Conservation implications of the reproductive ecology of *Agalinis acuta* (Scrophulariaceae)[1]

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Reproductive ecology of *Agalinis acuta* was investigated by examining potential for self-fertilization before and at anthesis, reproductive output from outcrossed vs. selfed matings, and effects of browsing, plant size, and conspecific plant density on seed and fruit production. These features of a plant species can provide indirect information pertinent to conservation such as patterns and maintenance of genetic diversity, risk associated with inbreeding depression, and changes in pollinator abundance or effectiveness. The species is self-compatible, with 97% of selfed flowers setting fruit; pollinators were not required for reproduction. However, seed set in self-pollinated fruits averaged 17–20% less than that in open-pollinated fruits. Geitonogamous and facilitated selfing are possible throughout anthesis and autonomous selfing is possible late in anthesis as corollas abscise. Delaying self-pollination until after outcrossing opportunities likely limits selfing rates and thus reduces risks associated with inbreeding but allows reproduction in absence of pollinators. Supplementing pollen on open-pollinated flowers yielded no additional seed set over controls. Neither early-season browsing of primary stems nor conspecific plant density had significant effects on number of fruits per plant, on fruit size, or on number of seeds from open-pollinated flowers. Currently, reproduction appears to be high (about 2400 seeds/plant), and future risks due to lack of genetic diversity are likely low.

Key words: *Agalinis acuta*, endangered species, mating system, reproductive ecology, Scrophulariaceae.

The purpose of this research was to investigate the reproductive ecology of the federally listed endangered plant, *Agalinis acuta* Pennell. Specifically, I investigated potential for self-fertilization before and at anthesis, reproductive output resulting from outcrossed and selfed matings, as well as effects of browsing, plant size, and conspecific plant density on reproduction. Further, insect visitors to *A. acuta* were identified. Knowledge of each of these features of a plant species can provide indirect information pertinent to conservation efforts such as patterns of genetic diversity, threats of diversity loss, risk of inbreeding depression, and risks associated with changes in pollinator abundance or effectiveness. Data on basic reproductive ecology can be collected relatively cheaply and easily in the field and can help determine if more intensive and expensive investigations are warranted.

For example, knowing the mating system of a plant provides indirect information on patterns of genetic diversity because of the important role mating systems play in structuring that diversity (Hamrick, Linhart, and Mitton, 1979; Brown, 1989; Hamrick and Godt, 1989; Loveless and Hamrick, 1989; Ritland, 1989). Predominantly outcrossing taxa typically have high levels of diversity within and relatively little differentiation among populations; highly selfing species show just the opposite pattern. Thus, genetic diversity in highly outcrossed taxa is thought to be effectively conserved by maintaining relatively few populations of sufficient size to be protected from genetic drift. Additional populations provide redundancy but are not likely to capture substantial additional diversity (e.g., Brown, 1989). In contrast, capturing the range of genetic diversity present in differentiated populations of a highly selfed taxon requires protection of a larger number of populations, even if those populations are somewhat smaller. Of course, other factors such as founder effects, bottlenecks, and evolutionary history are also important in determining levels of genetic diversity (e.g., O'Brien, Wildt, and Bush, 1983; McCauley, Raveill, and Antonovics, 1995).

Mating system characteristics also strongly affect maintenance of diversity and potential effects of inbreeding. The degree of nonrandom matings in a population (e.g., self-fertilization, biparental inbreeding, or correlated paternity) directly affects rates of inbreeding (reviewed in Ellstrand, Torres, and Levin, 1978; Ritland, 1984, 1989; Barrett and Kohn, 1991). Inbreeding decreases the frequency of heterozygotes in a population and ultimately can result in inbreeding depression (lowered fitness of inbred individuals relative to individuals from outcrossed matings) (e.g., Wright, 1965; Charlesworth and Charlesworth, 1987). Correlated matings can also increase rates of loss of diversity due to genetic drift by decreasing effective population size. Consequences of such losses are debated and include possibly limiting the evolutionary potential of a taxon (Barrett and Kohn, 1991) and increasing extinction probability (Newman and Pilson, 1997). Regardless of mating system, risks from inbreeding and drift are higher in small than in large populations and are especially high in populations that have been reduced in size. Fragmentation will exacerbate these effects by isolating remaining populations and limiting gene flow (Young, Boyle, and Brown, 1996). However, specific manifestations of inbreeding risks will differ among selfing vs. outcrossing taxa. In general, we expect higher selfing rates and thus inbreeding depression in small than large populations in the same taxon, particularly if the taxon is highly self-fertile.

However, populations that typically self and that naturally occur in small populations may have purged their genetic load and thus experience less inbreeding depression than species that typically outcross (Charlesworth and Charlesworth, 1987) or occur in populations that have been reduced in size (Weller, 1994) (except see Ågren and Schemske, 1993). In contrast, actual selfing rates in self-incompatible taxa will not change as population size changes, although the apparent selfing rate may increase due to biparental inbreeding (Ellstrand, Torres, and Levin, 1978). Outcrossed taxa can harbor many more deleterious alleles than self-compatible taxa and thus are at higher risk from inbreeding depression when biparental inbreeding is increased in small or reduced populations (Charlesworth and Charlesworth, 1987). Small or reduced populations of plants with sporophytic incompatibility systems are at additional risk of losing compatible mating types (DeMauro, 1993; Young and Brown, 1999). Clearly, knowledge of mating system can provide information for selecting populations for conservation and for determining as well as preventing effects of changes in population size and degree of population fragmentation.

In self-compatible taxa, timing of self-pollination strongly affects the amount of selfing that actually occurs. Lloyd and Schoen (1992) have identified five types of self-pollination in chasmogamous flowers: geitonogamy (transfer of pollen between flowers on the same plant), facilitated selfing (vector mediated selfing within a flower), and three types of autonomous selfing that differ in relative timing of self and outcrossed pollination (prior, competing, and delayed). Geitonogamous, facilitated, prior, and competing selfing can reduce the amount of outcrossed pollinations in a plant because they occur before or at the same time as outcrossed pollinations and can clog stigmas with self-pollen. In contrast, delayed selfing can provide reproductive assurance when pollination is not adequate without affecting outcrossing potential.

Understanding dependence on pollen vectors also has important conservation implications. Obviously pollinator-dependent taxa, regardless of self-compatibility, will suffer reproductive failures if pollinators are ineffective or absent (Kearns, Inouye, and Waser, 1998). Pollinators are more likely be absent or ineffective in small than large plant populations (Jennersten, 1988; Rathcke and Jules, 1993; Van Treuren et al., 1993, 1994; Karron et al., 1995; Ågren, 1996; Cunningham, 2000). However, due to need for pollen from compatible donors, self-incompatible taxa will likely suffer more than self-compatible taxa when pollinators are simply reduced (Byers and Meagher, 1992; DeMauro, 1993; Young and Brown, 1999). Comprehensive species recovery plans need to address maintenance of all parts of the ecosystem on which an endangered species depends, including pollinator communities. The first step is to identify pollen vectors for a species and subsequently to maintain conditions that will allow continued pollination. If the vector is an animal, ensuring that habitat requirements are met is essential. Of course part of a pollinator's habitat requirements may include sufficient numbers or densities of the rare plant itself to provide pollen or nectar rewards. Thus, as populations of a rare plant decline, pollinators may be unable to locate the remaining plants or may move to more profitable foraging areas. Changes in pollination due to these effects can in turn affect mating patterns in populations (Ellstrand and Foster, 1983; Murawski and Hamrick, 1992; Karron et al., 1995; Kennington and James, 1997). In summary, it is particularly important to understand the reproductive ecology of rare species in which populations have been fragmented and population sizes have been reduced, such as *A. acuta*.

Prior work on the reproductive ecology of other *Agalinis* species indicate a range of mating systems and thus is not helpful in predicting the mating system of *A. acuta*. For example, *A. strictifolia* (Dieringer, 1991) is self-incompatible, while *A. skinneriana* (Dieringer, 1999), *A. neoscotica* (Stewart, Stewart, and Canne-Hilliker, 1996; Stewart and Canne-Hilliker, 1998), *A. paupercula*, and *A. maritima* (unpublished data) are self-compatible. Furthermore, potential for autogamous selfing in *A. skinneriana* varied among populations, being higher in a small than in a large population (Dieringer, 1999). *Agalinis neoscotica* is interesting in that it self-pollinates in bud as the anthers dehisce precociously (1–2 d before anthesis), and at the same time, germination of selfed pollen is delayed until anthesis (Stewart, Stewart, and Canne-Hilliker, 1996). Stewart and Canne-Hilliker (1998) consider self-compatibility to be derived within the genus.

MATERIALS AND METHODS

Study species—*Agalinis acuta* is an annual herb endemic to the coastal plain of eastern North America. It has thin, angular stems with up to 2.5 cm long, linear-filiform shaped leaves (Pennell, 1935). Individual plants grow to 10–40 cm high (Gleason and Cronquist, 1991). Its cup-shaped, slightly zygomorphic, 10–15 mm long flowers are pink with two yellow nectar guides and reddish-purple spots in the tubular throat (Pennell, 1935). Stamens are epipetalous; anthers, filaments, and the upper corolla lobes are densely covered with nonglandular trichomes. This species typically blooms between mid-August and mid-October with peak bloom in early September. Plants can bloom for up to several weeks; individual flowers, however, open for several hours on one day only. Fruits are ovoid, bicarpellate, many-seeded capsules; seeds are irregularly triangular to elliptical and measure between approximately 0.1 mm in width and approximately 0.4–0.6 mm in length (Canne, 1979).

The type specimen for *A. acuta* was collected in 1901 at Edgartown, Massachusetts, USA, on the island of Martha's Vineyard (Pennell, 1935). This federally listed endangered plant species is currently known from a total of 13 naturally occurring sites in five states: Massachusetts (4), Connecticut (1), Rhode Island (1), New York (6), and Maryland (1) (Neel and Somers, 2001), although the population at the type locality no longer exists. Throughout its range this species grows in open vegetation such as sandplain grassland and in openings in pine–oak forest on nutrient poor soils such as glacial outwash or serpentine. Major threats include loss of suitable habitat due to anthropogenic conversion of the coastal plain to agricultural, residential, industrial, and commercial development (U.S. Fish and Wildlife Service, 1988). Vegetational succession from open habitats to closed-canopy forest has also caused habitat loss and is a continuing threat at all remaining sites (U.S. Fish and Wildlife Service, 1988). As such, remaining populations of this species persist only at sites with continued management to reduce cover of competing vegetation through mowing or prescribed burning. For example, the two remaining naturally occurring populations on Cape Cod, Massachusetts, USA, occur in cemeteries that have been mowed regularly and thus maintain unforested sandplain grassland communities. Large population fluctuations are typical for this species; censuses in the two naturally occurring populations on Cape Cod between 1980 and 2000 ranged from 1 to 4253 plants at one site and from 0 to 3674 plants at the other (Neel and Somers, 2001). Fluctuations are associated with weather conditions (e.g., lowest population sizes followed Hurricane Bob) and with mowing regimes (e.g., populations increased dramatically after altering the seasons in which mowing occurred at one site; Neel and Somers, 2001).

Study site—The research was conducted in an artificially established population of *A. acuta* at the Waquoit Bay National Estuarine Research Reserve on Cape Cod, Massachusetts, USA (Fig. 1). This population occurs in sand-
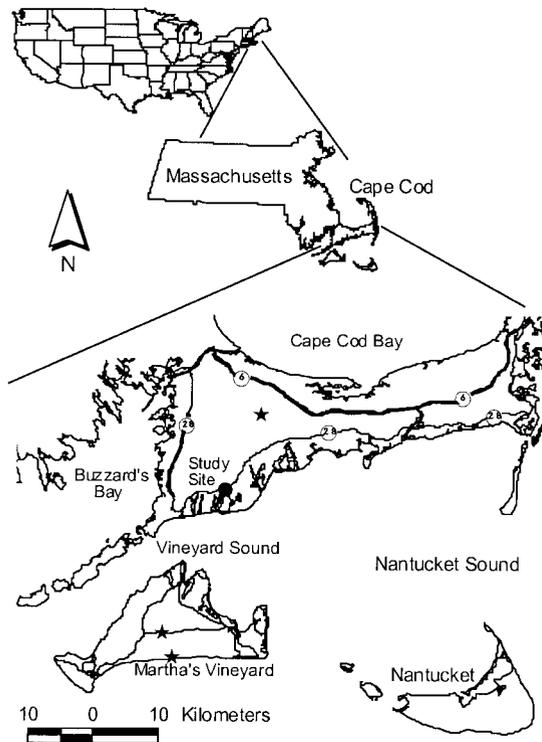
Fig. 1. Study site location on Cape Cod, Massachusetts, USA (●), and other nearby *Agalinis acuta* populations (★).

plain grassland habitat dominated by *Schizachyrium scoparium*, *Arctostaphylos uva-ursi*, *Solidago nemoralis*, *Ionactis linariifolia*, *Danthonia spicata*, *Festuca filiformis*, and *Cladonia* sp. Within this general habitat, *A. acuta* occurs in areas of relatively low plant and litter cover and during this research occupied an area totaling approximately 600 m². This population is located within 300–400 m of the naturally occurring Bay View Cemetery population and was established between 1994 and 1998 through a series of experiments to determine appropriate methods for establishing and maintaining this species (Neel and Somers, 2001). Tens of thousands to hundreds of thousands of seeds from the Bay View Cemetery site were used to start this population, which consisted of an estimated 30 000–60 000 individuals in the year 2000. This population is considered to be representative of natural populations for the purposes of this research and was chosen over the adjacent naturally occurring population to avoid potential conflicts associated with conducting research that required marking and repeatedly visiting plants in an active cemetery.

Relative anther and stigma positions in bud and at anthesis—Potential for pre-anthesis selfing was assessed using a dissecting microscope to examine anthers for dehiscence and stigmas for apparent receptivity, as well as stigma position relative to the anthers in four buds each 1 d and 2 d prior to flowering. To evaluate potential for autogamous selfing at anthesis, progression of flowering, as well as anther and stigma maturation and their relative positions, were observed on three flowers each at four different times from 8 to 11 September 2000, the middle of the blooming period for this species.

Pollination treatments—Pollination treatments were applied to 40 individuals that were selected from throughout the population on the condition that they were large enough to have at least 4–6 flowers open at the same time, preferably on the day following bagging. Thus plants were not selected randomly. At least two buds per plant were bagged in fine tulle that prevented access to flowers by insects. Two buds on each plant were marked and left untreated to serve as controls. In some cases additional flowers were sampled to provide flowers in different treatments that were open on the same day. Equal numbers of plants were randomly assigned to two treatment groups. In

the first group, bagged flowers were not otherwise manipulated (47 flowers on 20 plants). This treatment tested for self-compatibility and a mechanism for transfer of pollen from anthers to stigmas in absence of pollinators (autogamy). Agamospermy could also result in seed set without pollination and cannot be distinguished from self-pollination with this experimental design. In the second treatment, bagged flowers were hand-pollinated with pollen from the same plant excluding the two control flowers (44 flowers on 20 plants). This treatment assessed ability to self with pollinator-mediated movement of pollen from anthers to stigmas. Seed set resulting from the second treatment but not the first indicates dependence on pollen vectors. Low or absent seed set in only the second treatment would most likely indicate ineffective hand-pollination. To test for pollen limitation to seed set in open-pollinated flowers, pollen was supplemented on one or two flowers on 27 of the 40 treatment plants that had a sufficient number of flowers for all treatments on the same day. Pollen from four donor individuals was applied to the stigma of each flower by first brushing the open anthers of a donor flower with a paintbrush and then brushing the stigma of the recipient flower. Self-pollen was also added to the stigma using this procedure. It was not possible to control for the amount of pollen each flower received. Donor plants were chosen from the vicinity of the target plant but typically were not chosen from the closest neighbors to reduce potential for crossing close relatives. All flowers were marked to identify the day on which they were bagged, the day they were actually open, and the treatment group to which they belonged. All bags were removed from plants within 3 d after blooming to reduce effects from the bags themselves.

Fruits were collected upon ripening but prior to dehiscence. Upon collection, length and diameter at the widest point of each fruit was measured to the nearest 0.1 mm using Mitutoyo dial calipers (Mituyoyo Corp., Aurora, Illinois, USA). Numbers of seeds in each fruit were counted under a dissecting microscope once fruits had dried completely. Time to fruit maturity was calculated as the number of days between anthesis and collection for all fruits reaching maturity (i.e., aborted or eaten fruits were not included in totals).

Data from all fruits in each treatment were used to summarize mean values and associated error estimates for fruit length and width, time to maturity (in days), and number of seeds per fruit. Prior to further analysis, the average value for each treatment from each individual was calculated to eliminate problems resulting from dependent observations. Data were not normally distributed, and distributions were not improved with transformations, so nonparametric statistics were used for all analyses. Specifically, I used Wilcoxon matched pairs tests to assess significance of differences in fruit length, width, and time to maturity, as well as seed number between each of the three treatments and controls within plants. The matched pairs test controls for among plant variation in response variables. Size of and number of seeds produced in hand-selfed fruits were compared with simply pollinator-excluded fruits from different plants using Kruskall-Wallis analysis of variance (ANOVA). All analyses were conducted in Statistica 5.1 for Windows (Statsoft, 1997).

Insect visitors—All insects observed visiting *A. acuta* individuals during this work were noted and representative specimens were collected for identification.

Relationships of plant density and size with reproduction—Height and width of each of the 40 target plants were recorded as were total number of live, aborted, and browsed fruits produced by each plant. The number of seeds produced by open-pollinated fruits from the pollination treatments was also noted. To examine effects of plant density on reproductive success, the number of *A. acuta* in 1-m² plots centered on each of the 40 plants used in this experiment was counted. Relationships of conspecific plant density and plant height and width with number of mature fruits, percentage of aborted and browsed fruits, and number of seeds produced in open-pollinated fruits were examined using Spearman's rank correlation. Relationships between plant height and number of mature fruits, percentage of aborted and browsed fruits, and number of seeds produced in open-pollinated fruits were examined using Spearman's rank correlation.
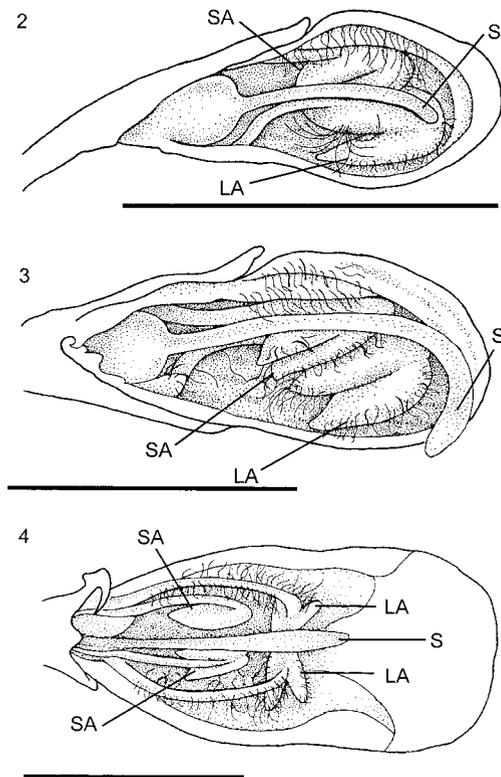
Figs. 2–4. Relative positions of *A. acuta* stigma (S) and anthers on long (LA) and short (SA) stamens (Fig. 2) in longitudinal section of bud 2 d prior to blooming; (Fig. 3) in longitudinal section of bud one day prior to blooming; and (Fig. 4) upon flower opening looking down from top of flower to the lower corolla lip with other four corolla lobes removed. Scale bar in all drawings = 5 mm.

Effects of browsing—Effects of browsing on *A. acuta* were assessed by comparing number of main and lateral stems with number of mature fruits and with percentages of aborted or eaten fruits produced by browsed vs. unbrowsed individuals. Main stems were defined as larger stems arising from or near the base of the plant. Lateral branches arose along a main stem; they tended to be smaller than main stems and they also bore flowers. Significance of browsing effects on total reproductive output was examined using presence of browsing of the primary stem as the independent variable and number of main stems and number of mature fruits and percentage of aborted or eaten fruits as dependent variables in separate Kruskall-Wallis ANOVAs.

RESULTS

Relative anther and stigma positions in bud and at anthesis—Two days prior to blooming, *A. acuta* buds were approximately 5 mm long from the calyx base to the distal end of the corolla. At this stage anthers were closed; the style lay along the dorsal side of the flower and was only slightly

curved ventrally (Fig. 2). The stigma lay amongst both sets of anthers, but none of them were fully developed: stigmas lacked the papillae that are characteristic at maturity, anthers were still closed, and filaments were not fully elongated. One day prior to blooming, buds were approximately 8 mm long. At this time the style was elongated and the stigma was dorsal and distal to the anthers (Fig. 3). Stamens were slightly differentiated into two long (held abaxially) and two short sets (held adaxially) (Fig. 3). Only one anther in one flower appeared to have dehisced in bud; this likely resulted from disturbance during floral dissection. At anthesis corollas were between 9 and 12 mm long; anthers were between 1.1 and 1.5 mm long and were 0.5 mm wide. Long and short stamens were strongly differentiated in length; anthers of the short pair were included in the throat while anthers of the longer pair were slightly exserted or held at the mouth of the corolla tube (Fig. 4). The style was dorsal to the filaments and anthers of the long stamens and passed between the filaments of the short stamens; however, the stigma was distal to both sets of anthers and was exserted from the corolla (Fig. 4). Anthers dehisced via longitudinal slits that faced away from the stigma for the duration of anthesis (Fig. 4).

On a given day, time of flower opening and pollination was related to temperature or sunlight, while cessation of flowering was also dependent on wind conditions. Flowers typically began opening at 0700–0730 and most were open by 0830. Anthers were dehiscing upon flower opening on warmer days, but on cooler days they could dehisce as much as 1 h after flowers opened. Corollas typically began falling off of plants by 1100 and finished dropping by 1400–1500, although corolla duration was highly dependent on wind conditions. As corollas abscised from flowers, they often remained hanging partially off, providing ample opportunity for contact at least between the short anthers and the stigma during a period when the stigma would still be receptive. Depending on wind conditions, anthers and stigmas could remain in contact for 1 h or more. No corollas of open-pollinated flowers were observed to remain on flowers for more than 1 d. Corollas of some bagged flowers were held in contact with the rest of the flower overnight due to physical constraints of the bags.

Pollination treatments—One plant (in the pollinator-excluded treatment) died during the experiment; both control flowers on one plant were browsed, as were both hand-selfed fruits on another plant, and both pollen-supplemented fruits on yet another plant. All results are based on the remaining 39 individuals and complete treatment-control pairs (actual sample sizes are provided for each comparison). All treated flowers bloomed between 9 and 13 September 2000. Mature fruits were collected between 23 October and 18 November. Mean fruit size, time to maturity, and seed number for each treatment are summarized in Table 1. Based on results of Wilcoxon

TABLE 1. Summary statistics for number of live, aborted, or eaten fruits and for mean values (1 SD) for fruit size, days to maturity, and number of seeds produced by mature fruits in each pollination treatment.

Treatment	No. mature fruits	No. aborted fruits	No. fruits eaten	Fruit length (mm)	Fruit width (mm)	Average no. seeds	No. days to fruit maturity
Open-pollinated control	84	1	3	3.52 (0.59)	2.42 (0.34)	74.8 (26.0)	55.2 (0.51)
Pollinator excluded	46	1	2	3.21 (0.62)	2.31 (0.37)	61.8 (24.9)	55.3 (0.78)
Pollinator excluded, hand-pollinated	36	2	2	3.25 (0.69)	2.31 (0.34)	59.6 (28.3)	53.8 (1.11)
Open-pollinated, pollen supplemented	48	0	0	3.63 (0.38)	2.45 (0.27)	72.8 (22.2)	53.8 (0.53)

matched pairs tests there were no significant differences between control and the pollinator-excluded treatment ($N = 19$) in fruit length ($T = 49.5$, $Z = 1.83$, $P = 0.07$) or width ($T = 54.0$, $Z = 1.37$, $P = 0.17$). Fruit length ($T = 134.5$, $Z = 0.44$, $P = 0.66$) and width ($T = 138.0$, $Z = 0.00$, $P = 1.00$) also did not differ significantly between pollen-supplemented and control treatments ($N = 27$). However, length ($T = 0.00$, $Z = 3.52$, $P = 0.0004$) and width ($T = 12.5$, $Z = 2.87$, $P = 0.004$) of hand self-pollinated fruits were significantly less than control fruits ($N = 17$). Time to maturity did not differ between control fruits and either pollinator excluded ($T = 41.0$, $Z = 0.31$, $P = 0.75$) or hand self-pollinated fruits ($T = 19.5$, $Z = 1.82$, $P = 0.07$), but pollen supplemented fruits matured in significantly fewer days than control fruits ($T = 35.0$, $Z = 2.61$, $P = 0.009$). Number of seeds produced by fruits in the pollinator-excluded ($T = 38.5$, $Z = 2.27$, $P = 0.02$) and the hand self-pollinated ($T = 19.0$, $Z = 2.72$, $P = 0.006$) treatments were both significantly less than control fruits. Number of seeds produced by pollen-supplemented fruits did not differ from controls ($T = 148.5$, $Z = 0.97$, $P = 0.33$). Further, based on Kruskall-Wallis ANOVA, there were no significant differences in fruit length ($H_{1,37} = 0.0002$, $P = 0.99$), fruit width ($H_{1,37} = 0.002$, $P = 0.96$), days to maturity ($H_{1,37} = 0.335$, $P = 0.56$), or number of seeds ($H_{1,37} = 0.002$, $P = 0.96$) between flowers that were hand self-pollinated and those in which pollinators were simply excluded.

Insect visitors—Insect visitors to *A. acuta* flowers included three members of the order Hymenoptera: *Agapostemon virescens* (Fabricius), *Lasioglossum* sp. (both in the family Halictidae), and *Bombus impatiens* Cresson (Family Apidae); as well as two common members of the family Syrphidae (Order Diptera) *Toxomerus marginatus* (Say) and *Eupeodes americanus* (Wiedemann). One adult lepidopteran, *Schinia septentrionalis* (Walker), was also observed visiting an *A. acuta* flower. The bumble bee *B. impatiens* was by far the most frequent visitor, was the only one large enough to reliably contact both the anthers and stigma during a visit, and was the only one observed to have abundant pollen on body parts. *Bombus impatiens* individuals approached *A. acuta* flowers in an erect position from below a flower and alighted for no more than 1–2 s. While no quantitative estimates were made, *B. impatiens* abundance was observed to vary substantially among days depending on the weather. They ranged from very abundant (hundreds of individuals) on warm, sunny days to nearly absent on cool, rainy days. Larvae of the forest tent caterpillar (*Malacasoma disstria*; Order Lepidoptera) were observed eating leaves and fruits of *A. acuta*. Although none of the 40 plants used in this study were so affected, in some cases, *Malacasoma disstria* ate all leaves and fruits on individual plants. Other fruit predation was noted; however, the responsible species were not observed.

Relationships of plant density and size with reproduction—At maturity, the 39 surviving plants were between 12.9 and 32.9 cm tall (mean = 23.2 cm, 1 SE = 0.64) and 3.5–24.0 cm wide (mean = 12.3 cm, 1 SE = 0.77). They produced an average of 32.2 (1 SE = 3.23) mature fruits/plant (ranging from 10 to 108 fruits/plant). Mature fruits represented an average of 85.3% (1 SE = 1.73) of all flowers on a plant (range = 47–100%). Not surprisingly, plant height and total number of fruits were highly correlated ($R = 0.68$, $P = 0.000002$) but plant height was not correlated with percentage of fruits aborted ($R = -0.12$, $P = 0.47$) or eaten by insects ($R = 0.04$, $P = 0.81$). On average, most fruit losses were due to abortion (mean = 11.0%/plant; 1 SE = 1.30). While the average percentage of fruits eaten by insects was low (3.6%; 1 SE = 1.50) and 27 plants had no fruits eaten, 50% of the fruits on one plant were eaten. Densities of *A. acuta* in plots centered on the 39 surviving target plants ranged from 1 to 305 plants/m² and averaged 51.6 plants/m² (1 SE = 9.7). There was no relationship of plant density to fruit length ($R = -0.03$, $P = 0.87$), width ($R = -0.15$, $P = 0.36$), or seed production ($R = -0.20$, $P = 0.23$) in open-pollinated flowers or in overall number of mature fruits produced on a plant ($R = -0.04$, $P = 0.79$).

Effects of browsing—There were between 1 and 10 main stems/plant with an overall average of 1.6 stems (1 SE = 0.24). Twenty-seven individuals were unbrowsed and thus had the primary stem intact; these individuals never had more than one main stem. The number of lateral stems on unbrowsed plants ranged from 0 to 10 and averaged 5.4 (1 SE = 0.53). The 12 browsed individuals had a mean of 3 main stems/plant (1 SE = 0.66), while the number of lateral stems ranged from 0 to 11 and averaged 4.8 (1 SE = 1.25). The number of main stems on browsed plants was significantly higher than on unbrowsed plants ($H_{1,39} = 36.83$, $P = 0.00001$), but there was no significant difference in number of lateral stems ($H_{1,39} = 0.16$, $P = 0.69$). There was also no significant difference in the number of fruits produced by browsed (mean = 37.1, 1 SE = 7.9) and unbrowsed (mean = 30.1, 1 SE = 3.1) plants ($H_{1,39} = 0.45$, $P = 0.50$).

DISCUSSION

Fruit production and seed set in both pollinator-excluded treatments indicated that *Agalinis acuta* is self-compatible, as 97% of the 86 self-pollinated flowers that were not eaten by insects developed fruit and set seed (Table 1). The 98% fruit set in the pollinator-excluded treatment without hand-pollination indicated that this species is not dependent on pollinators for successful reproduction (Table 1). There is a possibility that seed set in absence of pollinators resulted from agamospermy, but agamospermy is not known from the genus (Dieringer, 1991, 1999; Stewart, Stewart, and Canne-Hilliker, 1996) and is relatively rare in angiosperms (Richards, 1999). Additionally, agamospermy is strongly associated with polyploidy, a condition not known from the genus (Canne, 1981, 1984). Further, self-compatibility has been documented in two other species in the genus, *A. skinneriana* (Dieringer, 1999) and *A. neoscotica* (Stewart, Stewart, and Canne-Hilliker, 1996; Stewart and Canne-Hilliker, 1998). As a result, I concluded that seed set in absence of pollinators was due to selfing.

As mentioned previously, timing and mechanism of self-pollination have important implications for relative amounts of inbreeding and outcrossing in self-compatible plants (Lloyd and Schoen, 1992). These features varied substantially among previously studied self-compatible *Agalinis* species. *Agalinis neoscotica* exhibits a combination of prior and either competing or delayed selfing; self-pollen is deposited on stigmas in bud, but germination of that pollen is delayed until anthesis (Stewart, Stewart, and Canne-Hilliker, 1996; Stewart and Canne-Hilliker, 1998). Selfing rates in that species ranged from $s = 0.356$ to 0.581. In contrast, timing of pollen tube growth indicated that selfed pollen was deposited on stigmas only late

in anthesis in *A. skinneriana* flowers excluded from pollinators, an example of delayed selfing (Dieringer, 1999). This late pollen deposition supports the idea that the anthers come into contact with stigmas as corollas fall from flowers ("corolla dragging"). Corolla dragging has also been observed to contribute to delayed selfing in some populations of *Mimulus guttatus* (Dole, 1990, 1992) but not others (LeClerc-Potvin and Ritland, 1994).

Pre-anthesis pollination in *A. acuta* is unlikely, given that during the time when the unlobed stigma is in close contact with the anthers in bud, the anthers are immature (Figs. 2 and 3). This morphology and development pattern are similar to those in *A. densiflora* (Canne-Hilliker, 1987) and *A. purpurea* (Stewart and Canne-Hilliker, 1998) but are in contrast to those in *A. neoscotica*, in which the lobed stigma is among the open anthers in bud (Stewart, Stewart, and Canne-Hilliker, 1996). At anthesis *A. acuta* is similar to *A. skinneriana* (Dieringer, 1999), *A. strictifolia* (Dieringer, 1991), and *A. purpurea* (Stewart and Canne-Hilliker, 1998), with the stigma dorsal and distal to the anthers and exserted from the corolla tube (Fig. 4). Interestingly, both obligately outcrossed (*A. strictifolia*) and self-compatible (*A. acuta* and *A. skinneriana*) taxa have similar floral morphology in this regard. While herkogamy in *A. acuta* prevents autogamy during early anthesis, there is ample opportunity for insect-mediated geitonogamy and facilitated selfing. *Bombus impatiens* individuals were frequently observed visiting multiple flowers on an individual plant before moving to another plant. In addition they were large enough to contact anthers and the stigma in a single flower, and given their approach pattern they would typically contact anthers first. There was also ample opportunity for delayed autogamous selfing due to corolla dragging. Depending on wind conditions, corollas (and more importantly their adnate anthers) may stay in contact with stigmas for extended periods of time, easily facilitating autogamous self-pollination. The densely matted trichomes on the corolla and stamens may hold corollas in place after they have abscised. In a rare, annual species in which each flower only blooms for 1 d, such a mechanism that promotes outcrossing and yet assures reproduction through delayed selfing is clearly advantageous.

These results indicate that fluctuations in pollinator effectiveness could have a moderate but not severe immediate effect on seed production and thus fitness in *A. acuta* populations. While small and low-density plant populations (Van Treuren et al., 1993, 1994; Karron et al., 1995; Ågren, 1996) and populations in fragmented habitats (Jennersten, 1988; Rathcke and Jules, 1993; Ågren, 1996; Cunningham, 2000) are known to have reduced pollinator visitation, autonomous selfing in this species appears to compensate and allow high levels of seed set. While fruit and seed set will not be strongly affected, there is likely to be a strong relationship between pollinator abundance and outcrossing rates, both from season to season and day to day. A cool or rainy day on which pollinators were not active could yield reduced reproduction and a large proportion of selfed seeds. More significant effects could result from years in which pollinators are absent or in low abundance. While a single generation without cross-pollination would not eliminate reproduction in *A. acuta*, it would be significantly reduced as seed set from selfed matings averaged 17–20% less than that from open-pollinated matings (Table 1). It is possible that levels of seed production due to corolla dragging indicated by bagged flowers are higher than they would be under natural conditions. Seed production in

unbagged flowers without pollinators might be lower than observed here because bags often prevented corollas from falling from the plant and thus increased contact time between anthers and stigmas during corolla dragging. In presence of pollinators, delayed selfing in unbagged flowers might be lower than in bagged flowers in which the amount of pollen remaining in anthers at the end of anthesis was unnaturally high because none of it had previously been removed by pollinators. In both cases, in addition to reduced seed set, the proportion of selfed offspring in the population would also be lower than would be anticipated by the current results.

The significant reduction in seed number in selfed compared with open-pollinated fruits may indicate insufficient pollination to sire all ovules, inbreeding depression manifested as differential abortion of selfed seeds early in development, or selection against selfed pollen through interactions between pollen tubes and stigmas or styles. Distinguishing these causes was beyond the scope of the present research. Selfing could have additional negative consequences not detected here. Effects of inbreeding depression later in the life cycle of a plant including reduced germination rates, delayed germination time, reduced reproduction in selfed relative to outcrossed individuals, and reduced autogamous selfing in selfed relative to outcrossed progeny are well documented (Karron, 1987, 1989; Johnston, 1992; Karoly, 1994; Nason and Ellstrand, 1995). Reduced seed set or selfing ability could exacerbate problems associated with small populations and could accelerate population decline due to interactions between small population size, pollinator effectiveness, and reductions in self-compatibility. However, under circumstances in which population sizes are sufficiently large, a mechanism that limits potential for selfing could have the positive effect of preventing excessive inbreeding. Relative risks and benefits depend on long-term population sizes, the proportion of time populations are small vs. large, on actual selfing rates, and on the severity of inbreeding depression due to selfing.

In contrast to the obligately outcrossing *A. strictifolia*, (Dieringer, 1992a, b), *A. acuta* was not pollen limited. Pollen limitation can be expected in both outcrossed and mixed-mating species (Karoly, 1992) but may be more easily detected in highly outcrossed or self-incompatible species. In the case of *A. acuta*, either pollination was sufficient for full seed set or autogamous selfing compensated for shortages in pollinator-mediated reproduction. Such compensation has been found in a number of other self-compatible taxa (Jennersten, 1988; Vaughton, 1988) and has been found to be related to patch size (Sih and Baltus, 1987). During the season in which this research was conducted, the *A. acuta* population at Waquoit Bay National Estuarine Research Reserve (as well as the other populations on Cape Cod) was exceptionally large and dense, and pollinators were abundant, circumstances under which pollen limitation might not be detected.

The range of *A. acuta* densities within 1 m of target plants had no effect on seed production in 88 open-pollinated fruits or on total fruit production, abortion, or predation on 39 plants. These results contrast with numerous studies demonstrating relationships between plant density and reproductive success (Jennersten, 1988; Van Treuren et al., 1993, 1994; Karron et al., 1995; Ågren, 1996; Bosch and Waser, 1999, 2001). Lack of local density effect in *A. acuta* could be because overall plant densities and pollinator abundance were high enough to facilitate pollination in all plants regardless of local density.

Alternatively, as discussed above, autogamy may compensate for low pollinator service of isolated individuals.

Early season browsing of primary stems increased the number of main stems produced by *A. acuta* individuals but had no significant effect on flower or fruit production. A similar response to herbivory was found in *Epilobium latifolium* (Doak, 1991), while browsed *Ipomopsis aggregata* are known to overcompensate in both seed production (Paige and Whitham, 1987) and paternity (Gronemeyer et al., 1997) when flowering stalks are browsed. In other circumstances, Juenger and Bergelson (2000) found that early season browsing (as simulated by clipping) significantly reduced flower, fruit, and seed production in *I. aggregata*. The apparent browsing in *A. acuta* was potentially due to rabbits but could also have resulted from mowing in the population. The area supporting this population is mowed several times each year, primarily in the spring. Mowing is typically stopped by 1 July each year to prevent damage to young *A. acuta* individuals but could have clipped stems of some plants. Additional negative effects of herbivory from the forest tent caterpillar, *Malacasoma disstria*, were noticed in this population but not in sampled individuals. While a number of individuals were killed outright by herbivory, most affected individuals sustained much less damage. For example, on the individuals studied here, on average only 3.6% of all fruits produced on a plant were eaten by insects. Thus, this season, herbivory and fruit predation did not have a significant impact on reproduction. Heavy predation has been noted in other years (P. Somers, Massachusetts Natural Heritage and Endangered Species Program, personal communication).

Conservation and management significance—Historical population sizes for *A. acuta* are unknown, but it is clear that there were a larger number of populations than currently remain (U.S. Fish and Wildlife Service, 1988). It is likely, however, that populations of this species were always patchily distributed in recently disturbed grasslands. As with most rare species, primary threats include habitat loss and subsequent degradation of remaining habitats through fragmentation and other effects on habitat quality. Extirpation or extinction from environmental stochasticity is always a major concern in these situations. Rare taxa that naturally occur in or have been reduced to small, isolated populations, such as *A. acuta*, are often at increased risk of extirpation and extinction due to such factors as inbreeding, genetic drift, and unreliable pollinator service (Barrett and Kohn, 1991; Ellstrand and Elam, 1993; Kearns, Inouye, and Waser, 1998). Actual risks from each of these factors are related to reproductive ecology of a species, including mating system. Conservation and management implications of the reproductive ecology of *A. acuta* are discussed below.

Given high levels of self-compatibility and other ecological characteristics of this species (floral life of only a single day, daily fluctuations in pollinator abundance, and dramatic annual fluctuations in *A. acuta* population sizes) it is possible that *A. acuta* selfs regularly. If selfing is frequent, this taxon is at risk of having low levels of within-population genetic diversity in terms of heterozygosity and potentially low numbers of alleles per locus (e.g., Hamrick, Linhart, and Mitton, 1979; Brown, 1989). Further, populations may be more inbred than they were historically if population sizes have been greatly reduced and fragmentation greatly increased compared with historical levels. Of course actual levels of diversity depend on long-term

effective population sizes and evolutionary history in addition to selfing rates. Genetic diversity patterns have been examined in two other members of the genus and support the importance of mating system in affecting these patterns. The self-compatible *A. skinneriana* was found to have little diversity in five examined populations, as it was found to be monomorphic at 59 of 60 randomly amplified polymorphic DNA loci (Kercher and Sytsma, 2000). In contrast, the outcrossing *A. strictifolia* had high levels of genetic diversity and low levels of population differentiation at five allozyme loci. While *A. acuta* can self, actual selfing rates under natural conditions are unknown and may not be excessive due to the delayed selfing mechanism that does not reduce outcrossing opportunities prior to corolla abscission. Further, loss of genetic diversity even under periodically high levels of selfing could be ameliorated by a seed bank, which *A. acuta* is known to possess (P. Somers, personal communication). Regardless of the potential risks, current levels of reproduction indicate that the population is not suffering from extreme lack of diversity. Based on averages of 74.8 seeds/open-pollinated fruit and 32.2 fruits/plant we can crudely estimate that plants in this population produce an average of approximately 2400 seeds/plant. It is also possible that frequent selfing over long periods of time may have purged deleterious alleles and reduced the genetic load in this species thus it may not suffer from severe inbreeding depression even if it is genetically depauperate (Lande and Schemske, 1985). There is no way to know if current levels of reproduction are lower than historical levels; however, reproductive failure is clearly not a current threat. Lack of seed germination is potentially a concern but is thought to be related to dormancy mechanisms rather than seed inviability. All in all, potential risks related to lack of genetic diversity and inbreeding depression within populations are likely low in this species.

In summary, risks associated with loss of genetic diversity or lack of pollination appear to be low for this species. Even if genetic diversity in *A. acuta* populations is low, lack of diversity is not currently impairing reproduction, at least in the population studied here. Additionally, because reproduction in this species is not completely dependent on pollination, reductions in pollinator abundance or effectiveness will not have immediate dire consequences. Thus, while it is important to maintain conditions that foster large population sizes and pollinator communities, occasional small population sizes or reduction in reproduction for limited periods will not likely have drastic effects. Population sizes are known to fluctuate with annual weather conditions and with mowing and burning regimes. Obviously the first factor cannot be controlled. Beneficial effects of mowing and prescribed burning on population size have been documented (Neel and Somers, 2001). There is, however, risk of clipping *Agalinis* plants if mowing occurs too late in the growing season. Lack of effect of browsing on reproduction indicates that risks associated with early-season mowing are small and will not likely significantly affect reproduction in this species. In contrast, not mowing or otherwise clearing vegetation significantly and immediately reduces *A. acuta* populations.

While investigations of reproductive ecology allow only general predictions of genetic diversity patterns, they allow assessment of current effects on reproduction and risks of future genetic diversity loss. More information regarding patterns of genetic diversity within and among populations would be interesting but because there appear to be no current det-

rimental effects and future risks are low, such information would not likely change suggestions for management and recovery of this species. Further investigation of mating patterns in relation to pollinator abundance would provide insight into actual levels of selfing and potentially into mechanisms of genetic diversity maintenance. Such information would be much more useful than a basic genetic diversity survey but is still not likely to be essential for management. Recovery activities should continue to focus on protecting existing sites and managing habitat conditions to promote large population sizes. Current management techniques of mowing and burning appear sufficient to accomplish this goal if they are done consistently from year to year.

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