

INBREEDING DEPRESSION AND SELFING RATE OF
IPOMOEA HEDERACEA VAR.
INTEGRUSCULA (CONVOLVULACEAE)¹

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Inbreeding depression and selfing rate were investigated in the self-compatible vine *Ipomoea hederacea* to assess the variability of the breeding system. Inbreeding depression differed between populations and the magnitude varied at germination, growth (as measured by aboveground biomass), and reproductive potential. Plants from Macon County, Alabama, USA, had significant inbreeding depression (31%) at germination, but no significant inbreeding depression for aboveground biomass or number of reproductive structures (buds and flowers) at 45 d post germination in the greenhouse or in the field. Plants from Morgan County, Alabama, however, had significant inbreeding depression (>50%) for all three stages in the greenhouse. In allozyme comparisons, five of the 11 *I. hederacea* populations surveyed had high selfing rates (66.66–92.53%) and high levels of homozygosity ($F_{IS} = 0.500$ – 0.861) in 2003, and three of four populations surveyed in 2004 had selfing rates that exceeded 50%. High selfing rates, high levels of homozygosity, and low levels of inbreeding depression suggest that inbreeding depression may not present a significant barrier to the transmission of selfing alleles in some populations of *I. hederacea*, but does not account for the maintenance of a mixed mating system in other populations.

Key words: allozyme; inbreeding depression; *Ipomoea hederacea*; mixed mating system; self-compatible.

Mating systems are a central component of the incredible floral diversity of higher plants (Barrett, 2003). The attraction and use of vectors to perform outcrossing remains the most significant adaptation for plant radiation (Mayr, 1942; Thompson, 1994; Schluter, 2000). However, most angiosperms bear hermaphroditic flowers that contain both anthers and stigmas and a significant proportion retain the ability to self-pollinate (Stebbins, 1974). Understanding the evolution and maintenance of plant mating systems has been of major interest to botanists since Darwin (1876, 1877).

There are two major hypotheses for the evolution of self-pollination (Jain, 1976) and both of these hypotheses predict that the selfing allele should become a fixed trait in plant populations (Charlesworth and Charlesworth, 1990). The “automatic selection” hypothesis states that if a gene promoting selfing arises in a population of outcrossers, then this gene will have a 50% transmission advantage to the next generation. The “reproductive assurance” hypothesis states that the selective advantage for self-pollination lies in the assurance of seed production when pollinators are limited, resulting in stable mixed mating systems with variable selfing rates dependent upon levels of pollen limitation (Baker, 1955; Schoen et al., 1996).

Inbreeding depression is the loss of fitness as a result of

self- or within-family fertilization (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996) and is considered the primary force that opposes the transmission advantage associated with selfing alleles (Darwin, 1877; Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Charlesworth, 2003). According to theoretical models, inbreeding can be maintained up to a threshold level of 50% (Lloyd, 1979; Charlesworth, 1980; Lande and Schemske, 1985). When inbreeding depression is above 50%, selfing alleles are lost from the population because the fitness of plants possessing these alleles is lower than the plants possessing alleles for outcrossing (Charlesworth and Charlesworth, 1987; Keller and Waller, 2002, for review). According to these models, the expected frequency distribution of selfing rates should be bimodal, with one mode corresponding to complete selfing when inbreeding depression is <50% and complete outcrossing when inbreeding depression is >50%. Paradoxically, most plant species have an intermediate, mixed mating system in which they outcross most of the time (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Barrett and Eckert, 1990; Vogler and Kalisz, 2001). Vogler and Kalisz (2001) suggest that intermediate rates of outcrossing are common and that pollinator unpredictability, and thus the need for reproductive assurance, could influence the evolution of outcrossing rates.

However, inbreeding depression is not a fixed state, and the genetic basis may vary across different life stages (Husband and Schemske, 1995, 1996). In a review of inbreeding depression in plants, Husband and Schemske (1996) found that primarily outcrossing populations had higher inbreeding depression values (δ) for seed production, germination, and survival, but had similar values to primarily self-fertilizing populations for growth and flower production. Inbreeding depression is also expected to vary as a function of inbreeding history with populations that habitually self-fertilize and have a lower level of inbreeding depression (Lande and Schemske,

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1985; Husband and Schemske, 1996). Latta and Ritland (1994), for example, found a negative relationship between prior inbreeding in populations of *Mimulus* sp. (Scrophulariaceae) and the magnitude of inbreeding depression for five fitness traits.

Likewise, inbreeding depression may have some phenotypic plasticity and vary between different environments (Dudash, 1990; Cheptou et al., 2002). Drought stress in *Crepis sancta* (Asteraceae) increased inbreeding depression for growth and flower production, but did not affect survival (Cheptou et al., 2000). In *Cucurbita pepo* subsp. *texana* (Cucurbitaceae), nitrogen stress increased inbreeding depression for flower, pollen, and fruit production (Hayes et al., 2005), but inbreeding depression was also shown to vary within the same field site between years. Rankin et al. (2002) found that the expression of inbreeding depression in *Schiedea menziessi* (Caryophyllaceae) varies dramatically between life stages and habitats and suggest that a habitat shift may have influenced the evolution of other *Schiedea* sp. Therefore, any study that examines the role of inbreeding depression in the maintenance of mixed mating systems should also examine the impact of environment across life stages.

In this study, the potential for inbreeding depression as a barrier to selfing is examined in *Ipomoea hederacea* var. *integriuscula* Gray (Convolvulaceae), the entire-leaf morning glory. Plants of the genus *Ipomoea* have been developed as a model system for the study of mixed mating systems (Ennos, 1981; Ennos and Clegg, 1983; Schoen and Clegg, 1985; Elmore, 1986; Chang, 1997; Chang and Rausher, 1998, 1999; Mojonner, 1998), and *I. hederacea* specifically has been used to study the effects of inbreeding on plant and herbivore interactions (Hull-Sanders and Eubanks, 2005). *Ipomoea hederacea* is a self-compatible annual vine that is believed to have a mixed mating system (Ennos, 1981; Elmore, 1986) due to its ability to produce viable seeds from autogamous fertilizations and hand outcrosses. This species is frequently found in cultivated gardens and disturbed areas and is a serious pest species in agricultural crops such as soybean (Thullen and Keeley, 1983; Klingaman and Oliver, 1996), cotton (Klingaman and Oliver, 1994), and peanuts (Bailey et al., 1999) throughout the southeastern United States. Germination occurs between mid-May and August, and flowering in east-central Alabama (AL) begins in early August. Individual flowers are only open for a few hours and wither by mid-morning, creating a small window of opportunity for pollinators. The fruits are dehiscent capsules containing 1–6 hard-coated seeds that mature 4–6 wks after pollination (Mojonnier, 1998).

An increased selfing rate in morning glories is associated with under-visitation by pollinators (Epperson and Clegg, 1987). Primary pollinators documented are bees of the genus *Bombus* (Ennos, 1981); however, hawkmoths (Lepidoptera: Sphingidae) and sulfur butterflies (Lepidoptera: Pieridae) have been frequently observed on flowers in AL (H. Hull-Sanders, personal observation). The morphology of *I. hederacea* flowers ensures pollination by forcing the anthers to glide over the stigmas as the flower closes (Elmore, 1986). In this way, *I. hederacea* flowers have the opportunity to be pollinated by insects when they first open, but, if pollinators do not visit the flower within a few hours, the flower will self-pollinate before senescence. Therefore, in morning glories, selfing may provide reproductive assurance.

In this study, the maintenance of a mixed mating system was examined in *I. hederacea*. The objectives of the present

study used multiple populations to determine (1) the occurrence of inbreeding depression, (2) the timing of inbreeding depression relative to plant phenology, (3) if inbreeding differed between the greenhouse and the field, and (4) the relative levels of genetic variation using allozyme loci.

MATERIALS AND METHODS

Inbreeding depression—In August 2000, seeds were collected from plants in a cotton field at the Auburn University EV Smith Research Center, Macon County, Alabama and from plants in a cornfield in Morgan County, Alabama. Seeds were collected at random from >50 plants. The two populations were approximately 265 km apart and were chosen because of the large population size and association with crops not sprayed with insecticide. Seeds were sown in 20-cm-diameter pots with Pro-Mix potting medium (Premier Horticulture, Dorval, Quebec, Canada). Flowers were allowed to self-fertilize without manipulation to produce inbred seed. To produce half-sibling, outbred seeds, we emasculated buds in the afternoon before they opened using a pair of sharp-tipped forceps to slit open flowers from the side and remove all the anthers. The anthers do not dehisce before 2200 hours and can be removed without the possibility of pollination (S. Chang, University of Georgia, personal communication). Flowers were allowed to develop overnight, and pollen was removed from undisturbed flowers of other plants and placed on the stigma of the emasculated flower. Seeds developed within 6 weeks and were harvested by removing seed capsules.

In 2001, inbred and outbred seed from Macon Co. and Morgan Co. cornfield populations were sown in the greenhouse as described earlier. The number of days to germination and percentage of seeds germinated were recorded. The plants were allowed to grow for 45 d and then harvested by cutting the plant from the roots at soil level. Length of plant (cm) and number of reproductive structures (flowers + buds) were recorded. The plants were then placed in foil packets, dried at 45°C for 7 d, and weighed to the nearest 0.01 g.

In 2002, inbred and outbred seeds from the Macon Co. population were germinated in 2 × 2 × 5 cm six pack cells with Pro-Mix potting medium. Ninety inbred and 90 outbred seedlings were transplanted to an experimental field plot on the Auburn University Agriculture Futures Park, Auburn, AL. Plants were placed 30 cm apart in blocks of 10 per row, and each row was placed 1 m apart. Bamboo garden stakes were inserted into the soil near the plants to support the climbing vines during growth. Plants were allowed to grow for 45 d and then harvested by cutting the plant away from the roots at soil level. Length of plant and number of reproductive structures were recorded. The plants were placed in paper bags, dried at 45°C for 5 d, and weighed to the nearest 0.01 g.

Allozyme analysis—To estimate the amount of inbreeding in multiple, isolated populations, seeds were collected from 11 sites in seven counties in AL in 2003 and four sites in three counties in 2004. Seeds were germinated in the greenhouse at the University of Virginia Blandy Experimental Farm, Boyce, Virginia. Whole seedling tissue was used for analysis. Seedlings were ground in needle extraction buffer (Mitton, 1979) with a mortar and pestle, centrifuged for 2 min at room temperature, and stored at –80°C for no more than 48 h before electrophoresis.

Cellulose acetate gels were used in conjunction with a Tris-glycine buffer system as outlined in Herbert and Beaton (1989), which enabled the resolution of five enzymes: isocitrate dehydrogenase (IDH, EC 1.1.1.42), glucosephosphate isomerase (GPI; EC 5.3.1.9), malate dehydrogenase (ME, EC 1.1.1.40), phosphoglucomutase (PGM, EC 2.7.5.1), and 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44). Supernatant from 272 individuals was loaded onto gels.

Data analysis—Population data were analyzed using SAS version 8.2 (SAS Institute, 2001). Percentage of seed germination differences was analyzed by chi-square. Number of days to germination and number of reproductive structures were log transformed prior to analysis to conform to the assumptions of analysis of variance (ANOVA). ANOVA was used to examine the effects of population, mating system, and the population by mating system interaction

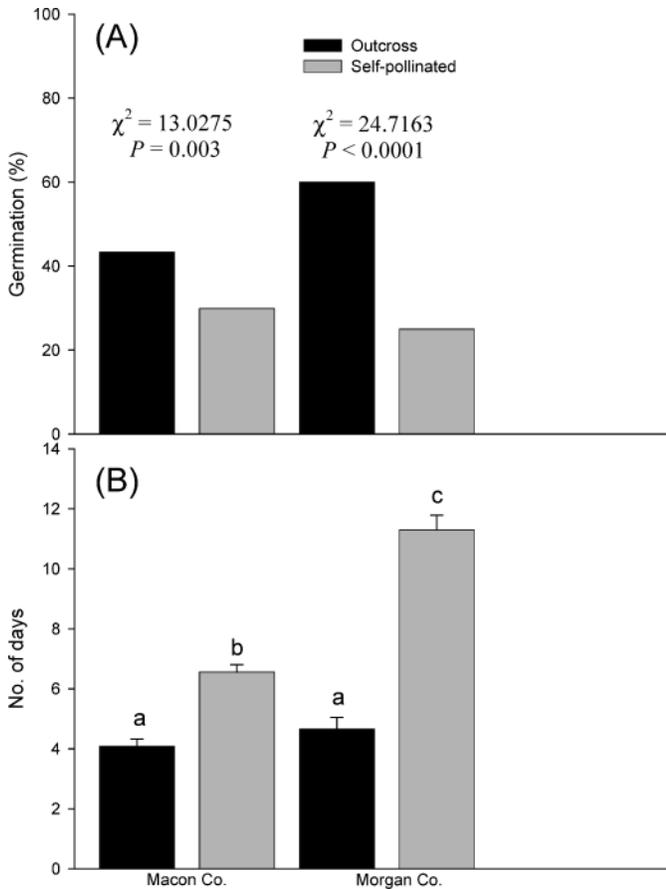


Fig. 1. Germination (%) of seeds (A) and the number of days required to germinate (\pm SE) (B) of progeny from outcross and self pollinations of *Ipomoea hederacea* from Macon County and Morgan County, Alabama, populations. Different letters indicate significant differences, $P < 0.001$.

on the number of days needed for seed to germinate, the number of reproductive structures produced, and aboveground biomass (AGB) in the greenhouse. Because only one population was germinated and transplanted into the field, ANOVA was used to examine the effects of block, mating system, and the block by mating system interaction on the number of reproductive structures produced and AGB in the field.

Inbreeding depression of self-fertilized progeny relative to outcrossed progeny was calculated with the equation

$$\delta = (W_o - W_s)/W_o = 1 - W_s/W_o,$$

where W_o is the mean of the measured fitness component of outcrossed progeny and W_s is the mean of the measured fitness component of self-fertilized progeny (see Keller and Waller, 2002, for a review). Our fitness components were height of plant, number of leaves, number of reproductive structures (flowers and buds), and aboveground biomass (AGB).

The allele fixation index (F_{IS}) per population was calculated using FSTAT software for Windows, version 2.9.3.2 (Goudet, 1995). Allele frequencies were calculated for five allozyme loci and 15 *I. hederacea* populations. From the allele frequencies, Nei's estimate of gene diversity (H ; Nei, 1973) was calculated as a measure of genetic variation among populations. Population selfing rate (SR) was calculated as

$$SR = 2 F_{IS}/(1 + F_{IS}) \times 100,$$

according to the methods outlined in Hartl and Clark (1997). Wright's F statistics (Wright, 1978), F_{IT} , F_{ST} , and F_{IS} per locus were calculated according to the methods outlined in Weir and Cockerham (1984) using FSTAT. These statistics represent reductions in heterozygosity expected under random mat-

ing in individuals relative to the total across all subpopulations (F_{IT}), the amount of variation distributed among subpopulations (F_{ST}), and the reduction in heterozygosity within a subpopulation relative to random-mating expectations (F_{IS}). Confidence intervals (95%) for F -statistics were estimated by bootstrapping over loci.

RESULTS

Significantly more outcrossed seed germinated than did those from self-pollinations ($\chi^2 = 30.69$, $P < 0.0001$; Fig. 1). Of the Macon Co. seed, 43.31% of the outcrossed seed germinated, whereas only 29.87% of the self-fertilized seed germinated. Of the Morgan Co. seed, 60% of outcrossed and 25% of the self-fertilized seed germinated. In addition, outcrossed seed took significantly less time to germinate than self-fertilized seeds in both Macon and Morgan Co. populations (ANOVA: $F_{3,311} = 69.23$, $P < 0.0001$; Fig. 1). Macon Co. outcrossed seed had a mean germination of 4.1 d, whereas self-fertilized seeds had a mean germination of 6.6 d. Morgan county outcrossed seed had a similar mean germination of 4.7 d, whereas self-fertilized seed had a mean germination of 11.3 d.

There was a significant difference between populations, between pollination treatments, and population \times pollination treatment interactions for AGB and reproduction when plants were grown in the greenhouse (Table 1). Overall, outcrossed plants had significantly greater AGB than self-fertilized plants when grown in the greenhouse (ANOVA $F_{3,102} = 18.01$, $P < 0.0001$); however, this was mostly due to the difference in the Morgan Co. population. Morgan Co. outcrossed plants had a 73.4% greater AGB than self fertilized plants (Fig. 2), whereas Macon Co. outcrossed plants had only an 18.3% greater AGB than self fertilized plants (Fig. 2). Overall, outcrossed plants also had significantly more reproductive structures (flowers and buds) after 45 d than self-fertilized plants (ANOVA $F_{3,177} = 9.65$, $P < 0.0001$). However, Morgan Co. outcrossed plants had 65.6% more reproductive structures than self fertilized plants (Fig. 2), whereas Macon Co. outcrossed plants has 6.5% fewer reproductive structures than self fertilized plants (Fig. 2).

In the field, there was a significant block effect, but no effect of pollination treatment (Table 1). Outcrossed plants and self-fertilized plants had similar AGB (ANOVA: $F_{1,176} = 0.74$, $P = 0.39$) (Fig. 3). Likewise, outcrossed plants and self-fertilized plants had a similar amount of reproductive structures in the field after 45 d (ANOVA: $F_{1,170} = 0.02$, $P = 0.88$) (Fig. 3).

Estimates of inbreeding depression in the greenhouse were greater for Morgan Co. than for Macon Co. (Table 2). Plants from both counties exhibited significant inbreeding depression at germination ($\chi^2 = 13.03$, $P = 0.0003$; $\chi^2 = 24.72$, $P < 0.0001$ for Macon and Morgan Co., respectively); however, only plants from Morgan Co. had significant inbreeding depression for growth and fitness estimates (ANOVA: $F_{1,17} = 32.67$, $P < 0.0001$; $F_{1,17} = 24.44$, $P < 0.0001$ for AGB and reproductive structure, respectively).

Interestingly, the expected heterozygosity across all populations and loci was calculated to be $H_t = 0.39$; however, electrophoretic data revealed that all populations of Alabama entire-leaf morning glory had a low level of heterozygosity (Nei's $H_o = 0.11$ and $H_o = 0.15$, in 2003 and 2004 respectively); with DeKalb, Baldwin, and Macon Co. populations having very high fixation indices (F_{IS}) in 2003 and Cullman and Macon Co. populations in 2004. Four of the eleven 2003

TABLE 1. Analyses of variance for (a) number of reproductive structures produced and (b) aboveground biomass for *Ipomoea hederacea* var. *integriscula* in Auburn, Alabama USA grown in the (A) greenhouse and (B) field. The number of reproductive structures was ln-transformed. Significant effects are in boldface.

Source	(a) Reproductive structure number				(b) Aboveground biomass			
	df	SS	F	P	df	SS	F	P
A) Greenhouse								
Population	1	41.19	10.38	0.0015	1	102.76	14.95	0.0002
Pollination treatment	1	30.79	7.76	0.0059	1	205.32	29.88	<0.0001
Population × Pollination	1	71.05	17.90	<0.0001	1	139.90	20.36	<0.0001
Error	177	702.64			102	701.01		
Corrected total	180	817.51			105	1072.28		
B) Field								
Block	17	225.71	17.50	<0.0001	17	8795.62	8.49	<0.0001
Pollination treatment	1	0.04	0.05	0.8264	1	71.21	1.17	0.2815
Block × Pollination	17	14.18	1.10	0.3607	17	1790.70	1.73	0.0439
Error	136	103.19			142	8651.92		
Corrected total	171	347.22			177	19 346.44		

populations and three of the four 2004 populations had an SR of 70% or higher (Table 3). The degree of differentiation among populations is very low. In 2003, the mean F_{ST} for polymorphic loci was 0.035 (95% CI, -0.024 – 0.138), and in 2004 F_{ST} was 0.014 (95% CI, -0.07 – 0.039), indicating that, in general, Alabama populations contain the same alleles (Table 4). The overall fixation index due to non-random mating

(F_{IS}) across all alleles was 0.456 (95% CI, 0.166–0.733) in 2003 and 0.615 (95% CI, 0.476–0.740) in 2004 (Table 4).

DISCUSSION

Ipomoea hederacea appears to have a mixed mating system. Populations of *I. hederacea* had selfing rates that ranged from

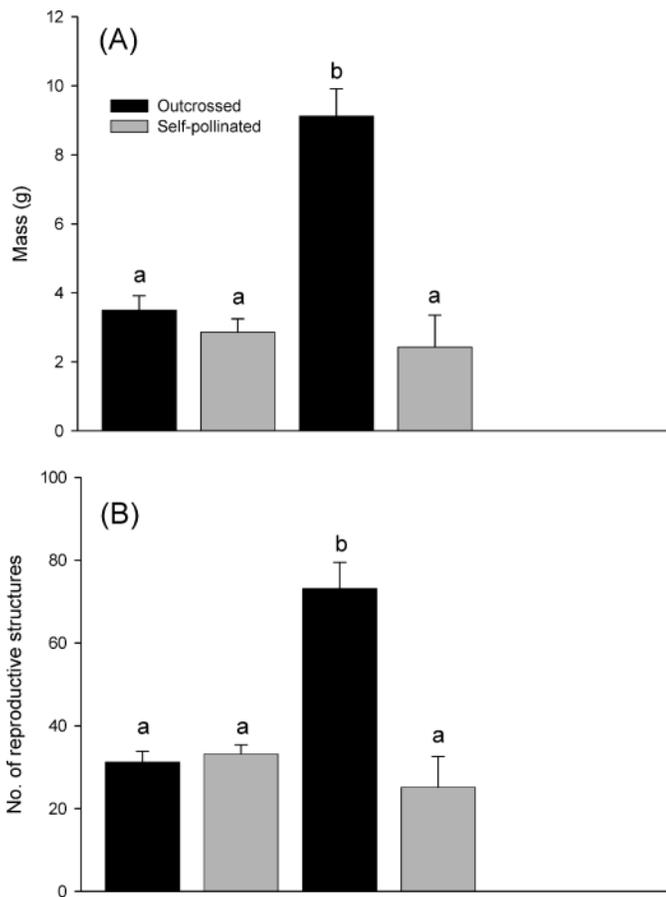


Fig. 2. The aboveground biomass (\pm SE) (A) and number of reproductive structures (\pm SE) (B) of *Ipomoea hederacea* plants grown in the greenhouse after 45 d. Different letters indicate significant differences, $P < 0.001$.

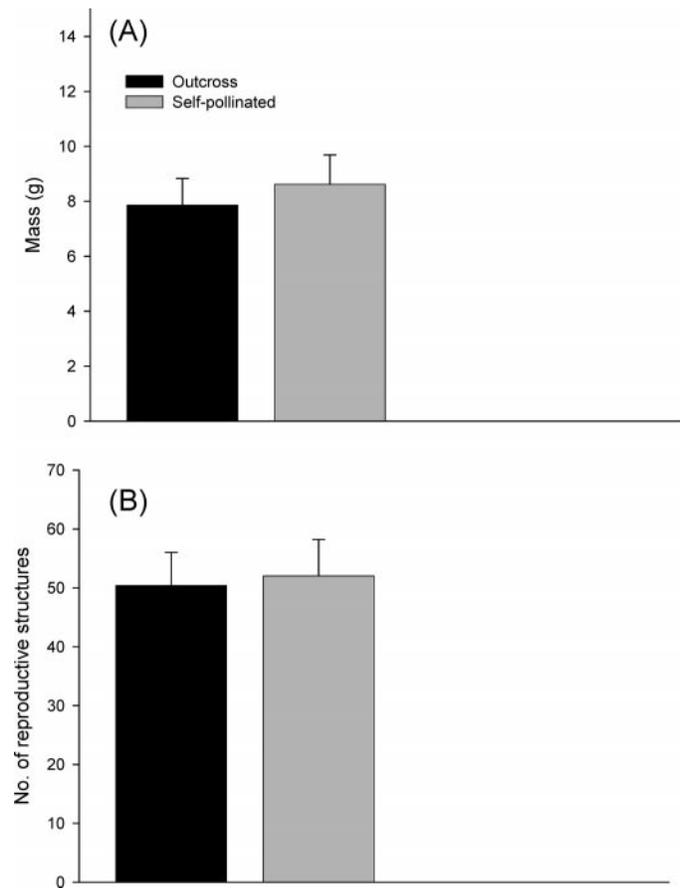


Fig. 3. The aboveground biomass (\pm SE) (A) and number of reproductive structures (\pm SE) (B) of *Ipomoea hederacea* from Macon County plants grown in the field after 45 d.

TABLE 2. Performance estimates (and +SE of means) of self and outcross progeny for three life history characters in *Ipomoea hederacea* var. *integriuscula* in two Alabama populations, Macon County (A) and Morgan County (B). Separate estimates of inbreeding depression (ID) [(1 – self mean/outcross mean) × 100] are given for each character.

	<i>N</i>	Self	Outcross	ID
A) Macon Co.				
Germination (%)	241	29.87	43.31	31.03%*
Biomass (g)	86	2.79 (0.39)	3.50 (0.42)	20.29%
Flowering (no.)	125	38.7 (2.13)	42.0 (2.78)	7.90%
B) Morgan Co.				
Germination (%)	78	25.00	60.00	58.33%*
Biomass (g)	19	2.43 (0.90)	9.13 (0.76)	73.38%*
Flowering (no.)	19	25.1 (6.84)	73.1 (5.83)	65.62%*

Note: *N* = no. of individuals contributing to each self-outcross comparison.

* Significant differences between progeny $P < 0.0001$.

19–92%, seeds were readily produced by self- and cross-pollinations, and outcrossed seed germinated at a significantly higher rate than selfed seed. Five populations in 2003 were found to have high levels of selfing (66.66–92.53%) and very high inbreeding coefficients (0.50–0.861). Of the two populations surveyed again in 2004, selfing and inbreeding coefficients increased, suggesting that selfing rates are highly variable in *I. hederacea* from year to year (Rankin et al., 2002).

Other species of *Ipomoea* also have varying selfing rates (Rausher et al., 1993; Chang and Rausher, 1998). However, in *I. purpurea*, flower morphology, anther–stigma distance, and frequency distribution of morphs have been shown to influence selfing rates (Ennos, 1981; Schoen and Clegg, 1985; Epperson and Clegg, 1987; Rausher et al., 1993; Chang and Rausher 1998, 1999). Although *I. hederacea* has two leaf morphologies, only a single flower morph occurs (Elmore, 1986), and the anthers occur in a whirled pattern around the stigma rather than at fixed distances (Ennos, 1981); therefore, the selfing rates are not expected to be influenced by these factors.

Many models of mating system evolution predict that inbreeding depression should decrease from generation to generation as the selfing rate in a population increases (Charlesworth et al., 1990; Uyenoyama and Waller, 1991). Selection through survival and fecundity differences should begin to eliminate low fitness genotypes, resulting in the purging of deleterious alleles in the population and thus lower levels of inbreeding depression. Results from Macon Co. follow this

TABLE 3. Genetic variation estimated from five allozyme loci for 11 Alabama populations of *I. hederacea* var. *integriuscula* in 2003 and four populations in 2004.

Population 2003	<i>N</i>	F_{IS}	SR	Population 2004	<i>N</i>	F_{IS}	SR
M	3	0.67	80.02%	M	33	0.76	86.36%
CS	20	0.50	66.66%	CS	23	0.81	89.56%
CP	20	0.27	42.02%	MgC	35	0.60	74.77%
CW	19	0.16	26.99%	MgH	24	0.29	45.08%
B	7	0.76	86.56%				
DA	11	0.33	49.74%				
DC	9	0.86	92.53%				
DF	11	0.34	50.41%				
E	14	0.66	79.30%				
H	7	0.11	19.17%				
L	26	0.33	49.96%				

Note: M = Macon Co., CS = Cullman Co. soybean field, CP = Cullman Co. peanut field, CW = Cullman Co. weed plot, B = Baldwin Co., DA = DeKalb Co. asparagus field, DC = DeKalb Co. corn field, DF = DeKalb Co. fenced plot, E = Elmore Co., H = Henry Co., L = Lee Co., MgC = Morgan Co. corn field, MgH = Morgan Co. USDA field garden.

N = number of individuals, F_{IS} = inbreeding coefficient due to non-random mating, SR = selfing rate calculated [$2 F_{IS}/(1 + F_{IS}) \times 100$].

pattern. Selfing rate was estimated to be >80%, while inbreeding depression remained under 50%. Therefore, inbreeding depression probably does not pose a significant barrier to the maintenance of selfing in that population. However, the Morgan Co. corn-associated population's selfing rate was estimated to be ~75%, but inbreeding depression was >50%. This combination of high selfing rates, high inbreeding depression, and early-stage specific inbreeding depression may suggest mating system instability (Rankin et al., 2002). Neighboring populations in Morgan Co. continued to have a relatively low selfing rate (~45%), suggesting that perhaps there may have been less pollinator service in 2004 than was typical; therefore, we would predict that should pollinator service remain low in the corn-associated Morgan Co. population, then inbreeding depression should decrease with successive selfed generations and significantly affect mating system alleles, perhaps causing a shift from a mixed mating system to a predominantly autogamous mating system (Crow, 1999).

Previous studies have suggested that populations of *I. hederacea* will be highly self-pollinating when they occur in conjunction with populations of *I. purpurea* due to pollen competition (Ennos, 1981; Elmore, 1986). Pollen from *I. purpurea* can grow down the style of *I. hederacea*, but it is incompatible

TABLE 4. Estimates of the reduction in heterozygosity due to inbreeding within populations and genetic drift among populations in Alabama of *Ipomoea hederacea* var. *integriuscula* (F_{IT}), the degree of differentiation among populations (F_{ST}) and the inbreeding coefficient due to non-random mating calculated for each locus (F_{IS}).

Locus	2003			2004		
	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}
IDH	0.779	0.197	0.724	0.514	-0.007	0.517
GPI	0.032	-0.004	0.035	0.649	0.045	0.632
ME	0.656	-0.050	0.673	0.805	-0.005	0.806
PGM	0.522	-0.010	0.526	0.331	-0.007	0.336
6PGDH	1.000	0.244	1.000	0.737	0.051	0.722
Overall	0.475	0.035	0.456	0.621	0.014	0.615
95% CI	0.194–0.767	-0.024–0.138	0.166–0.733	0.472–0.746	-0.007–0.039	0.476–0.740

Note: isocitrate dehydrogenase (IDH, EC 1.1.1.42), glucosephosphate isomerase (GPI, EC 5.3.1.9), malate dehydrogenase (ME, EC 1.1.1.40), phosphoglucosmutase (PGM, EC 2.7.5.1), and 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44).

with the ovule and hollow seeds are produced (Guries, 1978). Selfing in these populations would confer not only reproductive assurance, but also prevent heterospecific pollen from growing down the style. This mechanism, however, is unlikely to explain the results of this study. In AL, *I. hederacea* primarily occurs either in pure stands or in conjunction with *I. cordatotriloba*, rarely with *I. purpurea*, and none of the 11 populations in this study were sympatric with *I. purpurea*.

In other species of *Ipomoea*, inbreeding depression alone could not account for the maintenance of the mixed mating system (Chang and Rausher, 1999). High selfing rates should dramatically reduce gene flow among populations and populations should have increased genetic differentiation (Carr and Fenster, 1994; Latta and Ritland, 1994; Carr et al., 1997; Wallace, 2002). Given the high levels of inbreeding within a population, but relatively little population differentiation, gene flow may be maintained through seed distribution. One locus examined in this study (6PGDH) appeared to have reached fixation in 2003, but showed some variation in 2004. There is also some genetic variation among populations ($F_{ST} = 0.244$). The amount of gene flow is yet unknown, and seed dispersal may be facilitated by human transport of crops with which these plants are associated.

Stage specific inbreeding depression frequently occurs in plants with mixed mating systems (Dudash, 1990; Johnston, 1992; Husband and Schemske, 1996). Within the Macon Co. population, significant inbreeding depression only occurred at germination (31%). Within the Morgan Co. population, greater than 50% inbreeding depression occurred at all three life-cycle stages (germination, growth, and reproduction). While inbreeding depression occurs more often during growth and seed set in angiosperms, inbreeding depression is not uncommon at germination (Husband and Schemske, 1996). The timing of inbreeding depression in the life cycle may have substantial fitness consequences. Early stage inbreeding depression reduces the fitness of the progeny to 0, whereas with late stage inbreeding depression, the proportionate reproductive contribution to the next generation is >0 (Charlesworth, 1980; Husband and Schemske, 1996).

Husband and Schemske (1996) found that predominantly selfing plant species not only have less cumulative inbreeding depression than outcrossing plant species, but that inbreeding depression can be expressed at different times in the life cycle than in outcrossers. Variation in the timing of inbreeding depression between Macon and Morgan Co. populations suggests that the Macon Co. population of *I. hederacea* had a relatively high rate of selfing that may have purged deleterious alleles leading to a low inbreeding depression rate, whereas Morgan Co. plants may have a highly variable mixed-mating system that would allow recessive alleles to be maintained within the population. These results are consistent with other studies that have found inbreeding depression significantly varied between populations (Carr and Dudash, 1995; Cheptou et al., 2002; Wallace, 2003); however, they are counter to the expectations of Husband and Schemske (1996), that predicts inbreeding depression should occur at later life stages rather than at germination.

It is generally thought that inbreeding depression will be higher in field experiments than in greenhouse experiments because environmental conditions are expected to exacerbate the negative effects of inbreeding (Dudash, 1990). Some studies, however, have observed little variation in inbreeding depression between greenhouse and field-grown plants (Chang,

1997; Willis, 1993), and a small number of studies suggest that inbreeding depression can be higher in plants grown in the greenhouse than plants in the field (contrast Carr and Eubanks, 2002 with Ivey et al., 2004). In this study, inbreeding depression occurred in plants grown in the greenhouse. It is generally thought that the greenhouse provides the optimal growing conditions; however, within a greenhouse setting, season, diurnal temperature, and seed source have been found to influence germination, growth, and seed production in *I. hederacea* (Thullen and Keeley, 1983; Klingaman and Oliver, 1996). Therefore, the greenhouse may not provide optimal growing conditions. *Ipomoea hederacea* has been found to do best in cleared, disturbed fields in the absence of other plant competitors (Whigham, 1984), so the newly tilled field plot used for these experiments should have provided good conditions for growth. However, there was no detectable inbreeding depression for Macon Co. plants in either the greenhouse or in the field.

In conclusion, *I. hederacea* populations in Alabama had varying selfing rates. But even within a population that had high selfing rates, significant inbreeding depression occurred and was stage specific. Inbreeding depression in Macon Co. was observed at germination, but did not reach the threshold level of 50% and therefore was not expected to be high enough to present a barrier to the transmission of selfing alleles. However, while selfing rates were high, none of the allozymes were found to have reached fixation; the mixed mating system in *I. hederacea* may be maintained by some mechanism other than inbreeding depression.

LITERATURE CITED

- BAILEY, W. A., J. W. WILCUT, D. L. JORDAN, C. W. SWANN, AND V. B. LANGSTON. 1999. Response of peanut (*Arachis hypogaea*) and selected weeds to Diclosulam. *Weed Technology* 13: 771–776.
- BAKER, H. G. 1955. Self-compatibility and establishment after 'long-distance' dispersal. *Evolution* 9: 347–348.
- BARRETT, S. C. H. 2003. Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society London, B, Biological Sciences* 358: 991–1004.
- BARRETT, S. C. H., AND C. G. ECKERT. 1990. Variation and evolution of mating systems in seed plants. In S. Kawano [ed.], *Biological approaches and evolutionary trends in plants*, 229–254. Academic Press, San Diego, California, USA.
- CARR, D. E., AND M. R. DUDASH. 1995. Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. *Heredity* 75: 437–445.
- CARR, D. E., AND M. R. DUDASH. 1996. Inbreeding depression in two species of *Mimulus* (Scrophulariaceae) with contrasting mating systems. *American Journal of Botany* 83: 586–593.
- CARR, D. E., AND M. D. EUBANKS. 2002. Inbreeding alters resistance to insect herbivory and host plant quality in *Mimulus guttatus* (Scrophulariaceae). *Evolution* 56: 22–30.
- CARR, D. E., AND C. B. FENSTER. 1994. Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. *Heredity* 72: 606–618.
- CARR, D. E., C. B. FENSTER, AND M. R. DUDASH. 1997. Relationship between mating-system characters and inbreeding depression in *Mimulus guttatus*. *Evolution* 51: 363–372.
- CHANG, S.-M. 1997. Maintenance of a mixed mating system in the common morning glory, *Ipomoea purpurea*; the role of anther–stigma distance. Ph.D. dissertation, Duke University, Durham, North Carolina, USA.
- CHANG, S.-M., AND M. D. RAUSHER. 1998. Frequency-dependent pollen discounting contributes to the maintenance of a mixed mating system in the common morning glory *Ipomoea purpurea*. *The American Naturalist* 152: 671–683.
- CHANG, S.-M., AND M. D. RAUSHER. 1999. The role of inbreeding depression

- in maintaining the mixed mating system of the common morning glory, *Ipomoea purpurea*. *Evolution* 53: 1366–1376.
- CHARLESWORTH, B. 1980. Evolution in age-structured populations. Cambridge University Press, Cambridge, UK.
- CHARLESWORTH, D. 2003. The effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 358: 1051–1070.
- CHARLESWORTH, D., AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.
- CHARLESWORTH, D., AND B. CHARLESWORTH. 1990. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. *Evolution* 44: 870–888.
- 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.
- CHEPTOU, P.-O., A. BERGER, A. BLANCHARD, C. COLLIN, AND J. ESCARRE. 2000. The effect of drought stress on inbreeding depression in four populations of the Mediterranean outcrossing plant *Crepis sancta* (Asteraceae). *Heredity* 85: 294–302.
- CHEPTOU, P.-O., J. LEPART, AND J. ESCARRE. 2002. Mating system variation along a successional gradient in the allogamous and colonizing plant *Crepis sancta* (Asteraceae). *Journal of Evolutionary Botany* 15: 753–762.
- CROW, J. F. 1999. Dominance and overdominance. In J. G. Coors and Shivaji-Pandey [eds.], *The genetics and exploitation of heterosis in crops*, 49–58. 1997. American Society of Agronomy, Madison, Wisconsin, USA.
- DARWIN, C. 1876. The effects of cross and self fertilization in the vegetable kingdom. J. Murray, London, UK.
- DARWIN, C. 1877. The different forms of flowers on plants of the same species. 1986 reprint by University of Chicago Press, Chicago, Illinois, USA.
- DUDASH, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaaceae): a comparison in three environments. *Evolution* 44: 1129–1139.
- ELMORE, C. D. 1986. Mode of reproduction and inheritance of leaf shape in *Ipomoea hederacea*. *Weed Science* 34: 391–395.
- ENNOS, R. A. 1981. Quantitative studies of the mating system in two sympatric species of *Ipomoea* (Convolvulaceae). *Genetica* 57: 93–98.
- ENNOS, R. A., AND M. T. CLEGG. 1983. Flower color variation in the morning glory, *Ipomoea purpurea*. *Journal of Heredity* 74: 247–250.
- EPPELSON, B. K., AND M. T. CLEGG. 1987. Frequency-dependent variation for outcrossing rate among flower-color morphs of *Ipomoea purpurea*. *Evolution* 41: 1302–1311.
- GOUDET, J. 1995. FSTAT version 1.2: a computer program to calculate *F* statistics. *Journal of Heredity* 86: 485–486.
- GURIES, R. P. 1978. A test of the mentor pollen technique in the genus *Ipomoea*. *Euphytica* 27: 825–830.
- HARTL, D. L., AND A. G. CLARK. 1997. Principles of population genetics. Sinauer, Sunderland, Massachusetts, USA.
- HAYES, C. N., J. A. WINSOR, AND A. G. STEPHENSON. 2005. Environmental variation influences the magnitude of inbreeding depression in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *Journal of Evolutionary Biology* 18: 147–155.
- HERBERT, P. D. N., AND M. J. BEATON. 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis: a practical handbook. Helena Laboratories, Beaumont, Texas, USA.
- HULL-SANDERS, H. M., AND M. D. EUBANKS. 2005. Plant defense theory provides insight into interactions involving inbred plants and insect herbivores. *Ecology* 86: 897–904.
- HUSBAND, B. C., AND D. W. SCHEMSKE. 1995. Magnitude and timing of inbreeding depression in a diploid population of *Epilobium angustifolium* (Onagraceae). *Heredity* 75: 206–215.
- HUSBAND, B. C., AND D. W. SCHEMSKE. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50: 54–70.
- IVEY, C. T., D. E. CARR, AND M. D. EUBANKS. 2004. Inbreeding alters *Mimulus guttatus* tolerance to herbivory in natural environments. *Ecology* 85: 567–574.
- JAIN, S. K. 1976. The evolution of inbreeding in plants. *Annual Review of Ecology and Systematics*. 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.
- KELLER, L. F., AND D. M. WALLER. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17: 230–241.
- KLINGAMAN, T. E., AND L. E. OLIVER. 1994. Influence of cotton (*Gossypium hirsutum*) and soybean (*Glycine max*) planting date on weed interference. *Weed Science* 42: 61–65.
- KLINGAMAN, T. E., AND L. E. OLIVER. 1996. Existence of ecotypes among populations of entireleaf morningglory (*Ipomoea hederacea* var. *integrifolia*). *Weed Science* 44: 540–544.
- LANDE, R. S., 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. Relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. *Evolution* 48: 806–817.
- LLOYD, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *The American Naturalist* 113: 67–79.
- MAYR, E. 1942. Systematics and the origin of species. Columbia University Press, New York, New York, USA.
- MITTON, J. B., Y. B. LINHART, K. B. STURGEON, AND J. L. HAMRICK. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70: 86–89.
- MOJONNIER, L. 1998. Natural selection on two seed-size traits in the common morning-glory *Ipomoea purpurea* (Convolvulaceae): patterns and evolutionary consequences. *American Naturalist* 152: 188–203.
- NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.
- RANKIN, A. E., S. G. WELLER, AND A. K. SAKAI. 2002. Mating system instability in *Schiedea menziesii* (Caryophyllaceae). *Evolution* 56: 1574–1585.
- RAUSHER, M. D., D. AUGUSTING, AND A. VANDERKOOI. 1993. Absence of pollen discounting in a genotype of *Ipomoea purpurea* exhibiting increased selfing. *Evolution* 47: 1688–1695.
- SAS INSTITUTE. 2001. SAS, version 8.2. SAS Institute, Cary, North Carolina, USA.
- SCHLUTER, D. 2000. Introduction to the symposium: species interactions and adaptive radiation. *American Naturalist* 156(Supplement): S1–S3.
- SCHOEN, D. J., AND M. T. CLEGG. 1985. The influence of flower color on outcrossing rate and male reproductive success in *Ipomoea purpurea*. *Evolution* 39: 1242–1249.
- SCHOEN, D. J., M. T. MORGAN, AND T. BATAILLON. 1996. How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 351: 1281–1290.
- STEBBINS, G. L. 1974. Flowering plants: evolution above the species level. Belknap, Cambridge, UK.
- THOMPSON, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago, Illinois, USA.
- THULLEN, R. J., AND P. E. KEELEY. 1983. Germination, growth, and seed production of *Ipomoea hederacea* when planted at monthly intervals. *Weed Science* 31: 837–840.
- UYENOYAMA, M. K., AND D. M. WALLER. 1991. Coevolution of self-fertilization and inbreeding depression. I. Mutation-selection balance at one and two loci. *Theoretical Population Biology* 40: 14–46.
- VOGLER, D. W., AND S. KALISZ. 2001. Sex among the flowers: the distribution of plant mating systems. *Evolution* 55: 202–204.
- WALLACE, L. 2002. Examining the effects of fragmentation on genetic variation in *Platanthera leucophaea* (Orchidaceae): inferences from allozyme and random amplified polymorphic DNA markers. *Plant Species Biology* 17: 37–49.
- WALLACE, L. 2003. The cost of inbreeding in *Platanthera leucophaea* (Orchidaceae). *American Journal of Botany* 90: 235–242.
- WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.
- WHIGHAM, D. F. 1984. Effect of competition and nutrient availability on the growth and reproduction of *Ipomoea hederacea* in an abandoned old field. *Journal of Ecology* 72: 721–730.
- WILLIS, J. H. 1993. Partial self-fertilization and inbreeding depression in two populations of *Mimulus guttatus*. *Heredity* 71: 145–154.
- WRIGHT, S. 1978. Evolution and the genetics of populations, vol. 4. University of Chicago Press, Chicago, Illinois, USA.