REPRODUCTION AND INBREEDING IN *AGALINIS SKINNERIANA* (SCROPHULARIACEAE): FINAL REPORT

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ABSTRACT

Agalinis skinneriana is a rare annual plant native to Illinois prairie. Two populations were chosen to study the potential for inbreeding and inbreeding depression (the Site M population and the Revis Hill Prairie population). Breeding system experiments indicated that both populations experience a high level of autogamy. The two populations differed in size and reproductive characterisitcs. The Site M population population was the larger of the two, received numerous bee visits to the flowers, and produced a larger number of viable pollen grains, flowers, and fruits compared to the population at the Revis Hill Prairie Nature Preserve. No bees were observed at the Revis Hill Prairie population. These data are suggestive, but not indicative of inbreeding and inbreeding depression occurring for the smaller Revis Hill Prairie population.

Although plant species may be rare and endangered for a variety of reasons (Rabinowitz, 1981), one characteristic shared by many is small population size. Population size greatly influences breeding systems, population genetics and microevolutionary processes (Barrett and Kohn, 1991). Because of their size, small populations are subject to random fluctuations of genetic variation and inbreeding depression (reduced viability and fertility due to frequent mating between close relatives) which greatly reduces genetic diversity (Levin, 1989). Loss of genetic variation places small populations, and hence rare and endangered plants, at risk of extinction since it is genetic variation that allows a population to adapt to local environmental conditions. Simply producing lots of seed does not guarantee reproductive success.

Additional factors affecting small population size include reduced attractiveness to natural pollinators thereby effecting changes in genetic variability or maybe a complete loss of natural pollinators altogether resulting in the evolution of selfing (Simpson et al., 1986). Selfing in a previously outcrossing populations would also result in reduced genetic variation.

Agalinis skinneriana (Wood) Britt. (Scrophulariaceae) is a rare annual, native to prairies and is currently listed as a threatened plant species within Illinois (Illinois Endangered Species Protection Board, 1994). Within Illinois, it is only found in eight counties with population sizes ranging from approximately 100-1000 individuals (Robertson and Phillippe, 1993). The small isolated populations of A. skinneriana make them particularly susceptible to localized breeding patterns and eventually reduced genetic variability. In order to understand the breeding patterns and the potential for reduced genetic variability in populations of A. skinneriana, a study was undertaken to: 1) describe population reproductive characteristics, 2)

determine the breeding system using experimental crosses, and 3) estimate inbreeding depression and genetic diversity using seeds produced from breeding system crosses.

MATERIALS AND METHODS

Two populations of Agalinis skinneriana were chosen for study. One population was located at Site M near Chandlerville, IL in Cass county and the other was located at the Revis Hill Prairie Nature Preserve in Mason county. These two were chosen for the contrast they provide. The population at Site M was large relative to that at the Revis Hill Prairie Nature Preserve. Field observations began on 9 September 1994 and ended on 23 September 1994.

For each population, reproductive characteristics studied included: population size and density, flowering phenology, a list of visiting bees, floral ultraviolet (UV) reflectance, pollen viability, and the number of flowers and fruits produced by individual plants. Population size and density were estimated by using 15 randomly placed circular plots of $0.5m^2$. Bees observed visiting flowers were captured and identified. Flowering phenology and number of flowers and fruits produced by individuals were estimated by randomly tagging 25 individuals. Each week of the study, the number of flowers observed on those 25 plants was recorded. At the end of the flowering season, the number of fruits and non-fruiting flowers were recorded for each individual. Patterns of floral UV reflectance were determined using a Wratten 18A UV filter mounted on a Pentax camera. Photographs were taken using Kodak TMAX 400 film and an 8 second exposure. Pollen viability was estimated by the size, shape, and stainability of pollen using 1% aniline blue in lactophenol for 20 randomly chosen individuals. A minimum of 100 grains were observed for each individual.

The breeding system for each population was determined from experimental crosses using flowers enclosed with fine mesh cloth bags to exclude visiting insects. Types of crosses (treatments) made include: 1) no hand-pollination to test for automatic self-pollination or autogamy, 2) self-pollination to test whether self pollen will fertilize ovules, pollen being transferred between flowers of the same individual and 3) outcross pollination or xenogamy, pollen being transferred between flowers of different plants. Only one flower/plant was manipulated, sample sizes ranged from 11 to 31 flowers. Fruit production from these crosses was compared with that from open-pollinated flowers using the 25 plants tagged to record the flowering phenology. Statistical guidelines and procedures follow Sokal and Rohlf (1981).

Using the seeds produced from the breeding system experiments plus seeds from open-pollinated flowers (N = 30 plants/population), a number of population genetic parameters can be estimated. These include inbreeding depression, selfing rate, and inbreeding coefficient (Charlesworth and Charlesworth, 1987; Ritland, 1990). Seeds were to be germinated and plants grown in the greenhouse and performance recorded. Performance was to be recorded as seed weight, seed germination, growth rate, and size (biomass) taken at various stages of growth.

Seeds were prepared for germination following a modified procedure of Judith Canne-Hilliker (personal communication). Fruits were kept in cold storage (4^0C) until needed. Seeds were then removed and placed in coffee filters and washed with tap water overnight (approximately 12 hours). Seeds were then plated out on moist filter paper in petri dishes and refrigerated at 4^0C for 30 days. Seeds were then transferred directly to pots containing host plants in the greenhouse. Sorghastrum nutans (Poaceae), Indian grass, was used as a potential

host since it was very abundant at both localities. Grass is a frequently used host in many of the hemiparasitic Scrophulariaceae (Ter Borg, 1985) and the genus Agalinis is hemiparasitic (Baird and Riopel, 1984; Stewart et al., 1995; Dieringer, unpublished data).

RESULTS

The Site M population had an area of approximately 3750m^2 and the Revis Hill Prairie had an area of approximately 225m^2. The Site M population possessed approximately 2.5x more plants/m^2 and 41x more total number of plants than Revis Hill Prairie population (Table 1). Populations were checked on 4 August and plants had not yet produced flower buds. Breeding system crosses were performed on 2 September and the populations were either at or just past peak flowering. From 2 September to the end of the field observations (23 September), the number of flowers/individual steadily declined. Bees caught while foraging on flowers are presented in Table 1. Six species of bees were identified. All bee specimens are deposited in the Entomological Museum of Western Illinois University. It is interesting to note that bees were only observed visiting flowers at Site M, no bees were ever observed visiting flowers at the Revis Hill Prairie population. Flowers were UV absorptive; they do not produce any color patterns visible to bees and not humans.

Reproductive characteristics for plants at each population are presented in Table 2. Comparing the two populations, plants at Site M produced significantly more flowers and fruits that at Revis Hill Prairie ($t = 2.16$, $df = 48$, $P < 0.05$ and $t = 2.34$, $df = 48$, $P < 0.05$, respectively).

Pollen viability measured 86.6% (SE = 1.31) at Site M and 73.7% (SE = 4.06) at Revis Hill Prairie. The difference was statistically significant ($t = 3.11$, $df = 38$, $P < 0.01$; data arcsine square-root transformed).

Fruit production from breeding system crosses compared with open-pollinated flowers is presented in Table 3. In general, fruit production for all treatments was high (69.7% - 90.3%). Within each population, no significant differences were found in fruit production between any of the breeding system crosses or open-pollinated flowers ($G = 7.02$, $df = 4$, $P > 0.05$ and $G = 3.46$, $df = 1$, $P > 0.05$ for Site M and Revis Hill Prairie, respectively). Comparing fruit production from open-pollinated flowers between populations, the difference was significant ($G = 4.21$, $df = 1$, $P < 0.05$).

Seeds from the breeding system crosses and from open-pollinated flowers were treated as suggested by Dr. Judith Canne-Hilliker, and sown into pots containing Sorghastrum nutans. Number of seeds sown was not quantified, however the number sown was in the thousands (approximately 150 seeds/fruit x 179 fruits). Few seeds germinated (estimated at 15-20). Of those that germinated, none became established on host plants.

DISCUSSION

Agalinis skinneriana is a bee-visited prairie plant of Illinois flowering from mid-August through September. Breeding system results clearly indicate that local populations are autogamous (set fruit without the aid of any pollen vector). According to Robertson and Phillippe (1993), Agalinis skinneriana has always been a rare and difficult species to locate. However, it is not clear as to whether the rare populations have always contained few

individuals. Habitat destruction could have reduced population sizes. The autogamy observed in current populations may have been selected for as a mechanism to insure seed production in the face of declining population sizes and reduced pollinator attractiveness. This is suggested by the small population a Revis Hill Prairie and the complete lack of bee visitation.

Reduced bee visitation that could promote outcrossing among individuals and increased autogamous seed production can result in inbreeding depression (reduced fertility) and loss of genetic diversity. Again, data suggest this may be occurring for the Revis Hill Prairie population. Plants at this locality produced significantly fewer flowers, fruits, and viable pollen compared to plants at Site M. This lower fertility could not be a density effect since the Revis population was much less dense than the Site M population. Fruit production for open-pollinated flowers at Site M was significantly higher than Revis Hill Prairie and may be attributable to the bee pollination observed at Site M. Although these data are suggestive, a knowledge of the actual selfing and inbreeding levels for both populations is crucial before any strong conclusions can be made. Unfortunately, the experiments that could have provided this information failed. Research on these topics is currently in progress.

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Table 1. List of bees visiting the flowers of Agalinis skinneriana at Site M in 1994.[a]

Bee taxa	Number collected	My collection #
Hylaeus affinis	1	998
Megachile brevis	4	987, 988, 990, 997
Megachile mendica	1	989
Lassioglossum sp.	1	986
Bombus impatiens	5	985, 991, 992, 993, 996
Bombus pennsylvanicus	1	994

[a] All bee speciemens execpt the genus Bombus were identified by Dr. John Neff of Austin, TX. Species of Bombus were identified by the author.

Table 2. Reproductive characteristics for the Site M and Revis Hill Prairie populations of Agalinis skinneriana studied in 1994. Parentheses contain the standard error of the mean.

Population	Site M			Revis Hill Prairie		
Characters	N	Mean	Range	N	Mean	Range
Density (plants/m^2)	15	5.15		15	2.07	
Size (total no. of plants)		19,313			466	
Number of flowers	25	15.8 (2.1)	5-46	25	10.5 (1.2)	2-30
Number of fruits	25	12.2 (1.8)	4-41	25	7.4 (1.1)	0-15

Table 3. Fruit production from breeding system crosses compared with that from open-pollination for Agalinis skinneriana in 1994.

	Site M				Revis Hill Prairie			
Treatment	N_{plants}	$N_{flowers}$	N_{fruits}	% fruit	N_{plants}	$N_{flowers}$	N_{fruits}	% fruit
No cross (autogamy)	26	26	22	84.6	11	11	8	72.7
Selfed	30	30	27	90.0	21	21	17	80.9
Xenogamous	31	31	28	90.3	20	20	17	85.0
Open	25	398	306	76.9	25	264	184	69.7

PEOPLE / LIVING

Dieringer Receives Grant

Dr. Gregg Dieringer, plant ecologist at Western Illinois University, received a grant from the Illinois Wildlife Preservation Fund, awarded by the Illinois Department of Conservation. The project focuses on "Reproduction and Inbreeding in Aglaninis skinneriana," commonly known as pale false foxglove. It is a rare plant species that is showing evidence of vulnerability to possible extinction.

Pale false foxglove is distributed primarily in midwestern states. Within Illinois it is only found in eight counties, including nearby Mason county, with population sizes ranging from approximately 100-1000 individuals. Flowers of the annual species are pink/purple and pollinated by bees.

As is typical of many threatened species, pale false foxglove grows in a specialized habitat, loess hill and sand prairie in Illinois. This kind of habitat is increasingly difficult to find because of cultivation and fire suppression. Fire suppression of the prairie usually allows the invasion of woody trees and shrubs which ultimate brings an end to prairie habitat.

The small isolated populations of pale false foxglove make them particularly susceptible to localized breeding patterns which eventually reduced genetic variability. The loss of genetic variability places populations at risk of extinction.

The object of Dieringer's investigation will be to describe the pollination biology and determine the breeding system. This data will allow the assessment of the genetic variability present and aid in the determination of the kind of crossing program that will be needed to produce viable seed for reintroduction into other areas of suitable habitat.

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