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INBREEDING DEPRESSION, AUTONOMOUS SELF-POLLINATION, AND THE GENETIC AFFINITIES OF A RARE TAXON WITHIN THE ROSE-MALLows, HIBISCUS SECTION MUENCHHUSIA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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** * * * *

The Ohio State University

1995

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To the memory of my parents.
ACKNOWLEDGEMENTS

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FIELD OF STUDY

Major Field: Plant Biology
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INTRODUCTION

The taxa—The genus Hibiscus (Malvaceae), includes five species of "rose mallow," contained within the section Muenchhusia (Blanchard 1976) (Table 1). They are marsh herbs, exclusively eastern North American in distribution and comprise the only Hibiscus species in this area. Unlike some other sections of the genus which are more heterogeneous, the rose mallows are all diploid and have the same number of chromosomes (n=19). This lack of genomic differentiation and their confinement to a continuous range that was largely glaciated during the Pleistocene suggests that they may be very recently evolved (Wise and Menzel 1971). Four of the species have been divided into two informal natural groups based on morphology and cross-compatibility. (Wise and Menzel 1971). Each group consists of a closely related pair of species, of which one member has a wide geographic distribution and the other is restricted to the extreme southern edge of the section's range. The groups are: (1) the widespread H. moscheutos and the restricted southern Atlantic and Gulf Coastal H. grandiflorus, and; (2) the widespread H. laevis and the Florida endemic H. coccineus. The fifth species, H. dasycalyx is a rare endemic known only from a few
Table 1. Pertinent systematic and reproductive ecology traits of *Hibiscus* section *Muenchhusia* (from Blanchard 1976), five species of perennial herbs of freshwater and brackish marshes whose natural range appears to be confined to eastern North America. All are diploid with \(n=19\).

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<th>Species</th>
<th>Range</th>
<th>Flower color &amp; scent</th>
<th>Probable pollinators</th>
<th>Distinctive morphologic features</th>
<th>Included taxa</th>
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<tr>
<td>moscheutos</td>
<td>widespread: Mass., S. Ont., N. Iowa, &amp; E. Neb. s. to Fla. &amp; SW. Tex.</td>
<td>pink, or white, with or without red basal spot; scentless or faint &quot;mousy&quot;</td>
<td>mostly by specialist bee, <em>Ptilothrix bombiformis</em>, (N. pops are outside range of the bee.) <em>Bumblebees also are effective</em></td>
<td>closely related to <em>moscheutos</em> and similar in vegetative chars. Staminal column &gt; 5 cm, filaments sound</td>
<td>Subsp. <em>lacustris</em> (glabrous fruits, incl. <em>H. palustris</em>); Subsp. <em>laciscarpos</em> (hairy-fuited, incl. <em>H. incanus</em>)</td>
</tr>
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<td>grandiflorus</td>
<td>restricted, southern Atlantic and Gulf coast: s. S.C. to Fla., w. to La.</td>
<td>white to pale pink with red spot near base; night-blooming; heavily scented</td>
<td>sphinx moths, inferred from lg. fl size, night-blooming and aroma like that of <em>Datura</em>, a known sphinx moth flower</td>
<td>closely related to <em>moscheutos</em> cor pink or white; leaves hastately 3-lobed</td>
<td>no subsp. or previously recognized names of importance</td>
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<tr>
<td>laevis</td>
<td>widespread: s.e. Pa. thru n. Ohio and n. Iowa to e. Neb., s. to Texas, e. to n. Fla.</td>
<td>pink or white, with reddish basal spot</td>
<td><em>Ptilothrix</em>; butterflies may also be effective, esp. outside range of the specialist</td>
<td>plants glabrous, cor pink or white; seeds pubescent; leaves hastately 3-lobed</td>
<td><em>militaris</em> is the name employed in most manuals</td>
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<tr>
<td>cocineus</td>
<td>endemic: Northeastern Fl., mostly drainage of St. Johns R.</td>
<td>deep red, petals more wide-spreading than other species</td>
<td>swallowtail butterflies, based on very open flower that precludes effective insect poll., and direct observation</td>
<td>closely related to <em>laevis</em> and fairly sim. in veget. chars. lvs. deeply palmately lobed; corolla red</td>
<td>no subsp. or previously recognized names of importance</td>
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<td>dasycalyx</td>
<td>known only from eastern Texas (rare)</td>
<td>creamy white, with deep red basal spot</td>
<td>unknown: possibly <em>Ptilothrix</em>, which visits other local rose mallow flowers</td>
<td>hairy fruit; deeply lobed lvs., suggests <em>laevis</em> x <em>moscheutos</em> hybrid</td>
<td>no subsp. or previously recognized names. (described in 1958)</td>
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locations in eastern Texas. Based on the results presented in Chapter III of this dissertation, it is closely related to H. laevis and so belongs in group (2) above.

Flowers and pollinators—In addition to the differences in geographic distribution and genetic affinities within the group, members of section Muenchhusia are distinguished by their floral traits and pollination vectors. All species produce large bisexual flowers that normally bloom for one day. The stigmas and anthers mature simultaneously, but they are spatially separated, at least at first. The stamens and stigmas within each flower of H. moscheutos and H. grandiflorus are separated throughout the flower's one-day lifespan, so an insect vector is required for pollination. By contrast, the style-branches of some populations of H. laevis and probably all H. coccineus arch backwards at the end of the day and touch the staminal column, possibly effecting self-pollination if outcrossing has not already occurred. Although published accounts of pollination ecology are few and mainly incidental to studies of Hibiscus ecology or taxonomy, the following is known. Hibiscus moscheutos and H. laevis are both pollinated by bees. Over most of their range the primary pollinator is the "hibiscus bee," Ptilothrix bombiformis (Hymenoptera: Anthophoridae), a solitary bee whose life cycle is obligately tied to these flowers (Rust 1980). At the north portion of the range of H. moscheutos where Ptilothrix is
absent, and elsewhere during seasons when the specialist bee is scarce, bumblebees are the primary pollinator (Blanchard 1976, Spira 1989). *Hibiscus grandiflorus* flowers are most likely pollinated by night-active hawkmoths (Lepidoptera: Sphingidae). *Hibiscus coccineus* is serviced by day-active butterflies, including swallowtails (Lepidoptera: Papilionidae). Flower color partly parallels the pollinator distinctions: where *Ptilothrix* is present, i.e., much of the ranges of *H. moscheutos* and *H. laevis*, the predominant color is white with a dark magenta central spot. The occurrences of *H. moscheutos* in the bumblebee-only northern portion of its range consist almost exclusively of pure pink-flowered plants (Blanchard 1976). *Hibiscus grandiflorus* is white or pink and has a red center. It has an aroma identical to that of *Datura*, a known hawkmoth flower. The *H. coccineus* flower is crimson.

**Synonymy**—Variation in leaf pubescence, calyx texture, petal color and floral bract ciliation correlated with geographic location has caused the wide ranging of *H. moscheutos*, to encompass several forms that were earlier recognized as separate species. The names *H. moscheutos* (sensu stricta), *H. palustris*, *H. lasiocarpos* and *H. incanus* are encountered in most regional floras and hence may be more familiar to practicing botanists than the more conservative treatment of Blanchard (1976) that is followed here. In his view, *H. moscheutos* embraces all these forms
and is differentiated into two subspecies on the basis of fruit pubescence, namely H. m. moscheutos (glabrous) and H. m. lasiocarpos (pubescent). The subspecies lasiocarpos occurs in the southwestern portion of the range of the species. Hibiscus laevis is more commonly known as H. militaris. The remaining species are of restricted distribution and have no important synonyms.

Questions asked by the dissertation research—My research focussed on several representative causes and consequences of genetic variation in plants. The rose mallows were chosen as study subjects for the following reasons. Their production of especially large, insect-pollinated flowers which are nonetheless freely self-compatible suggested the possession of a mixed mating system. This rendered pertinent the question of whether or not inbred offspring are at a disadvantage, the topic of Chapter 1, Inbreeding Depression in Hibiscus moscheutos and H. grandiflorus, which also includes an estimate of the mating system at a natural population that confirms selfing and outcrossing co-occur. A remarkable taxis exhibited by the flowers of H. laevis in which the upper portion of the gynoecium arches basipetally, sometimes resulting in contact between stigmas and anthers sufficient to effect self-pollination, was studied in detail and is presented as Chapter 2, Facultative delayed autonomous self-pollination in Hibiscus laevis. That investigation included both an
assessment of the reproductive output of autonomous selfing compared with other modes of pollination, as well as a study of inbreeding depression to determine if there is an advantage to delaying selfing (and so give preference to insect-mediated outcrossing). The large ranges of two of the rose mallows, *H. moscheutos* and *H. laevis* allowed me to investigate whether the phenomena under consideration, namely inbreeding depression in *Hibiscus moscheutos* and autonomous selfing in *H. laevis*, vary substantially among populations. Finally, the occurrence of a very rare endemic rose mallow whose systematic relationship to its two widespread congeners had been questioned but never intensively studied prompted the research set forth as Chapter 3, *Genetic affinity of the rare eastern texas endemic, Hibiscus dasycalyx*. The core of that study is an isozyme analysis undertaken to test whether speciation may have occurred via diploid hybrid speciation, an uncommon process that involves the production of a derivative taxon having the same chromosome number as two parental taxa but is nonetheless reproductively isolated from them.

*Abstract of inbreeding depression study*—The reduced fitness of progeny produced from selfing is an evolutionary force that helps shape the mating system of many self-compatible plant species. The rose-mallows (genus *Hibiscus* section *Muenchhusia*) bear large, self-compatible, hermaphroditic flowers that bloom for one day and possess
simultaneously mature anthers and stigmas. An insect visitor is required for pollination and they appear to have a mixed mating system. In tests to determine whether inbreeding depression may have influenced the maintenance of features that foster outcrossing, progeny produced by inbreeding were compared to progeny derived from outcrossing in several populations of two *Hibiscus* species: *H. grandiflorus*, which is restricted in distribution to the southeastern Atlantic and Gulf coasts, and the widespread *H. moscheutos*. Both taxa exhibited inbreeding depression in two components of fitness expressed later in the life-cycle: seed germination (in a few instances only), and (more frequently) progeny growth. The selfing rate of one Ohio *H. moscheutos* population, estimated during 1993 and 1994 using introduced plants with a foreign electrophoretic genotype was 0.44 and 0.57 during the two years respectively. The populations that were more strongly affected by inbreeding tended to be the more southern ones, suggesting that the more northerly ones may have gone through bottlenecks and so been purged of the deleterious alleles and/or the beneficial polymorphisms as they re-established the region following glacial retreat. Inbreeding depression appears likely to be maintaining floral features which result in a substantial fraction of outcrossed offspring.
Abstract of autonomous selfing study—Autonomous self-pollination by plants that are also capable of outcrossing often supplements low levels of vector-mediated pollination without precluding outcrossing when pollinators are abundant. In most instances facultative selfing is a result of timing, wherein self-pollination takes place after the normal activity period of pollinators. Style recurvature that occurs during flower aging was seen in H. laevis. This appeared to effect selfing because the stigma lobes contacted the uppermost stamens. In a pollinator-free greenhouse all 12 plants from one representative population proved capable of selfing in this manner. Selfing was facultative not only through late timing but also because stylar movements were effectively absent if stigmas received pollen loads early in the day. Mean levels of autonomous fruit and seed set were substantially below those of hand-pollinated controls, a difference that may be partly due to the absence of any wind disturbance in the greenhouse. In a natural stand examined for five consecutive days about the middle of the flowering season, the mean seed set of flowers which were modified to prevent selfing was only slightly less than that of either unmanipulated or hand-pollinated controls. Thus autonomous selfing was relatively unimportant. The population exhibited a modest level of inbreeding depression in one of three tested components of fitness, consistent with the evolution of a mixed mating
system favoring xenogamy while assuring the potential to reproduce when pollinators are scarce. Small-scale greenhouse tests of additional populations revealed substantial variation in the ability to set fruit in the absence of pollinators, wherein representatives from six southern sites (among a total of 10 populations tested) had stamens and stigmas situated too far apart to allow unfacilitated pollination.

Abstract of endemic rose-mallow study—Hibiscus dasycalyx is a rare eastern Texas endemic that shares key morphologic traits with two widespread sympatric congeners, H. laevis and H. moscheutos. These taxa were initially suspected of being possible ancestors of the endemic through diploid hybridization speciation. A 10-enzyme electrophoretic screening was conducted to determine whether H. dasycalyx exhibits a hybrid genetic profile. The three taxa share predominant alleles for all enzyme systems except ADH, GPI and PGM, for which H. dasycalyx and H. laevis display generally identical banding patterns that differ from those of H. moscheutos. An analysis of diagnostic leaf shape and calyx pubescence features of H. laevis revealed substantial variation among populations, including forms intermediate between typical H. laevis and H. dasycalyx. In greenhouse hand-pollinations, H. dasycalyx plants serving as ovule parents were freely compatible with H. laevis. Owing to the possession of identical isozymes, the occurrence of
morphologically intermediate populations where the species co-occur, and the apparent lack of reproductive isolation, the endemic *H. dasycalyx* may be best regarded as a variety of *H. laevis*, the taxon it most resembles morphologically. Conservation efforts should consider the potential effects of contaminating gene flow with typical *H. laevis*. 
CHAPTER I
INBREEDING DEPRESSION IN HIBISCUS MOSCHEUTOS AND H. GRANDIFLORUS

Introduction

Inbreeding depression is typically defined as the reduced fitness of progeny derived from selfing compared to those derived from outcrossing (e.g. Dudash 1990). It can be a potent evolutionary force shaping the mating systems of plants. In the absence of inbreeding depression, it is expected that plant populations will evolve to be self-pollinated. This is because a gene for selfing will be strongly favored in a normally outcrossing population as selfing permits a doubling of the frequency of genome transmission through pollen, in comparison with a solely outcrossing member of the population (Lande and Schemske 1985). The evolutionary maintenance of floral traits that foster cross-fertilization in plants that are self-compatible is therefore attributed to a decrease in fitness if plants fertilize themselves. Of particular interest is whether a certain level of inbreeding depression is constant within a species or closely related species group, or whether it varies. Regardless of whether inbreeding depression is due to loss of heterosis (overdominance), or
to the fixation of recessive deleterious alleles (partial dominance), its magnitude can be expected to be greater in populations with a high overall level of heterozygosity (Charlesworth and Charlesworth 1987). Highly heterozygous populations tend to be those which have comprised large breeding units over long time periods. In contrast, populations that have gone through a "bottleneck" (severe reduction in numbers) may have been forced to inbreed through selfing, outcrossing with close relatives, or both (Leberg 1992). Consequently currently existing post-bottleneck populations, regardless of their size, are composed of surviving progeny which lack the polymorphisms required for heterosis (overdominance model of inbreeding depression), or that have been purged of deleterious alleles that would cause inbreeding depression (partial dominance model).

Empirical studies have demonstrated a correlation between inbreeding depression and mating system features that serve to reduce inbreeding. For example, Dudash (1990) found substantial levels of inbreeding depression in all life stages of *Sabatia angularis*, a few-flowered protandrous perennial herb that is presumably highly outcrossing. Holtsford and Ellstrand (1990) examined three populations of the annual herb *Clarkia tembloriensis* and discovered that inbreeding, which reduced fecundity, was greatest in the most outcrossing populations. Levin (1989) studied seed
abortion in both outcrossing and predominantly selfing annual Phlox species and determined that the number of lethal equivalent genes was substantially greater in the outcrossing taxa. Sakai et al. (1989) showed that inbreeding depression may be a primary selective force in the prospective evolution of complete dioecy in two partly dioecious woody shrubs (Schiedea spp.).

The present study compares the performance of self and outcross progeny from several populations of rose-mallows comprising two species. The purpose is to gain an overall measure of inbreeding depression in these taxa to afford a comparison with other species and determine whether its magnitude varies markedly between the species or among populations of a species. In three instances the seed set and progeny performance of open pollinated plants was assayed anticipating that it might be possible to infer the approximate natural selfing rate in these populations, wherein the fitness of open-pollinated progeny would more closely resemble the breeding system that gave rise to them (Charlesworth 1988).

An absolute measure of inbreeding depression is rarely achieved because of inherent difficulties in measuring fitness, although it would useful in testing evolutionary theory. The ability to transmit genes to subsequent generations includes components of survival and reproduction. To realistically represent natural
evolutionary conditions, survival would need to be tested under natural conditions including the stresses of environmental extremes, pathogens and predators that influence selection, while the assessment of fecundity requires growing plants at least until they reach reproductive maturity. To accomplish this economically within the time frame and resources generally available for evolutionary studies is often impracticable. Consequently most investigators have employed simulated environments (greenhouse and garden environments) and/or a surrogate measure of fecundity, biomass of the progeny, with an assumption that fitness values obtained from the simulations and surrogates replicate those that would occur naturally. The present study of *Hibiscus*, because it employs long-lived marsh perennials from several populations, required that progeny testing be carried out under fairly artificial conditions and so, rather than obtaining an absolute measure of inbreeding depression, I determined the stages of the life history at which readily detected inbreeding depression occurs, and, inasmuch as the test conditions were uniform, was able to compare populations.

The taxa--The genus *Hibiscus* (Malvaceae), includes five species of "rose mallow," contained within the section *Muenchhusia* (Blanchard 1976). They are marsh herbs, exclusively eastern North American in distribution and comprise the only native *Hibiscus* species over much of this
area. Unlike some other sections of the genus which are more heterogeneous, the rose mallows are all diploid, with \( n=19 \). This lack of genomic differentiation and their confinement to a continuous range that was largely glaciated during the Pleistocene suggests that they are very recently evolved. The two species studied here are believed to be closely related, based on morphologic evidence and cross-compatibility (Wise and Menzel 1971). They are the widespread \textit{H. moscheutos} and the restricted southern Atlantic and Gulf Coastal \textit{H. grandiflorus}. Both species produce large bisexual flowers that normally bloom for one day and are self-compatible (Blanchard 1976; Spira 1989). The stigmas and anthers become mature simultaneously but because of their spatial separation an insect visitor is required to effect pollination. \textit{Hibiscus moscheutos} is pollinated by bees. Over most of its range this is accomplished mainly by \textit{Ptilothrix bombiformis} Cresson (Hymenoptera: Anthophoridae), a solitary bee whose life cycle is obligately tied to these flowers (Rust 1980). However at the northern portion of the plant's range \textit{Ptilothrix} is absent. Here (and elsewhere when the specialist bee is scarce) bumblebees are the primary pollinators (Blanchard 1976; Spira 1989). \textit{Hibiscus grandiflorus} flowers open in the evening and are believed to be pollinated by hawkmoths (Lepidoptera: Sphingidae) (Blanchard 1976).
One population of *Hibiscus moscheutos*, located in Edgewater, Maryland, has been intensively studied in connection with an elaborate series of investigations into male gametophytic competition, one portion of which (Snow and Spira 1992) examined differences between selfed and outcrossed progeny. Studying the relative fitness of selfed progeny in four maternal families, significantly lower values were recorded for seeds per fruit (one family only, having about a 20 percent reduction), percent seed germination (the same one family, having about a 25 percent reduction), and seedling biomass (detected in three families, and ranging from approximately 20 percent to a 64 percent reduction). Moreover, except for one seed germination value that was equal to that of outcrossed seeds, all of the data for selfed progeny were lower than the corresponding ones for outcrossed progeny. Because it proved so effective, I adopted their progeny biomass assay technique of co-planting selfed and outcrossed seeds in small pots to better detect differences between them, a practice that would also allows a rough comparison of our results. Because the Mill swamp population is polymorphic in just one conveniently detectable electrophoretic enzyme (GPI), these investigators could not derive an estimate of the outcrossing rate either by a multilocus method (Shaw et al. 1982) or by the introduction of a foreign genotype (Schoen and Lloyd 1992). However, the high levels of
pollinator activity they observed at Mill Swamp, coupled with the limited potential for geitonogamy (typically only one or two flowers per branch bloom on a given day) fostered a belief that the outcrossing rates are probably high.

Materials and Methods

This investigation proceeded in two stages. The first, conducted 1991 through 1992, was an initial screening to determine which components of fitness are most subject to inbreeding depression in the two rose-mallow species, using *H. moscheutos* and two populations of *H. grandiflorus*. Second, during 1993 through 1994, I focused on the more widespread taxon, *H. moscheutos*, and compared five populations for differences in progeny growth, the component of fitness found to be most strongly and consistently influenced by breeding system (Table 2).

Initial screening pollination—Hand-pollination was conducted in July and August 1991 on naturally occurring plants in the following large populations of rose mallows: (1) *H. moscheutos* at the Winous Point Shooting Club in Ottawa County, Ohio, (2) *H. grandiflorus* at the Guana River State Park in Duval County, Florida and (3) *H. grandiflorus* at the Bayou Suavage National Wildlife Refuge in Tammany Parish, Louisiana. Over the course of four days at each site eight flowers per cross type were selected on 8-10 haphazardly chosen, widely separated ovule parents. Natural
pollinators were excluded from treatment flowers with nylon mesh bags placed over buds the day before anthesis. For outcross pollinations, flowers were hand-pollinated with a total of 2-4 pollen donors selected haphazardly from the population, by gently rubbing the stigmas with the staminal column of a flower picked from each pollen donor. Within-flower self pollination was performed on other flowers immediately afterward by gently rubbing stigmas with the anthers of several stamens excised with forceps. For the *H. moscheutos* population and the Florida *H. grandiflorus*, mesh bags were placed on the developing fruits to retain all of the seeds, and an open pollination treatment was added by bagging naturally-pollinated fruits of about the same age as the hand-pollinated ones. Fruits were harvested four weeks after the pollinations were performed. Data were collected on fruit set (the fraction of flowers that set fruit) and seed set (seeds per fruit).

**Initial screening progeny testing**—Seeds were planted for greenhouse and garden progeny testing in May 1992. For each ovule parent and cross type a total of 96 seeds, were nicked to effect rapid germination and planted in dry peat-based growth medium in 10 cm³ cubical plastic pots. The initial planting arrangement was four seeds per cell of each cross type, randomly arranged by cross type. The trays were continuously watered until initial seedling emergence, 48-72 hours after planting. Approximately 7 days after planting,
Table 2. Inbreeding depression population locations and their respective studies (IS= initial screening; COP= comparison of populations).

**H. grandiflorus**
- **Florida**: St. Johns County; Diego Plains. Riparian marsh adjacent to Guana River (IS).
- **Louisiana**: St. Tammany Parish; Slidell. Lacustrine bayou adjacent to Lake Ponchetrain (IS).

**H. moscheutos**
- **Texas**: Douglass County; Nacogdoches. Isolated wet pasture (COP).
- **Texas**: Trinity County; Riverside. Riparian marsh adjacent to Trinity River (COP).
- **Ohio**: Athens County; Waterloo. Riparian marsh adjacent to Rock Camp Creek (COP).
- **Ohio**: Ottawa County; Port Clinton. Lacustrine marsh adjacent to Sandusky Bay (IS, COP).
- **Ohio**: Pickaway County; Walnut. Lacustrine marsh adjacent to Stages Pond (COP).

When germination was complete but before competitive effects could take place, the seedlings were randomly thinned to one per cell and simultaneously counted to assess germination rates. After 6-8 weeks growth in the greenhouse (the population plantings were timed one week apart, but each population was completely planted in one day), 12 plants of each cross type per ovule parent were randomly selected and transplanted to an outdoor plot at the OSU Horticultural Farm in Columbus Ohio for the remainder of the growing season. Their above-ground parts were harvested in early October, dried, and subsequently weighed. Data were pooled from the 8-10 ovule parents of each population.

**Among-population comparison pollination**—For five **H. moscheutos** populations pollinations were performed either
in natural marshes (three Ohio populations: Winous Point Shooting Club, Ottawa County; Grubb Marsh, Athens County, and Stages Pond Nature Preserve, Pickaway County) or in a garden plot located in Columbus Ohio (comprising plants collected from two eastern Texas populations: bank of the Trinity River in Riverside, Trinity County, and an inland pasture in Nacogdoches, Douglas County). In the natural marsh populations the pollinations were carried out as described above for the initial screening. The two garden-grown populations consisted of 11-12 plants, each grown from seed harvested from separate wild individuals. Four plants served only as pollen parents for the remaining 7-8 plants which served as ovule (and self pollen) parents. In all other respects the garden pollinations were carried out in identical fashion to the initial screening described above.

Among-population comparison of progeny performance—Data pertaining to breeding system effects on the production of progeny were limited to seed set, as fruit set was essentially 100% and the few instances of fruit abortion appeared to be attributable to incidental damage to the flowers. For progeny testing, seeds of each ovule parent plant were pooled using 1-2 fruits from each outcross donor and an approximately equal number of selfed fruits. For each ovule parent and cross type a total of 96 seeds was nicked and planted as described above, except that (1) it occurred in late autumn and winter and in the greenhouse
(supplemented with high intensity artificial illumination) and (2) the initial planting arrangement of four seeds per cell included complementary cross types situated diagonally from one another to enhance, through competition, differences in growth between self and outcross progeny (as per Snow and Spira 1992). Approximately seven days after planting the self/outcross seedling pairs were randomly thinned to one pair per cell and simultaneously counted to assess germination rates. The plants required daily watering that included, every third day, fertilizing with an aspirated liquid NPK fertilizer plus micronutrients. After 53 days of growth in the greenhouse the above-ground parts were harvested and weighed immediately.

**Natural selfing rate**—At the Pickaway County population of *H. moscheutos* the natural selfing rate was determined by introducing mature plants having a unique GPI genotype and assaying electrophoretically the genotype of open-pollinated seeds. The source of the transplants was the Ottawa County population, where the predominant GPI isoform is the faster-migrating of the two cytosolic alleles that have been detected in Ohio populations. Five multiple-stemmed plants with abundant flower buds (later confirmed by electrophoresis to be homozygous for the fast allele) were dug up in mid-June 1993, and transplanted a few days afterwards into the Pickaway County marsh. The genetic constitution of the population there was determined the
previous autumn using an electrophoretic analysis of 50 seeds gathered from separate plants dispersed across the marsh. All were homozygous for the slower GPI allele, hence I assume the population is monomorphic for that enzyme system.

The introduced plants were placed in a densely populated portion of the stand and positioned greater than 30 m apart, hence their contribution to the pollen pool was negligible. Fruits were collected in October 1993. Simultaneous hand-pollinations were performed on the alien plants to test for cross-compatibility (see Results section for details).

Statistics—For categorical data (fruit set and germination frequency) the chi-squared statistic was employed. Differences between means (seed set and progeny mass) were analyzed using t-tests, either unpaired (as for seed set in both experimental stages and for progeny growth in the initial screening, where cross types were grown separately), or paired (for progeny mass in the comparison of populations, where individuals from both the cross types were grown together in pots to effect competition). Among-population differences in progeny biomass were tested with an analysis of variance followed by a Duncan comparison of means.
Results

Initial screening seed set--Breeding system did not significantly affect the number of seeds per fruit in any population (Fig. 1). Seed set was strongly and significantly pollinator-limited in the Florida *H. grandiflorus* population during the apparently normal (i.e., mid-season of blooming, equable weather) four-day period of study, with open pollinated fruits containing 62% as many seeds as the hand-pollinated ones (ANOVA: $F = 30.1, p < 0.0001$). During 8 early evening hours pollinating these flowers only one hawkmoth was observed, although small moths were frequently seen at the base of the corolla feeding on nectar, approximately six cm below the stigmas. It did not appear that these small moths were pollinating the flowers. However, at the Louisiana *H. grandiflorus* population as well only one hawkmoth visit was observed during about 8 hours of study, but here full seed set occurred. Bumblebees were observed as frequent nectar-feeding visitors to the *H. moscheutos* flowers and it was apparent they transferred pollen during a fraction of the visits by contacting the stigmas when approaching the blooms.

Initial Screening Seed germination--Germination frequency was generally high (89% or greater). In all instances self pollinated seeds germinated slightly less than the other treatments, but this difference was statistically significant only for the Florida
Figure 1. Performance of selfed progeny relative to those produced by outcrossing for Hibiscus grandiflorus (Tammany and Duval populations) and H. moscheutos (Ottawa population). Sample sizes, parameter values and significance levels denoted by asterisks are shown on Table 3.
**H. grandiflorus** population (Chi-square = 16.60, p = 0.0002) (Table 3, Fig. 1).

**Initial screening progeny growth**—The progeny of selfed seeds slightly but significantly under-performed outcross ones for both **H. grandiflorus** populations (ANOVA: Florida F = 6.45, p = 0.0018; Louisiana F = 2.39, p = 0.0944) but not

<table>
<thead>
<tr>
<th>Species and Population</th>
<th>Mean number of seeds per capsule (N, SE)</th>
<th>Percent of seeds (%)</th>
<th>Percent germination (N)</th>
<th>Mean biomass (N, SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. grandiflorus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOUISIANA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>self</td>
<td>ND ND</td>
<td>90 (540)a</td>
<td>14.8 (74, 0.9)a</td>
<td></td>
</tr>
<tr>
<td>outcross</td>
<td>ND ND</td>
<td>92 (480)a</td>
<td>17.5 (79, 0.9)b</td>
<td></td>
</tr>
<tr>
<td>natural</td>
<td>ND ND</td>
<td>93 (576)a</td>
<td>15.8 (70, 0.9)ab</td>
<td></td>
</tr>
<tr>
<td>FLORIDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>self</td>
<td>46 (76)</td>
<td>125 (30, 4.4)a</td>
<td>32.1 (106, 1.6)a</td>
<td></td>
</tr>
<tr>
<td>outcross</td>
<td>55 (83)</td>
<td>135 (43, 3.7)a</td>
<td>40.2 (96, 2.0)b</td>
<td></td>
</tr>
<tr>
<td>natural</td>
<td>ND</td>
<td>81 (30, 7.6)b</td>
<td>33.0 (101, 1.6)a</td>
<td></td>
</tr>
<tr>
<td><strong>H. moscheutos</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHIO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>self</td>
<td>97 (38)</td>
<td>115 (28, 4.6)a</td>
<td>16.8 (91, 0.9)a</td>
<td></td>
</tr>
<tr>
<td>outcross</td>
<td>98 (42)</td>
<td>123 (55, 2.8)a</td>
<td>18.7 (100, 0.9)a</td>
<td></td>
</tr>
<tr>
<td>natural</td>
<td>ND</td>
<td>122 (33, 5.8)a</td>
<td>16.6 (101, 0.8)a</td>
<td></td>
</tr>
</tbody>
</table>

for the **H. moscheutos** (Table 3, Fig. 1). The performance of open pollinated plants varied but was not significantly different from either self or outcrossed ones and so these data were not useful to infer natural selfing rates.
Initial screening overall performance—Inbreeding performance at each life stage is expressed as the self/outcross ratio of each pertinent fitness parameter. Total inbred progeny performance was measured by a multiplicative fitness function consisting of one minus the product of the inbreeding performance values from each life stage (Schemske 1983). The three populations were compared using only parameters for which statistically significant differences between self and outcross progeny were detected (non-significant self/outcross ratios were set to unity), and fruit set was excluded because it was not determined for the Louisiana H. grandiflorus population (Table 3). The multiplicative fitness values are: Florida H. grandiflorus, 0.76; Louisiana H. grandiflorus, 0.85; Ottawa County Ohio H. moscheutos, 1.00.

Comparison of five H. moscheutos populations—Self and outcross progeny performance was assayed and compared for seed set, germination frequency, and biomass achieved under modestly competitive conditions imposed by being situated in self/outcross pairs per pot. The two Texas populations showed a slight significant decrease in germination frequency caused by inbreeding, and all five populations showed breeding system effects on biomass, ranging from 92.9% (Ottawa) to 71.7% (Trinity), compared with outcrossing (Table 4; Fig. 2). In multiple means comparisons the reduction in biomass suffered by both Texas populations was
Table 4. Performance of progeny produced by self and outcross pollinations on five populations of *Hibiscus moscheutos*. Statistically significant effects are denoted by * = \( p<0.05 \), ** = \( p<0.01 \), and *** = \( p<0.001 \).

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean no. Germination, ( (N, SE) )</th>
<th>Mean Biomass, ( (N) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TEXAS</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>DOUGLASS</strong></td>
<td>self 90.7 (30, 3.0) 75.2 (659) 5.31 (421, 0.14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>outcross 87.3 (27, 2.8) 80.2 (880)* 8.86 (421, 0.18)***</td>
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</tr>
<tr>
<td><strong>TRINITY</strong></td>
<td>self 91.3 (45, 2.2) 80.0 (864) 7.21 (407, 0.15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>outcross 90.0 (47, 2.1) 85.8 (864)* 10.06 (407, 0.17)***</td>
<td></td>
</tr>
<tr>
<td><strong>OHIO</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ATHENS</strong></td>
<td>self 121.8 (58, 2.0) 87.8 (864) 6.22 (428, 0.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>outcross 122.3 (76, 2.7) 89.2 (864) 7.53 (428, 0.13)***</td>
<td></td>
</tr>
<tr>
<td><strong>PICKAWAY</strong></td>
<td>self 92.4 (45, 1.9) 95.0 (864) 9.43 (420, 0.16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>outcross 92.1 (41, 2.2) 96.8 (864) 10.66 (420, 0.15)***</td>
<td></td>
</tr>
<tr>
<td><strong>OTTAWA</strong></td>
<td>self 121.4 (50, 1.8) 97.9 (864) 12.37 (426, 0.20)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>outcross 122.1 (54, 1.9) 96.1 (864) 13.32 (426, 0.21)**</td>
<td></td>
</tr>
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</table>

significantly greater than that of all three Ohio ones \( (p<0.05) \), but within each state the among-population differences were not significantly different.

**Natural selfing rate**--From fruits produced during the 1993 flowering season, a total of 84 seeds from the five alien plants were assayed (one per fruit), and the selfing rate (proportion of homozygotes), was 0.44, with an asymptotic variance of 0.003, calculated using the methods of Schoen and Lloyd (1992). In 1994 122 seeds were similarly sampled from four of the plants, and the selfing rate that year was 0.57, with a variance of 0.002. Those
authors caution that, when using the unique genotype method for estimating breeding systems, there must be no background effects associated with the unique allele or cross-incompatibility that would influence the estimation. To determine whether any such confounding factors were in effect, I pollinated a total of 13 flowers distributed among three of the five foreign plants with equal mixtures of self (foreign) and outcross (alien) pollen and determined the electrophoretic genotypes of 293 of the resultant seeds. The homozygote/heterozygote ratio was 0.82, not significantly different from the expected value of unity (chi square = 1.42, p= 0.24), thus serving to confirm the absence of significant background effects or cross-incompatibility.

Discussion

In the initial screening modest levels of inbreeding depression were detected in the three rose-mallow populations studied, ranging from an approximately 20% average reduction in fitness upon selfing for H. grandiflorus, to a lack of detectable depression in the sole H. moscheutos population. The subsequent assay detected statistically significant levels of inbreeding depression in all five H. moscheutos populations, in some instances (the Texas populations) nearly approaching the 50 percent level considered sufficient to prevent the evolution
Figure 2. Performance of selfed progeny relative to those produced by outcrossing for five populations of Hibiscus moscheutos. Sample sizes, parameter values and significance levels denoted by asterisks are shown on Table 4.
of self-fertilization (Lande and Schemske 1985). Nevertheless I consider the observed levels of inbreeding depression to be comparatively minor because they are confined to later stages in the establishment cycle of plants --germination and growth --and so act mainly to diminish rather than eliminate an affected inbred individual's potential for establishment. Several other inbreeding depression studies have shown earlier acting and sometimes more multiplicative effects. In Allium schoenoprasum, for example, paternal fecundity, seed viability, seedling growth and seedling survival were all greater from outcross matings (Stephens and Bougourd 1988). In all three species of Costus that he tested, Schemske (1983) found that self pollinations resulted in lower seed production, which he attributed as probably due to the death of embryos homozygous for recessive lethals. Similarly, selfing caused moderately higher levels of seed abortion in selfed Phlox drummondii (Levin 1989), and Clarkia tembloriensis (Holtsford and Ellstrand 1990).

The restriction of inbreeding symptoms to later stages in a plant's life cycle appears to be another common pattern. In a study of Gilia achilleifolia that included data on six sequentially expressed life history parameters, Schoen (1982) found that seedling survivorship was the only one which contributed greatly to inbreeding depression. Similar findings were obtained for two species of Lobelia
(Johnston 1992), and *Hydrophyllum appendiculatum* (Wolfe 1993).

In these instances where breeding system mainly affects progeny vigor and associated fecundity, the environment may play a key role in governing the immediate impact of inbreeding. Many researchers have discovered that more stressful progeny growth regimes enhance the deleterious effects of selfing (Schemske 1983; Dudash 1990; Wolfe 1993). From this the converse can be deduced: for plants associated with particularly stress-free situations such as those having low levels of intra-specific competition, inbred progeny may have ample opportunity to transmit their genes to subsequent generations. Many wetlands are characterized by cyclical flooding that periodically eliminates much of the surface vegetation (van der Valk 1981). The comparatively few marsh species, including the rose mallows, which regenerate solely by seed may therefore typically become established, and live to reproductive maturity, under conditions of low competition. They have evolved a "ruderal" strategy (sensu Grime 1977) which emphasizes rapid growth and a short time to reproductive maturity, in preference to features which would strengthen competitive ability. Lacking strong competitive effects in the marsh environment, it is likely that the modest, late-acting effects of inbreeding that recorded in these short-term greenhouse assays is not an underestimate, and may in fact
be an over-estimate, of the effective levels of inbreeding depression which have evolutionary consequences.

Among the five *H. moscheutos* populations studied, significant reductions in biomass upon selfing were more pronounced for the two populations from the southwest portion of the range of the species. Moreover, these two populations were the only ones that displayed inbreeding depression for germination frequency. To these two sites may be added the results of the Edgewater, Maryland population similarly examined by Snow and Spira (1993) showing, for some maternal families at least, even greater inbreeding depression. The few previous studies that examined inbreeding depression in multiple populations, e.g. Holtsford and Ellstrand's (1990) *Clarkia* investigation and Levin's (1989) work with *Phlox*, there was no basis to infer the presence of a geographic pattern to the observed among-population variation.

For the subject *Hibiscus* populations (although the number of sites is too small to apply statistical tests), the distribution of results is consistent with a hypothesis that *H. moscheutos* populations north of the glacial boundary are generally less prone to inbreeding depression. Since a lowering of levels of inbreeding depression is one expected consequence of a reduction in overall genetic variation (Charlesworth and Charlesworth 1987), explanations of the pattern necessarily evoke a scenario of population
bottlenecks. Historical factors could account for this, as migration and establishment in colonizing species (marsh plants included) often involve a small number of immigrants, resulting in a loss of genetic variation through sampling effects (Barret 1989). The progenitors of the northern rose mallow populations may have undergone numerous bottlenecks in the process of colonizing the Great Lakes/midwest region after glacial retreat. For the purely pink-flowered plants which occur on the U.S. shores of the lower Great Lakes, a particular migration route is suspected. Those plants are morphologically identical with northern Atlantic Coastal ones, with which they were for several decades regarded as a separate species (H. palustris). A strikingly high number of shoreline plants similarly occur both along the margins of Great Lakes and the Atlantic coast, a fact which led Peattie (1922) to posit the existence, of a riparian corridor from the Atlantic Coast to the borders of the post-glacial Great Lakes along what is now the Hudson and Mohawk River systems. Peattie inexplicably omitted any mention of rose mallow in his discussion or list of species, but Blanchard (1976) regarded it a likely member of the referenced migration contingent. The ancestry of the southern Ohio populations is unknown, but inasmuch as they exhibit a variety of flower colors, it is apparent they are not simple direct descendants of the Great Lakes plants. Based on an examination of their first appearances in pollen
records, Vesper and Stuckey (1977) inferred two routes for the re-invasion of six selected submersed aquatic species -- the aforementioned eastern one and also a southern one from the Mississippi Valley. Perhaps the latter recolonization pathway was used by the progenitors of the southern Ohio rose-mallow populations.

During glacial maxima extensive areas similar to current rose mallow habitats may have existed in backwater marshes and embayments. However, as *Hibiscus* is primarily a tropical genus (Mabberly 1987) and the rose mallows in Ohio are near the northern limit of their range (despite the frequent occurrence of marshlands much farther north), it appears they would have been able to survive only at some considerable distance from the glacial boundary. The phytogeographic history of this species includes: (1) ice coverage of part of its range during the Wisconsin glaciation, (2) the contemporary restriction of southern Ohio marshes to fairly small and isolated areas, and (3) the likelihood, based on an apparent sensitivity to cool temperatures, that re-invading populations had to traverse a substantial distance south of the glaciated area (a region similarly poorly endowed with wetlands) in the process of returning to the region. Consequently it is probable the progenitors of the current Ohio populations encountered numerous sequential population bottlenecks during that time.
Shifts in a species range have a recognized power to alter gene diversity. Pleistocene migration may have played a significant role in the population genetics of many eastern North American and European plants. Lagercrantz and Ryman (1990) examined 22 electrophoretic loci in 70 populations of *Picea abies* and found central European populations to be consistently depauperate of genetic variability, which they attributed to severe restrictions in population size during the last glaciation. A similar pattern was found for a western North American conifer, *Pinus monticola* (Critchfield 1984). A well known extreme case is that of the *Pinus resinosa*, a fairly widespread tree of north-central to northeastern U.S. and adjacent Canada that possesses little or no genetic variation, an apparent consequence of confinement to a small isolated area during the Pleistocene Era (Fowler and Morris 1977). Among the several angiosperm examples of this pattern, Lewis (1991) compared allozyme variation between widespread and extreme southeastern U.S. endemic *Polygonella* species and discovered much less variation in the widespread ones. He attributed this disparity to substantial mass migrations undergone by the wide-ranging taxa (the endemics merely experienced range contractions). Other species with populations in glaciated areas that display comparatively low levels of genetic variation that have been attributed to postglacial migration from refugia in unglaciated areas are *Sullivantia* spp.
(Soltis 1982), *Cirsium pitcheri* (Loveless and Hamrick 1988) and *Silene regia* (Dolan 1994). In similar fashion the vegetational history of the northern populations of *H. moscheutos*, may have suffered a loss of genetic diversity that underlies the markedly lower levels of inbreeding depression reported here.

Several alternative or possibly additional factors may account for the perceived geographic distribution of variation in inbreeding depression. Being near the northern edge of its distribution, the Ohio marshes are likely ecologically marginal as well, with only a narrow range of genotypes adapted for life there (Mayr 1970). Observations of reduced genetic variation in ecologically marginal populations of *Pinus jeffreyi* (Furnier and Adams 1986), *Hordeum jubatum* (Schumaker and Babble 1980) and other species have been attributed to directional selection. *Hibiscus moscheutos* populations in eastern Texas, which may be considered ecologically central in this context, exhibit substantial morphologic variability lacking in the Ohio portion the species range (Blanchard 1976); some regional floras (e.g., Correll and Johnston 1970) have recognized three sympatric Texas species within the scope of modern *H. moscheutos*. Loss of the deleterious alleles responsible for inbreeding depression may have occurred concomitant with the overall reduction, by selection, of the genotype to the single form adapted for existence in these marginal
areas. Yet another factor might have been a shift in pollinators, or occasional scarcities of them. The specialist bee *Ptilothrix bombiformis* that is a primary pollinator over most of the range of *H. moscheutos* does not occur in Ohio, where instead bumblebees are the only important pollinators (Blanchard 1976). Data are lacking regarding the foraging patterns of the two types of bees, and there is no hard evidence for any autonomous selfing in this species. Nonetheless a generally higher level of inbreeding could result from sole reliance on a possibly scarce bee that has not co-evolved closely with the *Hibiscus* flower, which in turn may have caused decreases in heterozygosity that enhanced the purging of deleterious alleles from the affected populations.

Finally, the selected Ohio marshes may merely be more isolated and/or short-lived than the Texas ones, and hence their rose mallow populations may have passed through bottlenecks with the same genetic consequences as those described above, but of more recent occurrence and without the involvement of glaciation.

The finding of significant among-population differences in the degree to which fitness is reduced upon selfing depression may be associated with various historic and genetic phenomena. Direct measures of the levels of variation in these and other nearby populations are warranted to further our understanding of historic and
population genetic correlates of variation in this important trait.

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Johnston, M.O. 1992. Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and


CHAPTER II
FACULTATIVE DELAYED AUTONOMOUS SELF-POLLINATION IN HIBISCUS LAEVIS

Introduction

Mixed plant mating systems that combine selfing and outcrossing in substantial proportions are fairly widespread despite potentially great advantages to selfing. This is apparently so because inbreeding depression reduces the fitness of selfed offspring and therefore promotes the development of outcrossing. In these cases selfing occurs as an unavoidable accompaniment to outcrossing or as an alternative means of fertilization when pollinators are scarce (Lloyd and Schoen 1992). Geitonogamy, cleistogamy combined with chasmogamy, and vector-facilitated self-pollination are common mechanisms leading to mixed mating (Richards 1986). Apparently less frequent (judging by the scarcity of examples) is autonomous self-pollination, i.e., that which occurs in flowers that are also capable of outcrossing but in which the selfing occurs without the participation of an external agent. Autonomous selfing has been classified by Lloyd and Schoen (1992) into three modes, termed "prior," "competing," and "delayed," according to the timing of the intervals during anthesis when opportunities
for selfing take place relative to outcrossing. Each mode has typical mechanisms of action and an expected magnitude of selfing relative to outcrossing. Prior selfing, exemplified by bud pollination, results in the lowest outcrossing rates. Competing selfing, an intermediate mode with respect to outcrossing rate, can occur for example in flowers with anthers and stigmas in close proximity during the normal period of anthesis. Delayed selfing occurs at the end of anthesis and so is least likely to displace outcrossing. It occurs from flower movements such as (1) the late curling of stylar branches such that the stigmatic surface touches pollen deposited on non-stigmatic areas of the style, as in many Campanula species (Faegri and van der Pijl 1979), (2) stamens bending upwards late in flowering, causing anthers to collide with the stigma, as in Kalmia latifolia (Rathcke and Real 1993), and (3) stigmas being brushed by the anthers of epipetalous stamens during corolla abscission, as in Mimulus guttatus (Dole, 1990). Agren and Schemske (1993) present two unusual reports of selfing in monoecious species, Begonia hirsuta and B. semiovata. In these plants the male and female flowers are small and paired within the inflorescence, with each male flower positioned above a female one. Selfing in B. hirsuta is "active," wherein the dehiscing anthers are moved into direct contact with the receptive stigma, while in B. semiovata it apparently is achieved merely through pollen
falling onto stigmas from the anthers positioned directly above them.

Delayed autonomous selfing is advantageous in that it assures seed production when pollinators are scarce, yet allows outcrossing to predominate when they are abundant (Wyatt 1983). If vector-mediated outcross pollination occurs substantially before autonomous self pollination, outcross fertilization may predominate owing to the time advantage enjoyed by earlier-deposited pollen grains. In such a model autonomous delayed self-fertilization is facultative although the self pollination will occur regardless of prior outcrossing (assuming that ample amounts of pollen and receptive stigma area exist at the end of anthesis). A phenomenon in which the pollination per se is facultative can be imagined, namely one where the facilitating movements of flower parts take place only in the absence of prior vector-mediated pollination.

I tested for facultative delayed self-pollination in the smooth rose-mallow Hibiscus laevis All. Like many members of the mallow family, these flowers possess numerous stamens that are monadelphous, forming a tube, the base of which is united with the base of the corolla. The style ends in five short branches with capitate stigmas. After anthesis, which in this species lasts one day, the corolla, stamen tube and upper portions of the gynoecium are shed together. Older flowers often exhibit recurved styles, sometimes with
stigmas contacting stamens (Fig. 3, "Scioto") Stephens (1948) described the stylar movements of *Hibiscus laevis* as a mechanism to foster outcrossing by positioning the stigmas in the flight path of nectar-seeking bumblebees subsequent to a predominantly male phase of anthesis when the stigmas are held together, projecting forward. By contrast Blanchard (1976) interpreted the movement of the styles rose-mallow as a means to effect selfing, citing personal observations that the stigmas recurve into the anther mass toward the end of the day. During field studies at five populations in eastern Texas it became apparent to me that populations there generally have flowers which, owing to herkogamy (i.e., anthers and stigmas are spatially separated), probably cannot self-pollinate in an autonomous fashion (Fig. 3, "Neches").

The present set of experiments was undertaken to determine if autonomous delayed self-pollination is possible within a representative population of *H. laevis* in which it was suspected to occur, as well as to provide some details of its mechanism, reproductive potential compared with abundant hand-pollination, and possible importance during one part of a flowering season. Further, the performance of self and outcross pollination are compared with respect to seed production and progeny performance, to determine if the population is subject to inbreeding depression. The occurrence of inbreeding depression would affirm there is
Figure 3. Typical flowers of *H. laevis* about mid-day after anthesis. "Scioto" was photographed August 13, 1991 at a natural population along the Scioto River in Concord, Delaware County, Ohio from which the 1993 greenhouse study population was derived. "Neches" was photographed July 24, 1992 along the Neches River in Wakefield, Angelina County, Texas, one of the 10 populations examined in the 1994 greenhouse study of variation in the capacity for autonomous selfing.
a potential evolutionary advantage to the maintenance of aspects of floral biology that govern a shift toward the production of outcrossed offspring when pollinators are active. Finally, several populations were assayed to determine whether the ability to self is a uniform trait of the species or instead varies from place to place across the range of the species.

Materials and methods

Autonomous self-pollination: greenhouse tests--In 1993 an experiment was performed on 12 Hibiscus laevis plants situated in a pollinator-free greenhouse on the OSU campus. The test plants were grown from seed harvested off separate wild individuals occurring alongside the Scioto River in Concord, Delaware County, Ohio. Between 28 June and 22 July 1993 13-26 flowers on each test plant were assigned alternately to one of two experimental treatments: (1) pollinated by gently brushing all five stigma lobes with stamens excised with forceps from the same flower, and (2) unpollinated. Hand pollination was carried out between 9:00 a.m. and 1:00 p.m. To confirm that unpollinated fruit set was due to autonomous selfing, during the final week of treatments I removed pollen with a vacuum apparatus from all anthers situated in the distal one cm of the stamen-tube on 19 flowers distributed among most of the experimental plants. Fruits were harvested in mid-August and their seeds
were subsequently counted. During the experiment I noticed that curvature was more pronounced in the unpollinated styles than in those which were hand-selfed, i.e., delayed autonomous self pollination appeared to be facultative in these plants, occurring only in the absence of prior facilitated pollination. To confirm and quantify this phenomenon, on 46 flowers about equally divided between pollinated and unpollinated ones I inserted a pin through the calyx and base of the corolla, causing the latter to remain associated with its treatment flower while petals collapsed and abscission of the style and corolla proceeded normally. Late in the day after flowering, each pinned flower was removed and scored with respect to the number of stigmas touching anthers.

To assess whether the capacity for autonomous self-pollination is a constant feature of *H. laevis* or instead varies among populations, in 1994 1-2 plants each (a low sample size imposed by space limitations) from 10 populations of *H. laevis* were grown from seed to reproductive maturity in the greenhouse (Table 5). Throughout most of the flowering period (June through September for the group as a whole), flowers were alternately hand self-pollinated or unmanipulated and the resultant fruit set tabulated.
Table 5. Locations of the 10 H. laevis populations represented in the greenhouse test of variation in the capacity for autonomous selfing.

**OH**: Ohio; Ottawa County; Oak Harbor
**IL**: Illinois; Lawrence County; Lawrenceville.
**AR**: Arkansas; Faulkner County; Conway.
**OK**: Oklahoma; Tulsa County; Tulsa.
**LA**: Louisiana; Oachita Parish; Monroe.
**MS**: Mississippi; Sunflower County; Doddsville.
**T1**: Texas; Gregg County; Gregg.
**T2**: Texas; Angelina County; Wakefield.
**T3**: Texas; Lee County; Old Dime Box.
**T4**: Texas; Chambers County; Eminence.

**Autonomous selfing: field study**—To assess whether autonomous selfing may contribute to the reproductive output of a natural stand of plants, during August 1-5 1994 (about the peak of blooming) I manipulated and labelled flowers on 12 plants at the Delaware County, Ohio population. Each day a total of three flowers per plant was assigned to these treatments: 1) unmanipulated (natural pollination), 2) hand self-pollinated, and 3) emasculated (as described for the greenhouse study) to prevent autonomous selfing. Four weeks later, fruits were harvested and their seeds counted.

**Inbreeding depression**—Hand-pollination was conducted during June and July 1993 in the OSU greenhouse using 12 plants grown from seeds harvested off the same wild individuals which gave rise to the plants employed in the autonomous selfing experiment. Four plants served only as pollen parents for 8 plants which served as ovule (and self pollen) parents. For outcross treatments, flowers were hand-pollinated by gently rubbing the stigmas with the
staminal column of a flower picked from one pollen donor. Throughout the experimental period, each ovule parent received self and outcross pollinations alternately, and the outcross treatments rotated among donors. Within-flower self pollination was performed by gently rubbing stigmas with the anthers of several stamens excised with forceps. Mature fruits were harvested four weeks after the pollinations were performed. Data were collected on seed set (fruit set approached 100% and the few instances of fruit abortion appeared to be attributable to accidental injury to the flowers). For progeny testing, seeds of each ovule parent plant were pooled using 1-2 fruits from each outcross donor and an approximately equal number of selfed fruits. For each ovule parent and cross type a total of 96 seeds were nicked to effect uniform and rapid germination and planted in peat-based growth medium in 10 cm² cubical plastic pots located in the OSU greenhouse under artificial illumination supplemental to natural sunlight. The initial planting arrangement was four seeds per cell, with two self seeds and two outcross seeds in each pot. The trays were continuously watered until initial seedling emergence, 48-72 hours after planting. Approximately 7 days after planting, when germination was complete but before competitive effects could take place, the self/outcross seedling pairs were randomly thinned to one pair per cell and simultaneously counted to assess germination rates. After 53 days of
growth in the greenhouse the above-ground parts were harvested and weighed immediately.

Results

Autonomous selfing; greenhouse tests—None of the 19 emasculated flowers set fruit, indicating that fruit set by the experimental flowers resulted from the treatments rather than apomixis or accidentally introduced insect visitors. All but one of 104 hand-pollinated flowers set fruit, suggesting that complete fruit set is potentially possible in this species and that any observed failure to set fruit resulted from insufficient pollination rather than the production of excess of flowers as sometimes occurs in other taxa, for example Catalpa (Stephenson 1979) and Asclepias (Willson and Price 1977). All 12 experimental plants produced fruits autonomously, but fruit set and mean seed set were lower than the hand-pollinated controls. Fruit set ranged from 14% to 100% among the plants (Fig. 4), producing a total of 85 fruits from 132 unmanipulated flowers (64%). The average number of seeds within these fruits was moderately and significantly lower than that obtained from self pollination ($t = 8.29; P < 0.0001$) (Fig. 5). Contrary to expectations based on the fact that lower levels of fruit and seed set are both evidently caused by weak stigma-anther contact, there was no significant correlation between autonomous fruit set and seed set ($r = 0.29, P = 0.35$).
Figure 4. Percentage fruit set and mean seed set of pollinator-excluded flowers on 12 H. laevis plants grown in the 1993 greenhouse study of a Delaware County, Ohio population.
Figure 5. Percentage fruit set and mean seed set of pollinator-excluded flowers on 12 H. laevis plants grown in the 1993 greenhouse study of a Delaware County, Ohio population.
In the greenhouse, hand pollination strongly influenced curvature of the style branches (Figs 6, 7). Thirteen of the 22 hand-pollinated flowers which were scored had no stigma-anther contacts, and none of them had greater than three contacts (mean=0.73). By contrast, most of the 25 unpollinated ones which were scored had four or five contacts, and none had fewer than two (mean = 4.0) (Figs. 6, 7). Failure of a stigma to contact anthers was generally attributable to insufficient backwards curvature of the style branches; in this population the anthers were uniformly positioned sufficiently close to the stigmas that contact with stigmas would occur in the event of style curvature.

There appears to be variation among populations in the capacity for autonomous selfing. In the greenhouse test in which 1–2 plants from each of 10 populations across the range of the species were compared for this trait, five populations (mainly from the s. edge of the species range) yielded negligible fruit set from unmanipulated flowers, one (a northern Ohio population) yielded a minor degree of fruit set, and three (from midwestern locations), produced a substantial amount (Fig. 8). Style recurvature was noted in unmanipulated flowers from all populations, and the inability to self appears to be due to herkogamy (Fig. 9). Since only 1–2 plants were grown from each population, the inference that there is among-population variation in
Figure 6. The number of stigmas per flower that curved backwards and touched anthers, counted the day after anthesis on hand pollinated and unmanipulated flowers of 8 H. laevis plants grown in the 1993 greenhouse study of a Delaware County, Ohio population. Bars represent standard error; the differences are highly significant (P < 0.0001).
Figure 7. Stamen tube with associated style from pairs of simultaneously blooming flowers excised from *H. laevis* plants grown in the 1993 study of a Delaware County, Ohio population. Each member of a pair received a different treatment as labelled, wherein "X" and "S" signify unmanipulated and copiously hand-pollinated treatments, respectively.
Figure 7.
autonomous fruit set rests upon the untested assumption that experimental plants were representative of their respective populations. This seems reasonable in view of the observance of somewhat uniform results from the 12 experimental plants from Delaware County, Ohio, and personal observations at several *H. laevis* populations that flower morphology does not vary greatly among plants at a site.

**Autonomous selfing: field study**—Sixty flowers of each cross type yielded a total of 150 fruits; fruit abortion rates did not vary significantly by cross type. The mean number of seeds per fruit was greatest for hand pollinated flowers and the least for emasculated ones, and the modest difference between them was statistically significant (*p*<0.05). Unmanipulated flowers (i.e., those that were naturally pollinated, by whatever means) yielded an intermediate seed set that was not significantly different from the other cross types (Fig. 10).

**Inbreeding Depression**—Data were pooled from the 8 ovule parents. Breeding system did not significantly affect the number of seeds per fruit or germination frequency. However the average mass of inbred plants was only 87% of outcross plants; this difference was highly significant (Table 6).
Figure 8. Percentage of treatment flowers that set fruit on 1994 greenhouse-grown representatives of 10 *H. laevis* populations. Flowers were alternately subjected to either of two pollination treatments: copiously hand pollinated or unmanipulated. Where bars are absent, fruit set = 0. Population labels correspond to Table 5.
Figure 9. Stamen tube with associated style from unmanipulated flowers from *Hibiscus laevis* populations grown in the 1994 greenhouse study of variation in the capacity for autonomous selfing. Population labels correspond to Table 5.
Figure 9 (continued).
Figure 10. Mean number of seeds per fruit (with SE bars) produced in 1994 by *H. laevis* plants at the Delaware County, Ohio site, subjected to three pollination treatments during 5 sequential mornings about the middle of the blooming period. Initial sample sizes were 60 flowers per treatment; apparently random abortion resulted in the sample sizes shown in the chart bars.
Table 6. The performance of self and outcross progeny for 3 components of fitness. Differences in the numbers of seeds per capsule and the percent seed germination, determined by T-test and chi-square respectively, were not significant. The biomass difference was highly significant (paired test $T = 3.97, p < 0.001$).

<table>
<thead>
<tr>
<th>Type of Cross</th>
<th>Number of seeds per capsule</th>
<th>Percent seed germination</th>
<th>Biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean N SD</td>
<td>Mean N</td>
<td>Mean N SD</td>
</tr>
<tr>
<td>Self</td>
<td>61.9 36 7.26</td>
<td>93.4 864</td>
<td>8.8 420 3.74</td>
</tr>
<tr>
<td>Outcross</td>
<td>63.0 45 6.65</td>
<td>94.1 864</td>
<td>10.8 420 3.82</td>
</tr>
</tbody>
</table>

Discussion

This experiment confirms that *H. laevis* is capable of delayed autonomous selfing. Autonomous fruit set and seed set values of the Delaware County, Ohio population were lower than corresponding ones for hand-pollinated flowers, but still were capable of yielding about 29 seeds/flower. This level of reproductive success is probably a minimum value, and considerably lower than that which would occur under natural conditions when pollinators are scarce or absent. The greenhouse was free of wind, hence pollen grains that became situated on the stigmatic surface were limited to those that encountered the stigma as a direct result of style curvature. Greater pollen loads would probably be deposited under modestly windy conditions, as slight movements of stigmas already in direct contact with aggregated anthers could greatly increase the degree of contact between fresh stigma surface and pollen. Further,
autonomous pollination could supplement low vector-facilitated pollination levels. This was suggested by the results of the 1994 field study in which the seed set of naturally pollinated flowers was intermediate between hand-pollinated and emasculated flowers. However, inasmuch as the differences were not statistically significant they do not rule out the alternative and equally plausible hypothesis that moderate levels of prior insect mediated pollination which effect only a fraction of the maximum possible seed set may be sufficient, owing to the facultative nature of the autonomous selfing, to preclude its occurrence. Further studies of natural populations that include a bagging treatment to prevent all but autonomous selfing, might further serve to delineate its importance.

The occurrence of significant inbreeding depression is consistent with a mixed mating system in which outcrossing is favored but opportunities for reproduction are assured in the absence of pollinators. Because the emasculation treatment had little or no effect on the reproductive output of the test plants in the natural stand (compared with unmanipulated flowers), autonomous selfing may not function in the maintenance of existing populations. The riverine shoreline habitats frequented by H. laevis are probably quite unstable due to frequent flooding, erosion, siltation, drought, and plant succession. Some of these forces may create new areas suitable for H. laevis establishment. The
seeds of this plant, being similar in size and weight to those of *H. moscheutos* are probably, like them (Spira 1989), capable of water-dissemination. Having observed abundant plants on river sediment newly exposed by a draw-down to allow dam repairs at the Delaware County site in 1990, as well as on side-cast dredged material along two eastern Texas rivers in 1992, I believe that seeds remain viable in the soil for substantial periods. Accordingly the seed bank may be an important source of germplasm to found new populations. Both these methods, water-borne transport and seed bank "withdrawals" that depend upon the vagaries of microsite conditions to determine the timing of seed germination, and consequently that of subsequent flowering, may frequently result in the presence of solitary or very sparse blooming individuals on newly created suitable habitat. The newness of plants on such sites may cause them to be deprived of a contingent of pollinators accustomed to foraging on their flowers. The intuitive axiom referred to as "Baker's law" specifies that "self-compatible rather than self-incompatible taxa will be favored in establishment after long-distance dispersal" (Baker 1955; Cox 1989). A logical extension of that reasoning suggests a still greater advantage would be enjoyed by taxa which can carry out delayed autogamy, as this would maximize the capacity for rapid spread onto a newly available site without abandoning the ability to exchange genes with the population at large.
once one develops. Since this trait may be of critical importance in the unstable fluvial zone that comprises prime \textit{H. laevis} habitat, the autogamy elucidated in this study may primarily operate during the founding of new populations.

The facultative nature of the autonomous pollination due to recurvature only by unpollinated styles is novel and intriguing, but also problematic. Upon first consideration it seems an elegant adaptation to preclude self pollination when there are ample opportunities for outcrossing, and that it may be. But because the self-pollination that would be so avoided would also be delayed by perhaps as much as several hours, it is unclear if its absence makes any sizeable difference in the breeding system. In the common rose-mallow, \textit{H. moscheutos}, a 2-hour difference between the application of abundant pollen loads on separate stigmas caused approximately 90\% of seeds to be sired by the earlier-deposited pollen (A. Snow, personal communication). It is therefore possible the stylar branches in \textit{H. laevis} might simply have become stiff as an unavoidable accompaniment to the growth of pollen tubes within them, and any imagined suppression of autonomous selfing is incidental and inconsequential. Close examination of other species with autonomously selfing flowers may reveal further instances in which self pollination rather than merely self-fertilization is facultative. In these cases, careful study would be warranted of the timing of the two pollination
types particularly with respect to how it affects fertilization in relation to the other critical variable, pollen tube competition.

The finding of extreme among-population differences in the capacity for autonomous fruit set is an apt example of the need to avoid making generalizations about a species based on results from one or a few populations. Also, like the results presented in Chapter I which demonstrated that eastern Texas populations of *H. moscheutos* suffered greater fitness reductions upon selfing that Ohio ones, a similar geographic distribution pattern is evident. The floral morphology of deep-southern (Texas, Mississippi and Louisiana) *H. laevis* plants prevents autonomous selfing while more northern ones (to a variable extent) can so self. A genetic basis similar to that which I proposed for the *H. moscheutos* results might underlie these differences too, wherein populations which evolved the selfing trait were those that were less prone to inbreeding depression and so would be favored by the capacity to self. Historical bottlenecks that caused reductions in genetic variation with a concomitant purging of the deleterious alleles are frequently evoked to explain both the evolution of selfing and the absence of inbreeding depression (Charlesworth and Charlesworth 1987). Two of the more capable populations, Arkansas and Oklahoma, are situated far south of the southern edge of the Wisconsin glacial advance and so it is
unlikely that bottlenecks associated with glaciation are responsible for the pattern. The deep south populations may for some unknown reasons be more ecologically central and thereby harbor greater genetic variation (Mayr 1970), or their habitats may simply have greater long-term stability. Whatever the reason, these *H. laevis* populations may be so susceptible to inbreeding depression that traits which foster selfing are selected against.

Alternative possibilities for the possible pattern may be considered. Perhaps pollinators are more generally available at the southern sites owing to the more equable climate or the sympatry of plants species (like the closely related *H. moscheutos*) that help maintain pollinators during periods of low *H. laevis* flower density. Finally, the herkogamy trait may be inherited in introgressive fashion from *H. moscheutos*. In that species the stamens and stigmas are too located far apart to allow unfacilitated selfing (Spira 1989). At three locations in eastern Texas I observed the species growing together, a co-occurrence that is reported to be unusual (Blanchard 1976) despite their nearly identical ranges over much of eastern North America. They occasionally hybridize (Wise and 1971, Blanchard 1976), producing fertile offspring (personal observation). Thus the observed *H. moscheutos*-like trait in *H. laevis* may have become incorporated into populations of the latter through hybridization and repeated backcrossing at one or
more locations. Detailed investigation of rose mallow reproductive ecology in areas where the species co-occur, including molecular approaches, would be necessary to the discern whether the occasional hybridization events are of evolutionary importance. The observation of multiple populations able to self-pollinate, coupled with the fact that it is a variable trait and therefore is subject to selection, serves as strong evidence the phenomenon is an important part of *H. laevis* vegetation dynamics.

**List of References**


CHAPTER III
GENETIC AFFINITY OF THE RARE EASTERN TEXAS
ENDEMIC HIBISCUS DASYCALYX

Introduction

Many endemic plant taxa are closely allied to wide-ranging species that they resemble morphologically. Depending upon the degree, sharpness and nature of the differences that separate an endemic from typical members of related wide-ranging species, it may be variously regarded as a variety (or ecotype if the distinctive traits are adaptive), a subspecies, or a distinct species. Knowledge of the genetic affinities of endemic taxa can foster an understanding of evolutionary processes and also serve practical interests of plant conservation. Wisely, many of the state and Federal programs that protect rare taxa include infraspecific categories within their purview. The delimitation of correct species boundaries, particularly when the endemic is found to comprise a subspecies or variety, may aid conservation biologists by alerting them of the potential for contamination of the endemic gene pool by hybridization with the species at large.

The biological species concept which regards species as an interbreeding community of populations that is
reproductively isolated from other such communities (Mayr, 1992) may be useful on a local scale in spite of its being neither universally applicable nor often practical to employ (Sokal and Crovello 1970). The presence or absence of reproductive isolating mechanisms between sympatric taxa may assist in a determination of whether they represent the same or different species, particularly when used in conjunction with an analysis of patterns of morphological dissimilarity and the distribution of neutral genetic markers such as isozymes. Isozyme data may also be used to determine the most likely evolutionary mechanism by which divergence has taken place, with various possibilities being gradual divergence from a common ancestor, a progenitor-derivative pair, or saltational speciation that may or may not involve hybridization or changes in chromosome number. The present study examines isozymes, morphology and interfertility to determine the genetic relationship of a rare endemic to two wide ranging species with which it is sympatric.

The taxa—Hibiscus dasycalyx Blake and Schiller (Malvaceae: Neches River rose-mallow) is a rare marsh plant known only from a few locations in eastern Texas. Its current Federal status is a category 2 species under review for listing. The Texas Natural Heritage Program includes the Neches River rose-mallow in their Special Plant List with a ranking of G1S1 (both globally and locally rare) and the Texas Organization for Endangered Species, in their
Endangered, Threatened and Watch list of plants of Texas, lists this taxon as a category III (State Endangered) species (Kennedy and Poole 1990).

This endemic is a member of Section Muenchhusia, a group of five closely related and apparently recently evolved Hibiscus species uniquely designated "rose-mallows," whose collective range is confined mainly to marsh habitats in the eastern half of the United States (Blanchard 1976). All are diploid (n = 19) perennial herbs. Two of the species, H. moscheutos L. (common rose-mallow) and H. laevis All. (smooth rose-mallow), range widely over this area while two others, H. grandiflorus Michx. and H. coccineus Walt., are restricted mainly to interior and coastal Florida. Hibiscus grandiflorus also occupies a narrow band bordering the Atlantic and Gulf coasts of the southern U.S and Cuba.

The diagnostic morphological traits of the three taxa which have been considered to be closely allied to the endemic are described below, based upon O. Blanchard's classification. Only the first two taxa are sympatric with H. dasycalyx (Correll and Correll 1972; Blanchard 1976).

Hibiscus moscheutos subsp. lasiocarpos is characterized by vegetative structures that are pubescent to a variable degree and by a lower leaf surface that is densely stellate, often with simple hairs as well. The leaf is unlobed, lanceolate or elliptic-lanceolate to broadly triangular-ovate. The calyx is stellate-tomentulose. The fruit (a
capsule) is pubescent, bearing hairs that are simple, stellate or glandular, with the various types occurring singly or in combination. Flowers are white or pink, with a red center, moderately spreading beyond the calyx tube, and bee-pollinated. The seeds are glabrous. Within this hairy-fruited western subspecies are forms that have been regarded as separate species, namely: \( H. \) moscheutos, \( H. \) lasiocarpos, and \( H. \) leucophyllus (Correll and Correll 1972).

\( H. \) laevis (Fig. 11, "H.l." ) has vegetative portions glabrous. The leaves are triangular-hastately 3-lobed in general outline (lobes are occasionally five in number or lacking). The middle leaf lobe is long acuminate and 2-6 times as long as the width of the body of the leaf. The calyx and the capsule are glabrous or very nearly so. Flowers are pink or white, with a red center, moderately spreading beyond the calyx tube, and bee-pollinated. Blanchard (1976) states "within its comparatively wide distribution in North America \( H. \) laevis is quite uniform in character. The entirely glabrous herbage and reddish-pubescent seeds serve to distinguish it from the superficially similar \( H. \) moscheutos."

\( H. \) coccineus is a Florida species with vegetative and reproductive portions that are glabrous. The leaves are palmately 5-parted. The flowers are crimson with petals extremely wide-spreading at anthesis, and butterfly-pollinated. This taxon was shown in crossing experiments
Figure 11. Illustrations of eastern Texas smooth rose mallows. *Hibiscus laevis* is the typical form as depicted in a regional flora (Correll and Correll 1972). *Hibiscus dasycalyx* is from Blake (1958), in which the new species was described and the 1955 Schiller collection was designated the type specimen.
conducted by Wise and Menzel (1971) to be more closely related to *H. laevis* than to the other widespread species. The resemblance of *H. dasycalyx* to *H. coccineus* cited by Blake (1958, quoted below) is quite superficial, consisting only of their both having deeply lobed leaves. *Hibiscus coccineus* is probably unworthy of further consideration as an especially close relative of *H. dasycalyx* because (1) the number of leaf segments differs between the two species, (2) their natural ranges are 1000 km apart, (3) several traits which would probably be shared if there were close ancestry in fact differ, such as flower color, flower shape and surface features of calyx and capsule, (4) eastern Texas *H. laevis* also displays deep lobing (hence a more parsimonious view would look toward that taxon as one which shares the lobing trait by descent). Accordingly *H. coccineus* is not here further considered.

The Neches River rose-mallow (Fig. 11, "H.d.") was first collected by Ivan Schiller in 1955 at a shrubby marshland in Apple Springs, Trinity County, Texas. Soon afterward, S.F. Blake (1958) classified the specimen as a new and very distinct species which appeared to be allied to both *H. coccineus* and *H. laevis*. He noted that it had leaves "much like those of the former species and corolla more like that of the latter, but at once distinguished from both of them and from all other United States species by its densely spreading-hirsute calyx."
Orland Blanchard visited the type location of *Hibiscus dasycalyx* in 1968 and noted the following (Blanchard 1976) some specimen collection citations have been omitted for clarity).

"*Hibiscus dasycalyx* shows strong affinity with the widespread *H. laevis*, differing from it principally in its much more narrowly lobed leaves and its prominently pubescent calyx and fruit. Both *H. laevis* and *H. moscheutos* subsp. *lasiocarpos* grow in proximity to *H. dasycalyx*, the former along the banks of the Neches River about a mile to the east and perhaps closer, and the latter within a few meters. Were it to have been known only from the type collection *H. dasycalyx* might have been treated as a chance hybrid between *H. laevis* and the hairy-fruited subspecies of *H. moscheutos*, but the author has visited the type locality and has observed that the 100-plus individuals examined agreed with the type. Further evidence of the genetic stability of the species is the fact that the few plants that were grown in the garden from wild-collected seed came true to seed and on open pollination set abundant seed themselves."

"A specimen of *Hibiscus* from Brazos Co., Texas (Fryxell No. 1896) may represent a hybrid between *H. laevis* and *H. dasycalyx*. Its leaves have narrower lobes than on any specimen of *H. laevis* examined, and there is a trace of pubescence on the calyx. The plant was found more than 80 miles from the known *H. dasycalyx* locality, and if its origin is being correctly interpreted, one may expect to find additional stations of *H. dasycalyx* in that area and perhaps elsewhere in eastern Texas."

Recent study of *Hibiscus dasycalyx* by local botanists has consisted of documenting its reported occurrence, and searching for additional populations (Kennedy and Poole 1990). Until 1992, only the type location near Apple Springs was known with certainty, despite extensive fieldwork by Texas Natural Heritage Program staff that included searches at 12 sites of suitable habitat in the
region. The Apple Springs population has declined markedly. A conservation biologist from the San Antonio Botanical Garden who visited the site in 1988 to collect germplasm encountered only 13 plants (P. Cox, personal communication). I found only three plants in 1992. However in 1991, local botanists discovered near Lovelady, Houston County, Texas, a second population situated 54 km northeast of the type location. This population consists of approximately 50 plants located mainly along the margin of a small pond used by cattle. Additional specimens of typical H. dasycalyx filed in the herbaria of Sam Houston State University (SHST; Huntsville, Texas) and Stephen F. Austin State University (ASTC; Nacogdoches, Texas) suggest the existence of two other populations, but information on the labels is insufficient to allow revisiting the precise locations where they were collected, and searches in the general area conducted by the author and by others have failed to relocate them. In addition to these 2-4 stations for plants that are morphologically quite alike and typical for H. dasycalyx, D. Williams and I found a fairly uniform population of plants that appeared somewhat intermediate between typical H. dasycalyx and H. laevis in July 1992 at Conroe, Trinity County, Texas. These plants have leaves more narrowly lobed than those described for H. laevis and the calyx pubescence is stellate, substantially shorter and less abundant than the hairs on the densely hirsute calyx of
typical H. dasycalyx. This population seems to agree closely with the description of the specimen believed by Blanchard to be a H. dasycalyx x H. laevis hybrid (see above). Surprisingly however, a specimen from this population was identified by an authority on the Malvaceae as a clear example of Hibiscus dasycalyx (P. Fryxell, personal communication with D. Williams). Moreover along the Trinity River in Trinity County and in Chambers County I noted many plants bearing especially narrow leaves, and at one of these sites some plants have moderately hairy calyces. These several questionable plants are addressed below in connection with an assertion that eastern Texas H. laevis plants display among-population variability in leaf shape and flower part surface features which is so substantial that (among other reasons) H. dasycalyx may be better regarded as a variety of H. laevis rather than a distinct species.

The goal of the present study was to determine the evolutionary relationship of H. dasycalyx to the other eastern Texas rose-mallows. Several possibilities were considered: (1) it could be a true species of diploid hybrid origin involving H. laevis and H. moscheutos, (2) a derivative species, subspecies or variety of H. laevis, or (3) a separate species perhaps having an ancestor in common with one or both of the others (Grant 1981). In hybrid speciation, the F₁ or a subsequent generation shortly
descended from an interspecific cross becomes genetically stabilized in one of several ways, preventing the hybrid gene combinations from being broken up by segregation in later generations. Most frequently, the stabilization mechanisms are numerical alterations of the chromosome set such as polyploidy, or asexual reproduction such as vegetative reproduction and agamospermy. Alternatively, through an apparently uncommon process, a highly fertile sexually reproducing species can be produced by recombination among the hybrid progeny of two species without a change in ploidy level. Termed recombinational or diploid hybrid speciation, this process requires parental species that are moderately dissimilar to one another chromosomally. Briefly, the genetic model invoked to explain the process requires a sterility barrier consisting of two or more segmental chromosome rearrangements. Because of the rearrangements, the interspecific F₁ hybrid is chromosomally sterile, but only partially so, and crosses among the F₁ or later progeny can give rise to new recombination types that are fertile but reproductively isolated from others outside their recombination type and also from the parents. If natural selection favors a recombinant type and the breeding system allows its proliferation, a population may become established as a newly formed bona fide species.

By virtue of its (1) ploidy level identical with that of
both *H. moscheutos* and *H. laevis*, (2) high fertility, and (3) occurrence near those species, it seemed that *H. dasycalyx* might be a hybrid derivative of them. Its self-fertility, possibly aided by facultative self-fertilization (Blanchard, 1976) is a mating system feature that would support the hypothesis, since selfing among hybrid progeny enhances the production of genically balanced progeny (Grant 1981). Moreover, there are reports of strong but not absolute reproductive barriers between *H. laevis* and *H. moscheutos* (Wise and Menzel 1971).

Allozymic data can be used to infer evolutionary relationships if the putative parent species are extant and divergent at one to several loci encoding enzymes to be examined electrophoretically, and the species have not diverged or accumulated many mutations since the origin of the new species (Gottlieb 1984; Crawford 1990). A new taxon of diploid hybrid origin may be distinguished if it combines parental alleles in one or both of two ways. Alleles from each parent may occur together at individual loci, or the parental-specific alleles may occur at different loci. In either case there is a distinctive "hybrid profile" (Fig. 12). Enzyme electrophoresis has been used successfully in the confirmation of suspected cases of diploid hybrid speciation, as in *Stephanomeria diegensis* (Gallez and Gottlieb 1982) and *Helianthus paradoxus* (Rieseberg et al. 1990) and to refute a hybrid origin hypothesis for *Lasthenia*
burkei (Crawford and Ornduff 1989), Helianthus petiolaris subsp. fallax (Rieseberg et al. 1990) and Pentstemon spectabilis and P. clevelandii (Wolfe and Elisens 1993). A second type of relationship may also be distinguished, that of a progenitor-derivative pair. The derivative taxon typically contains a subset of alleles found in the progenitor, as for example was detected in the Great Lakes endemic Cirsium pitcheri in a comparison with the more widespread great plains C. canescens (Loveless and Hamrick 1988). Finally, the possession by two closely related species of essentially equal amounts of genetic variation, especially when accompanied by alleles unique to each taxon, provide evidence of having evolved in a divergent fashion from a common ancestor (Crawford 1990).

![Figure 12. Types of enzyme additivity that might occur in a species of diploid hybrid origin. Pattern "A" depicts the derivative taxon combining two parental alleles at an individual gene locus, whereas at "B" it has inherited an allele from one parent taxa at one gene locus and from the other parent at another gene locus. (Redrawn from Crawford 1990).](image-url)
Materials and Methods

Acquisition of plant material—During the summer of 1992 I visited 10 eastern Texas locations (Table 7; Fig. 13) and collected stem cuttings, seeds, and voucher specimens (deposited in OS). They were chosen mainly from an inspection of local herbaria and through the guidance of D. Williams, Director, Mercer Arboretum, Humble Texas. Stem cuttings from the Apple Spring H. dasycalyx population obtained from the San Antonio Botanic Garden were examined for evidence of natural hybridization with H. moscheutos. Orland Blanchard kindly provided H. dasycalyx seeds he collected in 1968; these were used in the isozyme survey and also grown into ovule parents employed in the cross-compatibility study.

Allozymes—Eight seeds (fewer in one population each of H. moscheutos and H. dasycalyx owing to small population sizes) gathered from separate individuals at each population were scarified with a razor blade, imbibed, and analyzed for allozyme variability using the horizontal starch gel electrophoresis methods of Percy and Wendel (1992). Newly emerged seedlings were ground in 1.5 ml microcentrifuge tubes using a power-driven teflon pestle (on ice) and 150 microliters of a 75 mM Na-phosphate, pH 7.5, extraction buffer containing 0.5% BSA, 5% (w/v) sucrose, 10% (w/v) polyvinylpolypyrrolidone, 14 mM mercaptoethanol, 100 mM ascorbic acid, 10 mM dithioerythritol, and 10 mM
diethyldithiocarbamate. Young leaf material from mature plants was used to increase sample sizes in selected instances and to substitute for seeds in one population where they were unavailable. The leaf material could not be successfully assayed for ADH or AAT. Extracts were frozen at -70 C until electrophoresis. A total of ten enzyme systems were consistently resolved using three buffer system combinations: 1) Electrode buffer -- 0.19 M boric acid adjusted to pH 8.3 with lithium hydroxide (final LiOH molarity approximately 0.038); gel buffer -- 1 part electrode buffer to 9 parts 52 mM TRIS-8 mM citrate, pH 8.3; used for triose-phosphate isomerase (TPI), alcohol dehydrogenase (ADH), and aspartate aminotransferase (AAT), 2) Electrode buffer -- 65 mM L-histidine--19mM citrate, pH 6.5; gel buffer -- 1:6 dilution of the electrode buffer; used for phosphoglucomutase (PGM), glucose-6-phosphate isomerase (GPI), phosphogluconate dehydrogenase (PGD) and shikimate dehydrogenase (SKD), and 3) Electrode buffer -- 40 mM citric acid titrated to pH 6.1 with N-(3-aminopropyl)-morpholine; gel buffer--morpholine; gel buffer -- 1:19 dilution of the electrode buffer; used for malate dehydrogenase (MDH), malic enzyme (ME) and NADP-isocitrate dehydrogenase (IDH). Enzymes were visualized using staining methods described in Wendel and Weeden (1989), except that
TABLE 7. Study populations of *Hibiscus dasyphyllum*, *H. laevis* and *H. moscheutos*. Site numbers are Fig. 13 map locations. Taxa are signified by first letter of the specific epithet.

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<th>DESCRIPTION</th>
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<td>Floodplain of Sabine River</td>
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<tr>
<td>2</td>
<td>Douglass; Nacogdoches</td>
<td>Inland wet pasture near Neches River</td>
<td>m</td>
</tr>
<tr>
<td>3</td>
<td>Redland; Angelina</td>
<td>Floodplain of Angelina River</td>
<td>l</td>
</tr>
<tr>
<td>4</td>
<td>Apple Springs; Trinity</td>
<td>Margin of swamp at roadside picnic area (type location)</td>
<td>d,m</td>
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<td>5</td>
<td>Wakefield; Angelina</td>
<td>Floodplain of Neches River</td>
<td>l</td>
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<tr>
<td>6</td>
<td>Lovelady; Houston</td>
<td>Seasonally inundated slough and margin of cattle pond</td>
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<td>Conroe; Montgomery</td>
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<td>Cove; Chambers</td>
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<td>Eminence; Chambers</td>
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Figure 13. Population locations. Numbers correspond to Table 7.
ADH required a substantially higher concentration of substrate.

**Leaf shape and calyx pubescence**—The principal diagnostic characters separating *H. dasycalyx* from *H. laevis* are its narrow, deeply lobed leaves and hirsute calyx. Because apparently intermediate plants were observed in the field, several populations were examined in detail with respect to these traits. Throughout spring and summer 1993 *H. laevis* and *H. dasycalyx* plants from nine populations (N = 6-22 per population) were grown in pots in the OSU greenhouse. In late September five consecutive leaves were removed from the midpoint of the main stem of each plant, pressed, and dried. Measurements were subsequently made of the length and width of the middle lobe and a lateral lobe of each leaf. These measurements supplied data to characterize leaf shape, and so assess the degree of within and among-population variation, including a comparison of *H. laevis* and *H. dasycalyx*. Also, several calyces from each population were sampled for microscopic examination of surface features, with particular attention given to anomalous pubescent calyces of *H. laevis*, and *H. dasycalyx* with fewer hairs (also of a different type) than the traits specified in previous descriptions (Blake 1958; Correll and Correll 1972; Blanchard 1976).

**Interfertility**—Hand-pollinations were conducted to detect whether there are post-pollination reproductive
isolating mechanisms between *H. dasycalyx* and its sympatric congeners and also assess the potential for automatic self-pollination. Two *H. dasycalyx* plants received pollen from the following sources: (1) *H. laevis* (Riverside and Wakefield populations); (2) *H. moscheutos* (Douglas and Riverside populations); (3) outcross *H. dasycalyx* (Apple Springs and Lovelady populations), (4) self and (5) unpollinated. The dependent variable is fruit set (fruits per flower); sample sizes are shown in Table 9.

As a further test of reproductive isolation that might function in mixed populations, simultaneous pollination was conducted using outcross *H. dasycalyx* pollen and pollen from one individual of *H. laevis* or *H. moscheutos*. The two pollen types were applied in concurrent fashion by coating two stigma lobes each with pure loads presumably resulting in strong competition among pollen tubes for ovules (Snow and Spira 1991). The dependent variable is the number of seeds sired by each pollen type, determined electrophoretically (the pollen parents differed in GPI alleles).

**Results**

*Allozymes*—Five enzyme systems were clearly resolved and polymorphic (Table 8). Three of them, ADH, GPI and IDH, displayed familiar dimeric enzyme banding patterns which allowed their genetic basis to be interpreted (Weeden and Wendel 1989). The monomeric enzyme PGM and the dimeric AAT
Table 8. Allele frequencies, by population, of six polymorphic loci in eastern Texas *Hibiscus* species. The alleles are listed in order of anodal migration distance on the gels, with lower numbers representing the farthest-migrating loci, and alleles within loci, of each enzyme system. ADH2, GPI2 and the PGM loci provided the most useful genetic markers.

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Table 8, continued

**H. laevis, continued**

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**H. dasycalyx**

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were also polymorphic, but could not be confidently described in terms of the numbers of loci and alleles expressed. The consistently observed dark-staining bands for these enzymes were putatively regarded to be individual loci. Five other enzyme systems were invariant: TPI, MDH, ME, SKDH and PGD.

Three of the five polymorphic enzymes, ADH, GPI and PGM, exhibited substantial among-taxon differences in allele distribution (Table 8; Fig. 14). ADH occurred as two loci (probably both cytosolic, as organelle-located enzymes in these extracts tended generally to be very faintly expressed). One locus (ADH1) was monomorphic and invariant. The second locus (ADH2) was slightly variable within and substantially variable among taxa, and included two alleles: allele 1 occurred solely in \textit{H. moscheutos}, while allele 2 was fixed in all \textit{H. laevis} and \textit{H. dasycalyx}, and occurred very rarely in \textit{H. moscheutos}. The cytosolic GPI locus included three alleles. Allele 1 was the most common isoform in \textit{H. moscheutos} yet was rare in \textit{H. laevis} and absent from \textit{H. dasycalyx}. Allele 2 was infrequent in all three taxa. Allele 3 occurred in all three taxa but was distributed in an unequal manner; it was found in only three \textit{H. moscheutos} populations where it was usually less frequent than allele 1, but by contrast it was the dominant allele (in some instances fixed) in all populations of \textit{H. laevis} and two of the three populations of \textit{H. dasycalyx}. 
Figure 14. Horizontal starch protein electrophoresis gels showing predominant banding patterns for the taxon-associated enzyme systems as labelled: PGM, ADH and GPI. The order of the lanes is: H. laevis (Redland population) H. moscheutos (Trinity population), and H. dasycalyx (Lovelady population); this pattern is repeated four times.
Phosphoglucomutase, despite the uncertainty of its genetic basis, displayed clear and consistent differences in among-taxon banding patterns that made this enzyme especially informative. A putative locus, PGM4, that was fixed for all *H. moscheutos* was not expressed in any *H. laevis* or *H. dasycalyx* (or, alternatively, these taxa possess a locus having an electrophoretic migration rate indistinguishable from the other allozyme).

The electrophoretic data permit some inference of evolutionary relationships of these taxa. Within the 14 rose mallow populations herein examined, five enzymes are polymorphic. Three of them, ADH2, GPI2, and PGM4, are marker enzymes, i.e., they contain alleles (or in the case of PGM4 the presence or absence of a fixed locus) that are found exclusively or predominantly within one or the other of the two wide-ranging species that were considered as possible progenitors of the endemic taxon. For each of these the banding pattern of *H. dasycalyx* is identical to that of *H. laevis*, comprising strong evidence that *H. dasycalyx* is not the product of a hybridization event but rather is more closely allied with *H. laevis*. Within *H. dasycalyx* only one enzyme (GPI2) was found to be polymorphic and no unique alleles were detected, whereas within *H. laevis* four enzyme systems were variable. Although the samples sizes for variable loci, populations of each species, and in some instances the number of individuals within population are
quite small, thus rendering any inference tentative, the endemic appears to possess a subset of the genetic variation found within *H. laevis*. This suggests that *H. dasycalyx* is a recent evolutionary derivative of *H. laevis*.

Leaf shape and calyx pubescence—Leaf shape data for individual plants consisted of the mean of five mid-stem leaves. Because the endemic displays leaves that are exceptionally narrow and deeply lobed, selected traits were: (1) "broadness," i.e., proportional width of the middle lobe, calculated as middle lobe width/middle lobe length, and (2) "lobedness" i.e., the length of the longest lateral lobe compared with the leaf blade length, calculated as lateral lobe length/middle lobe length.

Substantial difference in leaf shape was noted between *H. laevis* and *H. dasycalyx* and also among populations of *H. laevis* (Figs. 15-18). As expected, the three *H. dasycalyx* populations possess, on average, the most narrow and deeply lobed leaves. However, three populations of *H. laevis* situated near and along the Trinity River also have rather narrow and deeply lobed leaves, with population means for the measured traits only slightly different than the *H. dasycalyx* populations. Moreover one of them (Riverside) is more variable than the other eight populations and includes several plants with leaves apparently identical with those of typical *H. dasycalyx*. (Despite these anomalies it is clear these populations are
Figure 15. Silhouettes of representative leaves from three populations of *H. dasycalyx*. The two right-hand Apple Springs leaves are probable *H. dasycalyx* x *H. moscheutos* hybrids. Population names are the nearest town or city as indicated on Table 7.
Figure 16. Silhouettes of representative leaves of narrowly lobed *H. laevis* from near and along the Trinity River. Population names are the nearest town or city as indicated on Table 7.
Figure 17. Silhouettes of representative leaves of typical *H. laevis* from populations along separate major eastern Texas rivers. Population names are the nearest town or city as indicated on Table 7.
Figure 18. Means of two leaf shape parameters for 9 eastern Texas rose mallow populations (N = 6-22 plants per population; error bars are SD). The mean of five mid-stem leaves represented each plant. "Broadness" is the width of the middle lobe/leaf length. "Lobedness" is the length of one side lobe/leaf length. Populations are designated by the first three letters of the site name (refer to Table 7).
correctly identified or at least are consistent with local concepts of the taxon, as they are represented by modern labelled specimens on file in the herbarium at Stephen F. Austin State University. The recently retired curator there, E. Nixon, has extensive field experience with local rose mallows, and the collections have been scrutinized by persons searching for additional H. dasycalyx populations.) The samples from sites along three other major eastern Texas rivers have leaves that are typical for H. laevis.

In conformity with the description of H. dasycalyx, calyces of plants from the Apple Springs and Lovelady populations are densely hirsute, without distinguishable among-plant variability (Fig. 19, "H.d. APP" and "H.d. LOV"). The Conroe H. dasycalyx calyces are not densely hirsute but rather are short-stellate, with substantial among-plant variability in the density of these hairs, ranging from moderate density to glabrate (Fig. 19, "H.d. CON1" and "H.d. CON2"). The calyces in all but one population of H. laevis are uniformly glabrous. The exceptional population is Riverside, in which most plants are glabrous but a few have a moderate density of short stellate hairs (Fig. 19, "H.l. RIV1" and "H.l. RIV2"). Moreover, the hairy-fruited Riverside H. laevis plants tended to have narrower leaves (Spearman rank-order correlation of the "broadness" leaf trait with ordinally ranked calyx pubescence as observed under 10x magnification,
Figure 19. Scanning electron micrographs of fruiting calyces of eastern Texas smooth rose mallows grown in the greenhouse. "H.d. APP" and "H.d. LOV" were obtained from Apple Springs and Lovelady H. dasycalyx, respectively, populations which were noted to be uniformly densely hirsute. "H.d. CON1" is from Conroe H. dasycalyx, the moderately stellate prevalent form in this population and "H.d. CON2" is a sparsely stellate form from Conroe. "H.l. RIV1" is a glabrous individual of Riverside H. laevis, a form that is prevalent in this somewhat variable population, and "H.l. RIV2" is an uncommon moderately stellate form from Riverside (bar = 500 micrometers).
$N = 17$, is $r = -0.70$, $P = 0.002$). A new observation for *H. dasycalyx*, and also for the anomalous Riverside *H. laevis* plants, is that flowers with a non-glabrous calyx bear a strigose rather than glabrous fruit, as is typical for *H. laevis*. Some eastern Texas rose mallow populations of *H. laevis* and *H. dasycalyx* are composed wholly or partly of plants that are morphologically intermediate between the two species.

**Interfertility**—A total of 231 single-donor pollinations were performed on two *H. dasycalyx* ovule parent plants (Table 9). Fruit set after hybrid pollination with *H. laevis* was 78% (nearly identical to that of outcross *H. dasycalyx*) and approximately 50% with *H. moscheutos*. All of the 19 self pollinated flowers set fruit, showing that, like the four other rose-mallow species, *H. dasycalyx* is fully self-compatible. The negligible fruit set of unmanipulated flowers demonstrates that, in contrast to some populations of *H. laevis* and also *H. coccineus* (Blanchard 1976 and personal observations), *H. dasycalyx* is incapable of autogamous selfing. Because some of the pollination treatments were undertaken at different times during the 6-week flowering period of the test plants, and because the plants seemed to abort more fruits towards the end of that period, tests of the significance of fruit set differences among pollination treatments were not conducted.
Table 9. Fruit set from various types of within-species and hybrid pollinations on two greenhouse specimens of *H. dasycalyx*.

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</tr>
<tr>
<td><em>H. laevis</em></td>
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<td>64</td>
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<tr>
<td><em>H. moscheutos</em></td>
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<td>1.00</td>
<td>19</td>
</tr>
<tr>
<td>no pollination</td>
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<td>37</td>
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</table>

In simultaneous pollinations combining outcross *H. dasycalyx* with *H. laevis*, 88 seeds pooled from a total of four fruits were examined electrophoretically to determine pollen parentage. The hybrid combination appeared in 32% of the seeds. A similar treatment set using *H. moscheutos* as the hybrid pollen source yielded, among 39 seeds pooled from two fruits, 44% with a hybrid genotype. The finding of a substantial fraction of hybrid seeds further suggests that *H. dasycalyx* lacks post-pollination mechanisms that would reproductively isolate it from either of its sympatric congeners. Because of the small number of fruits employed, these data were not analyzed statistically. One individual of each hybrid type was grown to maturity in the greenhouse; they were intermediate in morphology, vigorous and appeared fully fertile, abundantly setting when hand-pollinationed.
Additional observations—The pollination biology of *H. dasycalyx* has not been described (Kennedy and Poole 1990), but by virtue of its similarity with other rose mallow in floral traits, it is reasonable to expect a similar pollination syndrome. Over most of their range, including the southwest, these plants are pollinated mainly by *Ptilothrix bombiformis* Cresson (Anthophoridae), a non-social bee that is a specialist pollinator of *Hibiscus* (Blanchard 1976; Spira 1989). On July 24, 1992 I observed several individuals of *Ptilothrix* foraging among and resting within flowers of *H. dasycalyx* at the Conroe population and collected a mating pair. The identification was confirmed by an OSU entomologist (G. Keeney, personal communication).

Hybridization appears to have occurred in nature. Among the eight live plants obtained from the Apple Springs *H. dasycalyx* type location two exceptional specimens closely resembled each other but differed from the rest by being more robust, with larger flowers with shorter and denser calyx pubescence, and bearing leaves that are moderately stellate-pubescent and possess only a few short broad shallow incisions at the lower corners (Fig. 15). In sum, their morphology was intermediate between *H. dasycalyx* and *H. moscheutos* subsp. *lasiocarpus*. Moreover, these two plants differed in their pollen color (pink or white) suggesting that they are separate genetic individuals. Both were examined electrophoretically and, unlike the other six
plants, possessed the *H. moscheutos* PGM4 marker locus and also were the only GPI heterozygotes. Inasmuch as *H. moscheutos* and *H. dasycalyx* occurred close together at Apple Springs, this finding indicates that the two apparent hybrid individuals became naturally established there, perhaps coincident with a severe decline in the population size of the endemic.

**Discussion**

As noted by its discoverers and others, the Neches River rose mallow, *H. dasycalyx*, differs from typical *H. laevis* in leaf shape and calyx surface features, but is otherwise essentially identical to that widespread species. Both taxa differ from *H. moscheutos* in many vegetative and floral traits. *Hibiscus laevis* is generally regarded to be rather invariant across its wide range, but in eastern Texas I found substantial among-population variation in leaf shape and, to a lesser extent, calyx pubescence. This includes forms that closely resemble *H. dasycalyx*. Moreover there is a prior recorded instance of a morphologic intermediate between *H. laevis* and *H. dasycalyx* which was interpreted as a probable hybrid (Blanchard 1976). The endemic shares electrophoretically detectable enzyme alleles with *H. laevis* that distinguishes them both from *H. moscheutos*. Moreover it is freely interfertile with *H. laevis* (as well as with *H. moscheutos*), at least as an ovule parent, and hybrid
offspring are robust and fertile. These observations confirm the earlier assertions made solely on morphologic evidence that H. dasycalyx is closely allied to H. laevis, yet suggest moreover that the relationship is so close the endemic might better be regarded as a variety of H. laevis rather than a separate species. On the other hand, H. dasycalyx may be the product of introgression in which repeated backcrossing with H. laevis followed one or more hybridization events between the two wide-ranging species, but it is not possible to discern this using the available data.

The relegation of H. dasycalyx to a lesser taxonomic status would advance Hibiscus taxonomy because it is more consistent with a modern biological species concept (Mayr 1992). However this change should not impart any lesser recognition of its potential importance as an evolutionary unit. The distinctive morphology of so-called H. laevis plants with "dasycalyx" leaf and floral traits may have adaptive value; for example trichomes are believed to deter insect predation, aid in water absorption, or reflect sunlight (Uphof 1962). Moreover its occurrence in local isolated populations near the southwest margin of the range of the widespread species may provide conditions that are ideal for speciation to occur (Levin 1993). Accordingly these populations are at least as worthy of protection as if they were a rare bona fide species. Because of their
vulnerability to dilution by gene flow with typical *H. laevis* or *H. moscheutos*, isolation from other rose mallows may be helpful when establishing preserves or reintroducing this rare plant to former localities.

A puzzling observation that weakens the inference of an especially close relationship based on cross-fertility is the apparent lack of reproductive isolation between the endemic and the markedly dissimilar *H. moscheutos*, including the apparent occurrence of natural hybrids at the type location. Its significance could be ascertained only with simultaneous examination of whatever reproductive barriers might exist between *H. moscheutos* and typical eastern Texas *H. laevis*, knowledge of which is currently lacking. This may be especially important to understanding of eastern Texas rose mallows because mixed populations of the two widespread taxa such as those occurring at Riverside and Eminence are very unusual (Blanchard, 1976).

List of References


