amsinckia*, as a group, have significantly lower inbreeding

TABLE 5. ANOVAs for the effect of population and of population characteristics on level of inbreeding depression. The first analysis tests for differences among the nine populations in level of inbreeding depression. The second tests for a difference between the four highly selfing, small-flowered, homostylous populations and the five moderately selfing, large-flowered populations, one of which is homostylous. The third tests for a difference between the diploid and tetraploid highly selfing populations. The fitness component is the dependent variable in each ANOVA.

Model and source of variation	Fitness component	df	Mean square	F	P<	Multiple R-square	N	# populations
Model: *ln*(Fitness + 1) = Population + Cross + Cross × Population + Constant + Error								
Cross × Population	Flower number	8	1.37	1.57	0.13	0.42	1376	9
	Cumulative fitness measure 1	8	5.24	2.04	0.04	0.20	1445	9
Model: *ln*(Fitness + 1) = Cross + Mating system + Population (Mating system) + Cross × Mating system + Constant + Error*								
Cross × Mating system	Flower number	1	4.06	4.65	0.04	0.42	1376	9
	Cumulative fitness measure 1	1	16.58	6.43	0.02	0.20	1445	9
Model: *ln*(Fitness + 1) = Cross + Ploidy + Population (Ploidy) + Cross × Ploidy + Constant + Error								
Cross × Ploidy	Flower number	1	0.004	0.006	0.94	0.42	700	4
	Cumulative fitness measure 1	1	12.88	4.61	0.03	0.15	743	4
	Cumulative fitness measure 2	1	16.20	3.52	0.07	0.09	623	4

* Large-flowered, homostylous Lompoc 17 *A. spectabilis* grouped with the four large-flowered, heterostylous populations.
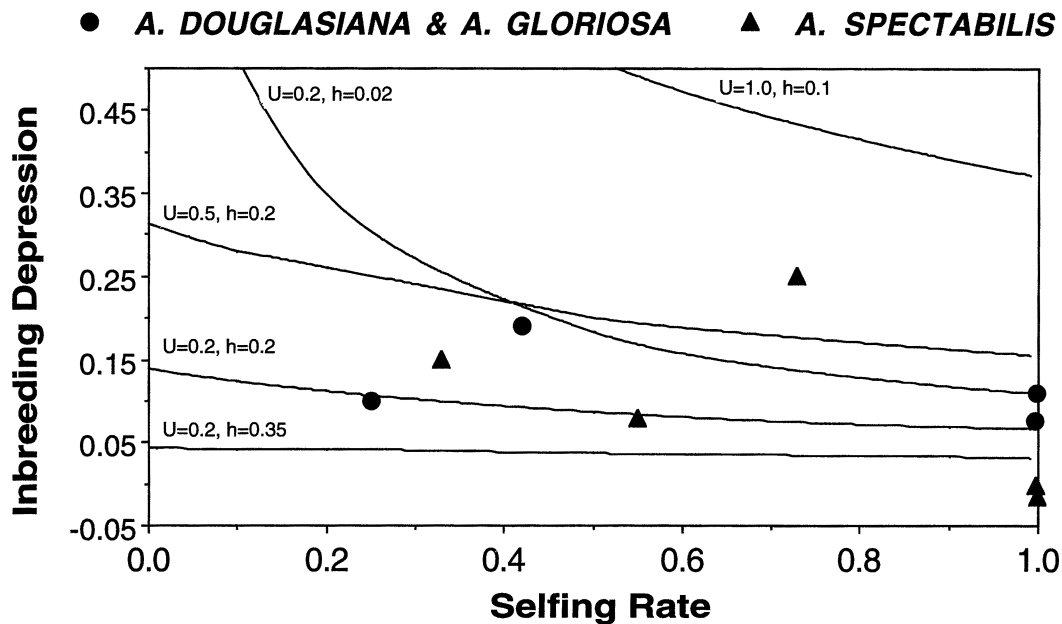
FIG. 5. Population inbreeding depression and the selfing rate: data and theory. Data points are from the 9 populations of *Amsinckia* in this study; fitness is measured from germination through flowering. Lines indicate the predicted effects of mutation rate per sporophyte genome, U, and dominance, h, on the equilibrium levels of inbreeding depression, under the assumption of unlinked loci. Lines were constructed by interpolating between the simulation results given in Charlesworth et al. (1990). Vertical and horizontal scales are equal.

depression than partially selfing ones, but the negative relationship between selfing and inbreeding depression is weak and nonsignificant (see Fig. 5). One can conclude that, in these nine populations, the levels of selfing and inbreeding depression are affected more by other factors than by each other. It is possible that populations are not in equilibrium. Lande and Schemske (1985) suggest that extreme reductions in population size or pollinator service, when occurring more often than about once every 100 generations, will decrease inbreeding depression and select for self-fertilization. The effect, however, will be most pronounced for lethal and sublethal mutations. Population size itself is expected to have little influence on inbreeding depression caused by mutational load (Charlesworth et al. 1992), and a study of the European grassland perennial *Scabiosa columbaria* found no relationship (van Treuren et al. 1993).

While one can conclude that selfing and inbreeding depression are determined more by other factors than by each other, one cannot conclude that the level of inbreeding depression is therefore unimportant in the evolution of selfing rates. First of all, many ecological and genetic factors may obscure the relationship (Uyenoyama et al. 1993), and, second, for realistic levels of dominance and mutation, the level of inbreeding depression decreases very little as a function of the selfing rate (Charlesworth et al. 1990). Figure 5 shows expected equilibrium levels of inbreeding depression as a function of selfing rate when the former is caused by mutations within an infinite number of unlinked loci without epistasis (lines show simulation results from Charlesworth et al. 1990). It can be seen that higher dominance coefficients not only lead to lower inbreeding depression, but also flatten its relation with the selfing rate. It thus appears that for dominance coefficients greater than about 0.2, there is little de-

crease in inbreeding depression with selfing rate, unless high rates of mutation are assumed. In a companion study of the four highly selfing populations, we (Johnston and Schoen 1995) estimated $U = 0.83$ per sporophyte genome per generation, $h = 0.32$ in *A. gloriosa* and $U = 0.32$, $h = 0.10$ for *A. spectabilis* (average of two populations for each species). The dominance coefficient of *A. gloriosa*, therefore, is sufficiently high that a causal relationship between the levels of selfing and inbreeding depression may be difficult to detect in many experiments of reasonable size. This is less likely the explanation for the weak relationship within *A. spectabilis*, with its low estimate of h. Unfortunately, we found no populations with very low selfing rates, where the inbreeding depression is expected to increase most dramatically (Fig. 5). The mutation and dominance estimates in *Amsinckia* are based on total fitness, from germination through autonomous seed production, while the *Amsinckia* data in Figure 5 extend only through flowering, in order to make the nine populations comparable. While it is likely that measuring the inbreeding depression through seed production would elevate the estimates, the effect would have to be proportionally greater in the more-outcrossing populations for it to strengthen the negative relation between selfing and inbreeding depression.

In sum, this study found evidence for the expected negative relationship between selfing rates and inbreeding depression. The weakness of this relationship, however, suggests the influence of other, currently unknown factors. A goal of future research should be to determine the relative importance of ecological and genetic factors in causing the levels of self-fertilization and inbreeding depression in natural populations.

Polyploidy

The well-known association of polyploidy with high selfing rates in annual plants might exist because a newly arisen

polyploid individual can mate only with itself (Stebbins 1971, p. 130); Grant 1981, p. 317; see also Barrett 1989) or because polyploidy lowers inbreeding depression. The relative importance of these two factors is unknown. Bennett (1976) predicted that autotetraploids should exhibit greater inbreeding depression than their diploid progenitors. Artificial induction of autopolyploidy by colchicine in inbred lines of diploid rye increased heterosis (Lundqvist 1966; Wright 1977). Naturally hexaploid *Sequoia sempervirens* (redwood) contains high levels of inbreeding depression in plant size (Libby et al. 1981). Considering outcrossing populations, Lande and Schemske (1985) concluded that tetraploidy should substantially reduce inbreeding depression caused by nearly recessive lethal and sublethal mutations. They further concluded that duplicate loci in outcrossing allotetraploids should not increase that portion of inbreeding depression due to partially dominant mutations or to quantitative variation. *Amsinckia gloriosa* is almost certainly an allotetraploid, as it displays disomic inheritance at the one variable electrophoretic marker locus in each population and is a fixed heterozygote at many of the other markers. In comparing diploid to tetraploid populations with selfing rates of very nearly one, we found that heterosis in tetraploid *A. gloriosa* exceeded that in diploid *A. spectabilis*. While these results contradict predictions of Lande and Schemske (1985), it is important to realize that they considered outcrossing populations and that the decrease was predicted primarily for lethal and sublethal mutations, which may not be an important source of inbreeding depression in highly selfing populations. Furthermore, in our study ploidy level is confounded with species. Factors associated with species might therefore have a greater influence on inbreeding depression. A better test of the effect of ploidy per se would compare populations within a species (e.g., Husband and Schemske, unpubl. ms.).

Inbreeding Depression at Different Life Stages

We detected no inbreeding depression in the period from seed maturation through flower production in any of the nine populations. Thus, nearly all inbreeding depression was expressed in the fertility rather than viability components of fitness. The consistency of this result across three species, two ploidy, levels and a range of selfing rates has several possible explanations. First, it may be truly representative of these species and part of a general pattern. In reviewing published data, Husband and Schemske (1996) found that inbreeding depression in primarily selfing taxa tended to occur at later stages than in primarily outcrossing taxa. In annual plants early fitness components are aspects of survival, and late components are aspects of fertility. The *Amsinckia* populations studied here, which have moderate to very high self-fertilization rates, thus conform to the pattern and can be contrasted, for example, with a highly outcrossing population of the annual *Gilia achilleifolia*, in which survival inbreeding depression greatly exceeded that in seed production (Schoen 1983). Studies of other partially selfing species have often found that inbreeding depression in fertility exceeds that in early viability (e.g., *Lupinus texensis* [Schaal 1984]; *Sabatia angularis* [Dudash 1990]; *Clarkia tembloriensis* [Holtsford and Ellstrand 1990]; *Lobelia cardinalis* and *L. siphilitica*

[Johnston 1992]; *Hydrophyllum appendiculatum* [Wolfe 1993]). Nevertheless, selfing species sometimes exhibit inbreeding depression in early survival (e.g., *Impatiens capensis* [Mitchell-Olds and Waller 1985]).

A second possible cause of equal survival of selfed and outcrossed offspring is the presumably benign growth environment of the phytotron, characterized by ample light and nutrients, low aboveground competition and no belowground competition. It is commonly stated that growth in higher-stress conditions will cause higher inbreeding depression, and several studies have indeed found increased inbreeding depression with lower light levels (Schemske 1983) or higher plant density (Schmitt and Ehrhardt 1990; Wolfe 1993). It is probably often the case, however, that outcrossed progeny will exhibit a proportionally greater response to increasing environmental quality. In this case, inbreeding depression will increase with environmental quality. For example, in *Lobelia siphilitica* and *L. cardinalis*, progeny flower number was much higher in the greenhouse than in the presumably more stressful native field environment (Johnston 1992). There was consequently more opportunity for progeny from selfing and outcrossing to differ, and inbreeding depression was therefore higher in the greenhouse. Nevertheless, other studies conducted in both environments have found higher inbreeding depression in the field (Dudash 1990) or no difference (Willis 1993a). The variety of results further highlights the dependence of inbreeding depression on environmental conditions (Griffing and Langridge 1963; Griffing 1990).

A third potential cause for the lack of fitness differences in early survival could be in the way fitness is measured. As a binary fitness measure, survival does not capture the close relationship between plant size and fertility: small and large living plants have equal survival but unequal fertility. Fourth, maternal effects may mask inbreeding effects early in life (Schaal 1984; Wolfe 1993). Fifth, partial selfing, at levels lower than in the nine studied here, is expected to largely rid populations of recessive lethals (Lande and Schemske 1985). Finally, in iteroparous species, selection may act more strongly at earlier stages, or the deleterious effects of mutations may increase with development or age (Kalisz 1989).

Meaning of Heterosis in Autonomous Seed Production

Higher seed abortion upon selfing than upon outcrossing is commonly severe in the conifers (Koski 1973) and is documented in several angiosperm species (Wiens et al. 1987, 1989; Levin 1989, 1991; reviewed in Johnston 1992). In partially or wholly outcrossing populations, lower seed production upon selfing does not indicate the presence of inbreeding depression unless differential effects of self- and outcross pollen are tested and accounted for (e.g., Montalvo 1992). In the present study, therefore, conclusions about inbreeding effects on seed maturation are limited to the four very highly selfing populations. In these populations we found no heterosis in seed development following hand-pollination, indicating that deleterious, partially recessive genes (and overdominant genes if present) do not affect offspring survival between fertilization and seed maturation. Both the pollen and ovules of these manual crosses were produced by highly

inbred plants collected as seeds from the field. Compared to outcrossed offspring, selfed offspring of *A. gloriosa* produced fewer seeds autonomously per flower (Fig. 4). Autonomous seed production is a composite character comprising not only the offspring genotype but also endosperm ability to nourish the developing embryo as well as three parental attributes: quality of pollen, quality of ovules, and ability to act as an environment to the developing seed. Heterosis (inbreeding depression) in autonomous seed production cannot be caused by heterosis in seed maturation, because we found no evidence for the latter. (Note also that autonomous seed production by both self and outcross offspring occurs by self-fertilization.) Therefore, inbreeding depression must exist in at least one of the three parental attributes mentioned above. It is known that endosperm quality may decrease with inbreeding (Brink and Cooper 1947; Brink 1952), and pollen viability has been found to decrease with the parental inbreeding coefficient in *Mimulus guttatus* (Willis 1993b).

Relative Variability

Plant and animal breeders have often noted increased variability among inbred as compared to outcrossed progeny (Wright 1977, ch. 4). Increased variability upon inbreeding may result from decreased ability of the developing individual to buffer environmental fluctuations or conditions. The diminishing of buffering ability has been hypothesized to result from a general loss of vigor (Rasmusson 1949) as well as from increased heterozygosity *per se* (Lerner 1954). Griffing and Langridge (1963), Wright (1977, p. 136) and Lande (1980) discuss several other potential explanations, including the possibility that inbred lines will often be fixed for genotypes exceeding the threshold of abnormality; that the two groups may differ in the amounts of genotype-by-environment interaction; that homozygosity will often be correlated with extreme phenotypes that lie outside a zone of canalization; and, finally, that inbreeding may cause fixation of genotypes that determine variability, change thresholds or react differently to the environment. Studies of inbreeding depression in wild populations have also found increased variability in selfed offspring. For example, without regard to statistical significance, coefficients of variation of selfed progeny exceeded those of outcrossed in two of four traits examined in *Collinsia verna* (Kalisz 1989); in three of four traits in *Begonia hirsuta* and in all four traits in *B. semiovata* (Ågren and Schemske 1993). In *Arabidopsis thaliana*, Griffing and Langridge (1963) found that the difference in coefficients of variation (*CV*) depended upon temperature, and in *Sabatia angularis* Dudash (1990) found that selfed *CV*s could be greater or less than outcrossed depending on whether the offspring were grown in the greenhouse or field.

The increased variability of selfed offspring is most often found in historically outbreeding populations and in characters exhibiting inbreeding depression (or heterosis) (Griffing and Langridge 1963; Wright 1977, ch. 4). Viewed from several angles (see Results), the *Amsinckia* data are in general agreement with both of these patterns. This study also found some support for an association between difference in *CV* and level of inbreeding depression. The *CV* of selfed progeny exceeded that of outcrossed for all six populations exhibiting inbreeding depression in flower number and for five of six populations exhibiting inbreeding depression in fitness through flowering (Figs. 2, 3; Table 4). Furthermore, populations with higher inbreeding depression tended to have greater differences between selfed and outcrossed progeny in *CV* of flower number (Spearman rank correlation, $r_s = 0.74$, $P < 0.05$). The correlation with fitness measure 1 was positive but not significant ($r_s = 0.52$, $P < 0.20$).

Literature Cited

ÅGREN, 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.

BAKER, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9:347–348.

BARRETT, S. C. H. 1989. Mating system evolution and speciation in heterostylous plants. Pp. 357–283 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

BARRETT, S. C. H., AND D. CHARLESWORTH. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature 352:522–524.

BENNETT, J. H. 1976. Expectations for inbreeding depression on self-fertilization of tetraploids. Biometrics 32:449–452.

BRINK, R. A. 1952. Inbreeding and crossbreeding in seed development. Pp. 81–97 *in* J. W. Gowen, ed. Heterosis. Iowa State College Press, Ames.

BRINK, R. A., AND D. C. COOPER. 1947. The endosperm in seed development. Bot. Rev. 13:423–541.

CAMPBELL, R. B. 1986. The interdependence of mating structure and inbreeding depression. Theor. Popul. Biol. 30:232–244.

CASPER, B. B., L. S. SAYIGH, AND S. S. LEE. 1988. Demonstration of cryptic incompatibility in distylous *Amsinckia douglasiana*. Evolution 42:248–253.

CHARLESWORTH, B. 1980. The cost of sex in relation to mating system. J. Theor. Biol. 84:655–671.

CHARLESWORTH, B., M. T. MORGAN, AND D. CHARLESWORTH. 1991. Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. Genet. Res. 57:177–194.

CHARLESWORTH, D. 1988. A method for estimating outcrossing rates in natural populations of plants. Heredity 61:469–471.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. Ann. Rev. Ecol. Syst. 18:237–268.

CHARLESWORTH, D., 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.

———. 1992. The effect of linkage and population size on inbreeding depression due to mutational load. Genet. Res. 59:49–61.

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.

FISHER, R. A. 1941. Average excess and average effect of a gene substitution. Ann. Eugen. 11:53–63.

FU, Y.-B., AND K. RITLAND. 1994. Evidence for the partial dominance of viability genes contributing to inbreeding depression in *Mimulus guttatus*. Genetics 136:323–331.

GANDERS, F. R. 1975a. Heterostyly, homostyly, and fecundity in *Amsinckia spectabilis* (Boraginaceae). Madroño 23:56–62.

———. 1975b. Mating patterns in self-compatible distylous populations of *Amsinckia* (Boraginaceae). Can. J. Bot. 53:773–779.

———. 1979. The biology of heterostyly. N. Zea. J. Bot. 17:607–635.

———. 1993. Amsinckia. Pp. 367–369 *in* J. C. Hickman, ed. The Jepson manual of higher plants of California. Univ. of California Press, Berkeley.

GANDERS, F. R., S. K. DENNY, AND D. TSAI. 1985. Breeding systems and genetic variation in *Amsinckia spectabilis* (Boraginaceae). Can. J. Bot. 63:533–538.

GRANT, V. 1975. Genetics of flowering plants. Columbia Univ. Press, New York.

———. 1981. Plant speciation. 2d ed. Columbia Univ. Press, New York.

GRIFFING, B. 1990. Use of a controlled-nutrient experiment to test heterosis hypotheses. Genetics 126:753–767.

GRIFFING, B., AND J. LANGRIDGE. 1963. Phenotypic stability of growth in the self-fertilized species, *Arabidopsis thaliana*. Pp. 368–394 *in* W. D. Hanson and H. F. Robinson, eds. Statistical genetics and plant breeding. Publication No. 982. Nat. Acad. Sci.-Nat. Res. Council, Washington, DC.

HAGERUP, O. 1951. Pollination in the Faroes—In spite of rain and poverty in insects. D. Kgl. Danske. Vidensk. Selsk. Biol. Medd. 17:1–10.

HOLLANDER, M., AND D. A. WOLFE. 1973. Nonparametric statistical methods. John Wiley and Sons, New York.

HOLSINGER, K. E. 1988. Inbreeding depression doesn't matter: The genetic basis of mating-system evolution. Evolution 42:1235–1244.

HOLSINGER, K. E., M. W. FELDMAN, AND F. B. CHRISTIANSEN. 1984. The evolution of self-fertilization in plants: A population-genetic model. Am. Nat. 124:446–453.

HOLTSFORD, T. P., AND N. C. ELLSTRAND. 1990. Inbreeding effects in *Clarkia tembloriensis* (Onagraceae) populations with different natural outcrossing rates. Evolution 44:2031–2046.

HUSBAND, B. C., AND D. W. SCHEMSKE. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54–70.

JAIN, S. K. 1976. The evolution of inbreeding in plants. Ann. Rev. Ecol. Syst. 7:469–495.

JOHNSTON, M. O. 1992. Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. Evolution 46:688–702.

JOHNSTON, M. O., AND D. J. SCHOEN. 1994. On the measurement of inbreeding depression. Evolution 48:1735–1741.

———. 1995. Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. Science 267:226–229.

KALISZ, S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. Evolution 43:1263–1272.

KIMURA, M. 1959. Conflict betwen self-fertilization and outbreeding in plants. Annu. Rep. Nat. Inst. Genet. Japan 9:87–88.

KOSKI, V. 1973. On self-pollination, genetic load, and subsequent inbreeding in some conifers. Comm. Inst. For. Fenn. 78.10:1–40.

LANDE, R. 1980. Genetic variation and phenotypic evolution during allopatric speciation. Am. Nat. 116:463–479.

LANDE, R., AND D. W. SCHEMSKE. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39:24–40.

LATTA, R., AND K. RITLAND. 1994. The relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. Evolution 48:806–817.

LERNER, I. M. 1954. Genetic homeostasis. John Wiley and Sons, New York.

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.

LEWIS, D., AND D. A. JONES. 1992. The genetics of heterostyly. Pp. 129–150 *in* S. C. H. Barrett, ed. Evolution and function of heterostyly. Springer-Verlag, Berlin.

LIBBY, W. J., B. G. McCUTCHAN AND C. I. MILLAR. 1981. Inbreeding depression in selfs of redwood. Silvae Genetica 30:15–25.

LLOYD, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. Am. Nat. 113:67–97.

———. 1992. Self- and cross-fertilization in plants. II. The selection of self-fertilization. Int. J. Plant Sci. 153:370–380.

LLOYD, D. G., AND D. J. SCHOEN. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. Int. J. Plant Sci 153:358–369.

LUNDQVIST, A. 1966. Heterosis and inbreeding depression in autotetraploids. Hereditas 56:317–366.

MITCHELL-OLDS, T., AND D. M. WALLER. 1985. Relative performance of selfed and outcrossed progeny in *Impatiens capensis*. Evolution 39:533–544.

MONTALVO, A. M. 1992. Relative success of self and outcross pollen comparing mixed- and single-donor pollinations in *Aquilegia caerulea*. Evolution 46:1181–1198.

NAGYLAKI, T. 1976. A model for the evolution of self-fertilization and vegetative reproduction. J. Theor. Biol. 58:55–58.

RASMUSSON, J. M. 1949. Inbreeding and hybrid vigor in improvement of Swedish sugar beets. Heredity 3:125.

RAY, P. M., AND H. F. CHISAKI. 1957a. Studies on *Amsinckia*. I. A synopsis of the genus, with a study of hererostyly in it. Am. J. Bot. 44:529–536.

———. 1957b. Studies on *Amsinckia*. II. Relationships among the primitive species. Am. J. Bot. 44:537–544.

RITLAND, K. 1990. A series of FORTRAN computer programs for estimating plant mating systems. J. Heredity 81:235–237.

RITLAND, K., AND S. K. JAIN. 1981. A model for the estimation of outcrossing rate and gene frequencies using *n* independent loci. Heredity 47:35–52.

SCHAAL, B. 1984. Life history variation, natural selection and maternal effects in plant populations. Pp. 188–206 *in* R. Dirzo and J. Sarukhan, eds. Perspectives in plant population ecology. Sinauer, Sunderland, MA.

SCHEMSKE, D. W. 1978. Evolution of reproductive characteristics in *Impatiens* (Balsaminaceae): The significance of cleistogamy and chasmogamy. Ecology 59:596–613.

———. 1983. Breeding system and habitat effects on fitness in three neotropical *Costus* (Zingiberaceace). Evolution 38:817–832.

SCHMITT, J. A., AND D. W. EHRHARDT. 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. Evolution 44:269–278.

SCHOEN, D. J. 1983. Relative fitnesses of selfed and outcrossed progeny in *Gilia achilleifolia* (Polemoniaceae). Evolution 37:292–301.

SCHOEN, D. J., AND D. G. LLOYD. 1984. The selection of cleistogamy and heteromorphic diaspores. Biol. J. Linn. Soc. 23:303–322.

SHULL, G. H. 1952. Beginnings of the heterosis concept. Pp. 14–48 *in* J. W. Gowen, ed. Heterosis. Iowa State College Press, Ames.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2d ed. W. H. Freeman, San Francisco, CA.

SOLBRIG, O. T. 1976. On the relative advantages of cross- and self-fertilization. Ann. Missouri Bot. Gard. 63:262–276.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern J. 73:9–27.

SORENSEN, F. C., J. F. FRANKLIN, AND R. WOLLARD. 1976. Self-pollination effects on seed and seedling traits in Noble fir. Forest. Sci. 28:283–292.

STEBBINS, G. L. 1950. Variation and evolution in plants. Columbia Univ. Press, New York.

————. 1957. Self-fertilization and population variability in the higher plants. Am. Nat. 91:337–354.

————. 1971. Chromosomal evolution in higher plants. Edward Arnold, London.

————. 1974. Flowering plants: Evolution above the species level. Belknap, Cambridge, MA.

SYSTAT. 1992. Statistics. Version 5.2 edition. SYSTAT, Inc., Evanston, IL.

UYENOYAMA, M. K. 1986. Inbreeding and the cost of meiosis: The evolution of selfing in populations practicing biparental inbreeding. Evolution 40:388–404.

UYENOYAMA, M. K., AND D. M. WALLER. 1991. Coevolution of self-fertilization and inbreeding depression. I. Mutation-selection balance at one and two loci. Theoret. Popul. Biol. 40:14–46.

UYENOYAMA, M. K., K. E. HOLSINGER, AND D. M. WALLER. 1993. Ecological and genetic factors directing the evolution of self-fertilization. Oxford Surv. Evol. Biol. 9:327–381.

VAN TREUREN, R., R. BIJLSMA, N. J. OUBORG, AND W. VAN DELDEN. 1993. The significance of genetic erosion in the process of extinction. IV. Inbreeding depression and heterosis effects caused by selfing andd outcrossing in Scabiosa columbaria. Evolution 47:1669–1680.

WALLER, D. M. 1979. The relative costs of self- and cross-fertilized seed in Impatiens capensis (Balsaminaceae). Am. J. Bot. 66:313–320.

WELLER, S. G., AND R. ORNDUFF. 1977. Cryptic self-incompatibility in Amsinckia grandiflora. Evolution 31:47–51.

WELLS, H. 1979. Self-fertilization: Advantageous or deleterious? Evolution 33:252–255.

WERTH, C. R. 1985. Implementing an isozyme laboratory at a field station. Virginia J. Sci. 36:53–76.

WIENS, D., C. L. CALVIN, C. I. DAVERN, D. FRANK, AND S. R. SEAVEY. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. Oecologia 71:501–509.

WIENS, D., D.L. NICKRENT, C. I. DAVERN, C. L. CALVIN AND N.J. VIVRETTE. 1989. Developmental failure and loss of reproductive capacity in the rare palaeoendemic shrub Dedeckera eurekensis. Nature 338:65–67.

WILLIS, J. H. 1993a. Partial self-fertilization and inbreeding depression in two populations of Mimulus guttatus. Heredity 71:145–154.

————. 1993b. Effects of different levels of inbreeding on fitness components in Mimulus guttatus. Evolution 47:864–876.

WOLFE, L. M. 1993. Inbreeding depression in Hydrophyllum appendiculatum: Role of maternal effects, crowding, and parental mating history. Evolution 47:374–386.

WRIGHT, S. 1977. Evolution and the genetics of populations. Vol. 3. Experimental results and evolutionary deductions. Univ. of Chicago Press, Chicago.

ZAR, J. H. 1984. Biostatistical analysis. 2d ed. Prentice-Hall, Englewood Cliffs, NJ.

Corresponding Editor: A. Snow