

# Reproductive biology and pollination ecology of the rare Yellowstone Park endemic *Abronia ammophila* (Nyctaginaceae)

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## Abstract

We examined the breeding system, reproductive output and pollination ecology of *Abronia ammophila* Greene, a rare and highly restricted endemic of Yellowstone National Park, Wyoming, USA. Floral morphology permits the automatic deposition of self-pollen on the stigma of individual flowers, and male and female reproductive functions temporally overlap. In controlled hand-pollination treatments, we found no significant difference among pollination treatments (unmanipulated, self-pollinated or cross-pollination). The species maintains a long reproductive season with high reproductive output (natural seed set ranged from 59 to 84%). Our results, along with pollinator observations, suggest that *A. ammophila* exhibits a mixed-mating system: the species can produce seed without pollinators (via either autogamy or agamospermy), but is also visited by an array of pollinating insects that included moths, butterflies and bumblebees. However, noctuid moths were the most abundant pollinators. In contrast, other *Abronia* species are obligate outcrossers. The mixed-mating system of *A. ammophila* may have evolved as a consequence of ecological pressures such as scarcity of mates or pollinators.

**Keywords:** autogamy, breeding system, moth pollination, Nyctaginaceae, rare plant.

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## Introduction

The need to gather information on the basic biology of rare species is vital to both species- and community-level conservation efforts. From a theoretical perspective, such information is also necessary to examine patterns of rarity in a broader sense, and to understand general differences (if any) between common and rare species (Kunin & Shmida 1997). Such information includes knowledge of the breeding system, natural levels of reproductive output and long-term demographic patterns. The reproductive biology of rare plants is of special importance for several reasons. First, reproduction may limit population growth, dispersal and colonization. Second, angiosperm reproduction often depends on interactions with animal pollinators. Factors such as population size and distribution may affect these pollinator relationships; pollinators

may be less attracted to small or fragmented host plant populations (Levin 1972; Tepedino 1979; Faegri & van der Pijl 1980). In addition, the same anthropogenic disturbances that threaten plant populations, such as habitat destruction or fragmentation, may also adversely affect pollinator populations.

Rare, self-incompatible plants may suffer depressed reproduction if they occur in small or sparse populations (Allee effect) because of a lack of mates and/or pollinator visits (Levin 1972; Feinsinger *et al.* 1986; Kunin 1992, 1993). Obligately outcrossing rare plants with specialized pollination relationships may be particularly likely to suffer decreased reproduction from a lack of either mates or pollinators (Levin 1972; Tepedino 1979). For example, *Abronia macrocarpa* L.A. Gal., a federal-listed rare species that exhibits low seed set, was found to be an obligate outcrosser dependant on moth pollinators for reproduction, and studies have suggested that reproduction in this plant may be pollinator limited (Williamson *et al.* 1996).

Levin (1972) predicted that one potential evolutionary outcome of this decrease in reproduction is selection for

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traits such as autogamy or apomixis that reduce dependence on mates and pollinators. Empirical evidence for such adaptive shifts in breeding systems is mixed. A number of studies have suggested that rare plants exhibit slightly higher levels of self-compatibility than common plants (Karron 1991; Kunin 1993; Kunin & Shmida 1997; Anderson 2001; Bernardello 2001). For example, Karron (1987, 1989) found that rare *Astragalus* species had a higher incidence of autogamy and lower pollinator visitation than common congeners. Similarly, some predominantly outcrossing species have isolated populations exhibiting autogamy or agamospermy near the limits of their geographical or ecological ranges (Baker 1955; Stebbins 1957; Bierzychudek 1985; Schmidt & Antlfinger 1992; Runions & Geber 2000). Nonetheless, many rare plants are obligate outcrossers, suggesting that adaptive shifts towards autogamy/agamospermy are not the only adaptive options available to rare plants.

Our objectives were to provide information about the life history traits of *Abronia ammophila* Greene, a rare plant endemic to Yellowstone National Park, Wyoming, USA. Specifically our goals were: (i) to describe its floral morphology and phenology; (ii) to identify its important pollinators; (iii) to describe its breeding system; (iv) to measure its reproductive effort and output at the popula-

tion level; and (v) to examine the implications of these characters for its conservation.

## Materials and methods

### Study species

Tweedy first collected *Abronia ammophila* Greene (Nyctaginaceae), the Yellowstone sand verbena, near Pelican Creek in Yellowstone National Park, Wyoming, USA in 1885, 13 years after the park opened to the public (Tweedy 1886). *Abronia ammophila* is currently known from only four populations along the shores of Lake Yellowstone (Fig. 1). Historically, *A. ammophila* had a broader distribution along the shores of Lake Yellowstone and at least three populations (last documented in the 1890s, 1920s and 1960s, respectively) are extirpated (Nelson 1899; Haynes 1928; Whipple 2001).

*Abronia ammophila* is a prostrate, mat-forming perennial. Leaves, stems and flowers exude a sticky secretion that causes sand to cling to the plants. The hermaphroditic, white flowers occur in a capitulum. As in other members of Nyctaginaceae, petals are lacking; the tubular perianth is composed of a fused calyx and is subtended by a calyx-like involucre (Galloway 1975; Welsh *et al.* 1987). The base of the perianth is constricted just above

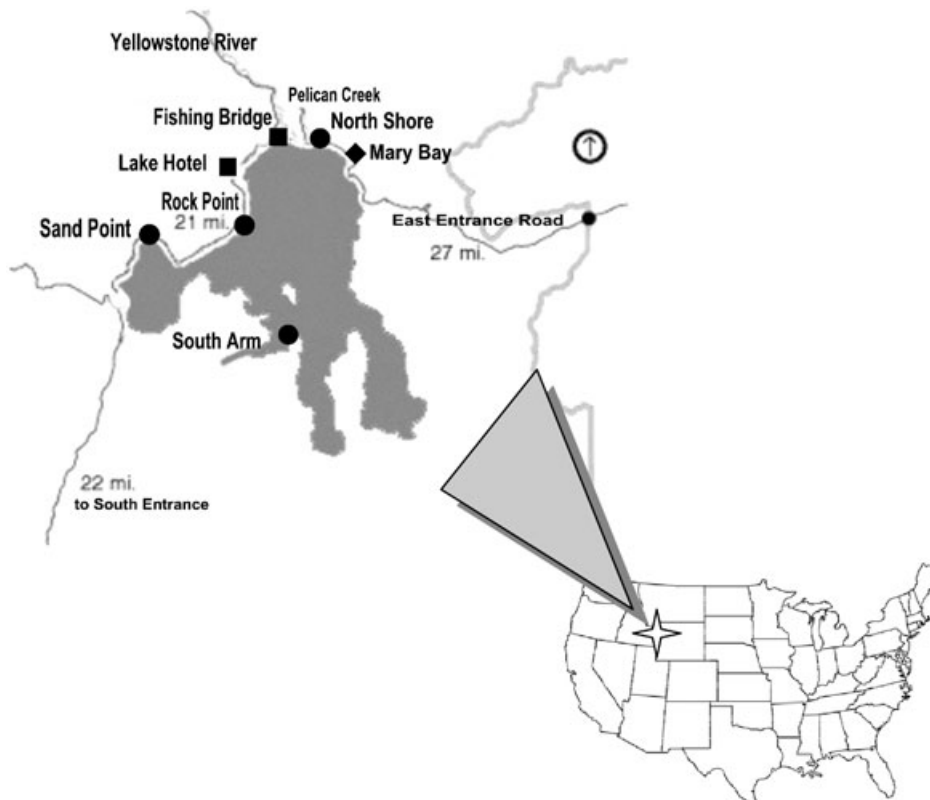


Fig. 1 Location of study sites near Lake Yellowstone in Yellowstone National Park, north-western Wyoming, USA. (●) extant populations; (■) extinct populations; (◆) suspected, but undocumented population site.

the ovary. The upper perianth is ephemeral; the lower perianth beneath the constriction persists in fruit, forming the hardened outer layer of a specialized achene called an anthocarp. The anthocarp becomes winged as it matures. The gynoecium is one-carpellate, with a single locule and basal placentation. The ovary is superior (flower hypogynous), appearing inferior by the constricted perianth (Galloway 1975; Welsh *et al.* 1987).

### Study site

Lake Yellowstone is a high-elevation freshwater lake, 2360 m a.s.l., in Yellowstone National Park, Wyoming, USA. The lake basin is a large caldera that was formed approximately 640 000 years ago when the magma chamber of two separate domes collapsed. The lake is 352 km<sup>2</sup> in area, although it was originally 32 m higher than current levels (Pierce *et al.* 2002; US National Park Service 2004).

We studied *A. ammophila* in the field at Yellowstone National Park at a location on the lake's north shore (NS) in 2003 and 2004. The NS population of nearly 8000 individuals occurs along a 2 km strand of shoreline. The primary habitat occurs in steeply sloping dunes bordered on the south by the lake and on the north by a belt of *Artemisia tridentata* Nutt. *Abronia ammophila* occurs less frequently in blowouts. This small group of 'blowout' plants within the NS population occurs in close proximity to two thermal vents. *Abronia ammophila* is commonly associated with *Phacelia hastata* Dougl. ex Lehm., *Polemonium pulcherrimum* Eastw., *Lupinus argenteus* Pursch and *Rumex venosus* Pursh. *Abronia ammophila* appears to be less competitive on more stable soils or in competition with *Artemisia tridentata* and *Eriogonum umbellatum* Torr., and is absent where these species occur. Additional pollinator observations were made at a smaller site near Rock Point (RP). RP is located on the north-west side of the lake in a north-facing cove. The RP population occurs in a small blowout with moderate slope. This site is also bordered on the south by the lake and on the north by *Artemisia tridentata*. There are no thermal vents associated with the RP population. The RP population is significantly smaller than the NS population, occupying an area of slightly less than 900 m<sup>2</sup>. This population had 325 individuals in the 1998 census (Whipple 2001) and a similar number was estimated in 2003 and 2004.

### Floral morphology

Flowers were field dissected to determine floral morphology, including position of stamens and stigma and location of nectaries. Flowers per inflorescence were counted in the randomly selected plants ( $n = 75$ ) used in the studies described below. Flowers were measured

within randomly selected inflorescences in the population to determine average floral tube length ( $n = 30$  plants).

### Timing of stigma receptivity and pollen viability

Pollen viability was estimated in the field using Alexander's stain, which differentially stains viable and non-viable pollen (Alexander 1980). On each of 10 plants, three flowers from a single inflorescence were collected on day 1, day 2 and day 3 of anthesis (29–31 July 2003). Most flowers had senesced by day 4. The anthers were removed and soaked in Alexander's stain and a pollen slide prepared. The slides were examined using a light microscope. Counts of viable and non-viable pollen grains were made from two randomly chosen fields of view at 10X. A range of 24–172 grains were counted within each field of view. Pollen that stained dark purple was scored as viable and pollen that stained any shade of green was scored as non-viable.

Stigma receptivity of plants was tested in the field by hand-pollinating stigmas of different ages. On each of 10 plants, three inflorescences were chosen and randomly assigned one of three treatments. All flowers of an inflorescence were hand-pollinated on day 1, day 2 or day 3 of anthesis with freshly dehisced pollen from a plant approximately 10 m from the recipient plant. Timing of anthesis within inflorescences generally varied by >1 day, so all flowers on an inflorescence were pollinated on the same day. Plants were covered to exclude pollinators prior to and during anthesis (see breeding system experiment below). Fruits were collected at maturity and viable seed set was used as a measure of stigma receptivity. To minimize destructive sampling of seeds of this rare species, seed viability was tested on a random sample of 10 seeds from each of three size/shape classes (Table 1) using tetrazolium chloride stain (Grabe 1970). We visually correlated seed size and shape of untested seed with viability as determined by the tetrazolium test. Viability by treatment of the remaining seeds was scored based on this visual assessment.

### Breeding system

Experimental hand-pollination tests were conducted in the field and complemented by observational data. Twenty-four plants were covered with pollinator-exclusion cages fashioned from a wire frame covered with nylon tulle. Cages were securely fastened to the ground using elastic straps and garden stakes driven into the sand. On each plant, four preanthesis inflorescences were marked and randomly assigned to one of four artificial pollination treatments: autogamy, geitonogamy, near xenogamy and far xenogamy (Table 2). For each treatment plant, an inflo-

**Table 1** Classes used in all tests with seed viability as a measure of reproductive success

	Description	Viable seeds (of 10)
Class 1	Full-size, optimal shape (endosperm fleshy, plump, fully hydrated), with bright, shiny color ranging from light brown to black	9
Class 2	Full-size, suboptimal shape (outer coat somewhat wrinkled, some dehydration, endosperm fleshy, but not plump), with either bright, shiny color or slightly flat color ranging from light brown to black	0
Class 3	Full- to less-than-full size, poor shape (outer coat highly wrinkled, seed severely dehydrated, endosperm not developed), color always flat ranging from opaque to black	0

Seeds were individually inspected and placed into one of the three categories. Viability of 10 random seeds from each category was tested using tetrazolium stain (Grabe 1970). In all tests, 90% of all Class 1 seeds, 20% of Class 2 seeds and 0% of Class 3 seeds tested viable. Visual assessment could not differentiate viable from non-viable Class 2 seeds, so all Class 2 seeds were scored as non-viable because the absence of a fully formed endosperm made germination unlikely.

**Table 2** Treatments used in the breeding system study

Treatment	Method	Tests for
Autogamy	Caged and unmanipulated	Automatic self-pollination within a single flower
Geitonogamy	Caged and pollinated with donor from same plant	Pollinator-mediated self-pollination among flowers on the same plant
Xenogamy—Near Outcross	Caged and pollinated with donor <10 m away	Pollinator-mediated cross-pollination
Xenogamy—Far Outcross	Caged and pollinated with donor ~10 m away	Pollinator-mediated cross-pollination
Control	Uncaged and unmanipulated nearby plant	Natural pollination

rescence on a nearby plant was marked as an open-pollinated control to measure the natural level of pollination.

Treatment plants were covered from preanthesis to fruit maturation except during pollination treatments. All flowers in a treatment inflorescence were pollinated on the first and second day of anthesis to insure proper pollen transfer. Hand-pollinations were carried out with a blunted Number 2 insect pin, with the aid of magnifying glasses. The pin was cleaned thoroughly between treatments and checked under magnification to insure that it was free of pollen before proceeding to subsequent treatment flowers. The presence or absence of fruits for each treatment inflorescence was recorded and mature fruits were collected.

In this study, treatment flowers were not emasculated. The only logistically feasible method to emasculate *A. ammophila* flowers would be to remove the entire top of the perianth along with the adnate anthers. Williamson *et al.* (1994) emasculated the larger flowers of *A. macrocarpa* in greenhouse pollination experiments in this manner. However, in our study the harsh, windy field conditions would have desiccated flowers treated in this way, and allowed the stigmas to become clogged with sand. As a result, our cross-pollination treatments may have resulted in a mixture of self- and cross-pollination. Close observation of the pollination technique under

magnification suggests that most pollen transferred to recipient flowers was the intended donor pollen. Our methods do not experimentally distinguish autogamy from agamospermy (asexual seed set) and pseudogamy (requiring pollination for asexual seed set).

Seed viability was tested using tetrazolium chloride stain on a representative subsample of 10 seeds from each of three size/shape classes (Table 1). We visually correlated seed size and shape of untested seed with viability as determined by the tetrazolium test. Viability by treatment of the remaining seeds was scored based on this visual assessment using appropriate percentages of seeds.

### *Reproductive success*

Natural reproductive output was estimated at NS. Two cohorts of plants ( $n = 14$  and  $n = 11$ ) were randomly selected and flagged. The first cohort was observed from 20 June to 1 August 2003, and the second cohort was observed from 7 July to 1 August 2003. On each observation plant, an inflorescence just beginning anthesis was marked and followed throughout the growing season without manipulation. Each subsequent week, an additional inflorescence just beginning anthesis was marked and followed. Fruits from all marked inflorescences were collected and later scored for fruit set and seed viability.

### Pollinators

A total of 69 pollinator observation sessions were conducted by either one observer, or two observers working in different areas of the population during each observation period. Observation sessions were 30-min long, during which observers watched a 10 m<sup>2</sup> area. Each insect was followed as it entered the observation area. Its actions and behaviors were recorded and it was followed until it left the observation area. As visitors were observed, their identity (to the taxonomic level possible in the field) and foraging behavior were recorded and used in conjunction with descriptions of floral morphology to evaluate their efficacy as pollinators. Representative floral visitors were collected and their bodies examined with a dissecting scope for the presence and placement of *Abronia* pollen. These data, along with field observations, were used to determine which visitors were likely to be pollinators, given their morphology. *Abronia* pollen was sufficiently distinct from synchronously flowering species that it could be identified on the insects with confidence. Of the subset of total floral visitors that met the criteria of pollinators, specimens were identified to species (when possible) by systematic experts. Vouchered specimens are deposited in the laboratory of S. Sipes at Southern Illinois University. We did not collect every pollinator observed visiting *Abronia ammophila* flowers because we did not want to adversely affect pollination of this rare species.

The collection of pollinator observation data at dawn and dusk was very limited because of the high level of grizzly bear activity in the immediate vicinity of the research site. For safety reasons and at US Park Service request, we conducted crepuscular and nocturnal observation sessions only when two or more persons could be present.

### Data analysis

For the stigma receptivity, breeding system and reproductive output tests, we calculated percent viable seed per inflorescence. Data were arcsine transformed, if necessary, to meet the assumption of normality of variance. For the stigma receptivity test, percentage of viable seed set by day following anthesis was averaged across the 10 plants, arcsine transformed and subjected to an ANOVA. Data were analyzed to determine if there was a significant difference among days following anthesis. For the pollen viability test, the percentage of viable pollen by day following anthesis was averaged across the 20 counts, and treated as the stigma receptivity test. For the breeding system study, an ANOVA of seed viability by artificial pollination treatment was used to test for significant differences among all of the artificial pollination treatments. In addition, a post-hoc, Tukey–Kramer pairwise comparison

was used to determine if there was a significant difference between any combinations of treatments. For the reproductive season study, an ANOVA of seed viability by week of anthesis was used to test for significant differences among weeks of the breeding season. All ANOVAs were carried out using SAS software (SAS Institute, Cary, NC, USA).

## Results

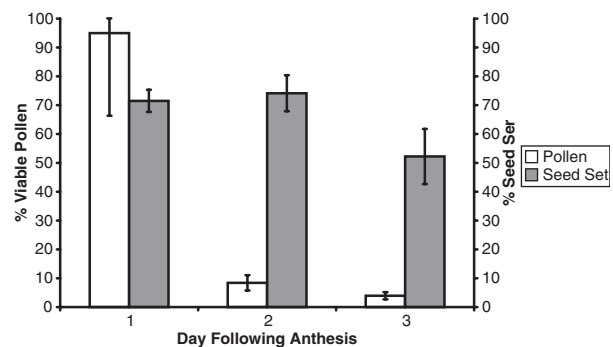
### Flower morphology

The number of flowers per inflorescence ranged from 4 to 21, with a mean  $\pm$  standard deviation of  $12.9 \pm 2.3$  ( $n = 75$  plants). Floral tube length ranged from 6 to 13 mm ( $n = 30$ , mean =  $9.8 \pm 0.08$  mm), with the majority falling within the 8–12 mm range.

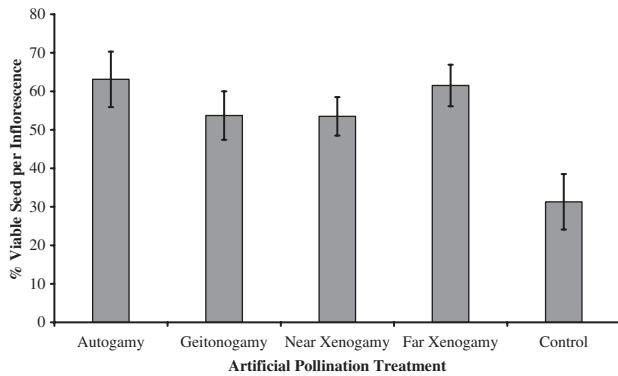
Flowers exhibit increased turgidity and more intense fragrance during the late afternoon and evening. Likewise, flowers appear to be less open (perianth lobes constricted) during the day. Mature flowers are held above or at the canopy level of the plant. As the infructescence ages it becomes increasingly decumbent, recurving into the soil. Primary seed dispersal appears to occur beneath the parent plant, but secondary dispersal may be achieved by the action of wind, which is prevalent, and water.

### Timing of stigma receptivity and pollen viability

Anthers dehiscence the first day of anthesis. Percent pollen viability was high (~95%) on the first day of anthesis, but dropped precipitously on subsequent days (Fig. 2). Stigmas were found to be receptive over the three days of



**Fig. 2** Timing of pollen viability and stigma receptivity for *Abronia ammophila*. Percent pollen viability for 1, 2 and 3-day-old flowers ( $\square$ ). ANOVA indicated that days differed significantly ( $n = 10$  plants, d.f. = 2,  $P < 0.0001$ ). Percent viable seed set per inflorescence for flowers pollinated on day 1, 2 and 3 following anthesis ( $\blacksquare$ ). ANOVA indicated no significant difference among days ( $n = 10$ , d.f. = 2,  $P = 0.09$ ). Error bars represent standard error.



**Fig. 3** Percent viable seed set per inflorescence for five breeding system treatments for *Abronia ammophila*. Treatments are outlined in Table 2. ANOVA indicated there was a significant difference among the treatments ( $n = 21$ , d.f. = 4,  $P = 0.0014$ ). A Tukey–Kramer pairwise comparison indicated that only the control treatment differed from the others. Error bars represent standard error.

anthesis and an ANOVA indicated no significant difference in percent viable seed per inflorescence among inflorescences pollinated on days one, two or three of anthesis (74–52%; Fig. 2).

#### Breeding system

Artificially pollinated *A. ammophila* flowers had a high level of fruit production; effectively 100% of flowers produced fruit, but not all fruit contained a viable seed. There was no significant difference in percent viable seed per inflorescence among the artificial pollination treatments: autogamy (63%); geitonogamy (54%); near xenogamy (54%); and far xenogamy (62%). However, the open-pollinated controls exhibited significantly lower seed viability (31.3%) (Fig. 3).

#### Reproductive output

Reproductive output under natural pollination was estimated at NS for inflorescences that began flowering between 20 June and 18 July 2003. Seeds were collected until 1 August, and seed viability was determined for each week of the field season. Overall, viable seed production was high (66–85%). An ANOVA indicated that there was no significant difference between viable seed production among weeks ( $n = 25$ , d.f. = 4,  $P = 0.14$ ). There were microsite differences between sites: plants occurring near a thermal vent (cohort 1) became desiccated and suspended growth and reproduction after mid-July. For these plants, reproductive effort was concentrated in the early part of the season (June through to early July), whereas reproductive effort continued throughout the field season at the dune site (cohort 2). Most plants at the

thermal site failed to produce flowers after 7 July 2003. In general, plants that did produce flowers beyond this date failed to produce fruits as the plants desiccated. The dune site (cohort 2) had slightly higher overall viable seed production, more consistent reproductive effort over the season, and a longer reproductive period. While reproductive effort appeared to slow at the dune site after 21 July, most of the dune plants still had buds and flowers when the study ended on 1 August 2003. The fruits initially examined at the thermal site took 14 days to mature, whereas those in subsequent weeks dispersed in as few as 8 days. Conversely, at the dune site, fruits marked later in the season appeared to take longer than 2 weeks to mature. These differences in the rate of fruit development may result from microsite environmental differences.

#### Pollinators

A total of 69 half-hour observation/collection sessions were conducted between 27 June and 31 July 2003, and between 6 July 2004 and 18 July 2004. Several orders of insects visited *Abronia* flowers, including lepidopterans (butterflies and moths), hymenopterans (bees), hemipterans (true bugs), coleopterans (beetles) and dipterans (flies). Crepuscular and nocturnal visitors included lepidopterans of the families *Noctuidae* (noctuid moths) and *Sphingidae* (hawk moths). Although crepuscular and nocturnal observations comprised only a fraction of total pollinator observation time, noctuid moths, particularly *Hada sutrina* Grote (nine specimens collected) and *Autographa pseudogamma* Grote (five specimens collected), were the most frequently collected floral visitors. Precipitation and extremely strong or gusty winds, which were common during the study period, appeared to have a negative impact on nocturnal pollination activity. In 2004, the floral display at NS was markedly reduced compared with the display in 2003, and fewer pollinators were observed both in the area and actually pollinating *A. ammophila*. During six evenings of pollinator observations conducted in July 2004, only two noctuid moths and a single sphingid moth were observed on *A. ammophila*.

Diurnal visitors were predominantly butterflies (*Lycaenidae*: *Glaucopsyche*; *Nymphalidae*: *Aglaia*, *Polygonia*; and *Pieridae*: *Pontia*) and bumblebees (*Apidae*: *Bombus*). Bees and butterflies were most prevalent on calm, warm days during the mid- to late-afternoon hours.

We evaluated the potential of floral visitors to serve as pollinators by the presence of *A. ammophila* pollen on their mouthparts and whether their behavior and morphology potentially allowed pollen transfer (Table 3). Both sphingid and noctuid moths had *A. ammophila* pollen on their mouthparts and mouthparts of sufficient length to effectively transfer pollen to the stigma of individual flowers. The butterfly visitors also had mouthparts of sufficient

**Table 3** Floral visitors identified as potential pollinators

Taxon	No. individuals collected
Lepidoptera	
Noctuidae	
<i>Autographa pseudogamma</i> Grote	5
<i>Copablepharon viridisparva</i> Dod	1
<i>Hada sutrina</i> Grote	9
<i>Papestra quadrata</i> Smith	3
Unidentified	2
Spingidae	
<i>Hyles lineata</i> Fabricius	1
Lycaenidae	
<i>Glaucopsyche lygdamus</i> Doubleday	1
Nymphalidae	
<i>Aglais milberti</i> Godart	1
<i>Polygonia faunus</i> W.H. Edwards	1
Pieridae	
<i>Pontia occidentalis</i> Reakirt	1
Hymenoptera	
Apidae	
<i>Bombus mixtus</i> Cresson	3
<i>Bombus fernaldae</i> Franklin	1
<i>Bombus sylvicola</i> Kirby	1

All pollinators were collected at the north shore site in 2003.

length to contact stigmas, and *A. ammophila* pollen was present on their mouthparts; therefore, butterflies are probable, if less frequent, pollinators. Butterflies typically visited only a few *A. ammophila* flowers before flying out of observational range. Bumblebees would typically visit several flowers per inflorescence and several *A. ammophila* plants successively. The bumblebees displayed nectaring behavior on the flowers, but none of the collected specimens had *Abronia* pollen in their scopae. Thus, it appears that the bumblebees visited *A. ammophila* primarily as a nectar source. The bumblebees had mouthparts of sufficient length to contact the stigma during these nectaring visits, and *A. ammophila* pollen was observed on three of seven bumblebee mouthparts collected. Other floral visitors (e.g. flies, beetles, homopterans) did not carry *A. ammophila* pollen on their bodies and were not considered to be potential pollinators.

Overall, rates of pollinator visitation were very low. Visitation by potential pollinators occurred on the average of 1.7 per hour per 10 m<sup>2</sup> ( $n = 69$ ). Each 10-m<sup>2</sup>-observation area contained hundreds of flowers and visitation rates per flower were extremely low.

## Discussion

Our study indicates that *A. ammophila* exhibits several fortuitous reproductive traits that may buffer the negative affects of rarity and small population size: high reproduc-

tive output, a diverse assemblage of effective pollinator species, the ability to produce seed even in the absence of pollinators, and a long reproductive season (June–September). We found high mean viable seed set (70% averaged across the reproductive season), a large number of flowers per inflorescence (~13) and a majority of flowers producing fruit (effectively 100%), which may buffer the effects of unpredictable pollinators and climate.

*Abronia ammophila* exhibits all of the floral traits specifically associated with moth pollination, including long corolla-tube length, white and off-white coloration, night blooming and intensely sweet nocturnal floral scents (Faegri & van der Pijl 1980). Indeed, *A. ammophila* is primarily visited by moths, as are some other *Abronia* (e.g. *A. fragrans*, *A. macrocarpa*, *A. pogonantha* and *A. umbellata*) and other members of Nyctaginaceae, including *Mirabilis* and *Acleisanthes* (Tillett 1967; Grant & Grant 1983; Williamson *et al.* 1994). Because noctuid and sphingid moths visited numerous flowers per inflorescence and many *A. ammophila* plants successively, and because *A. ammophila* exhibits a mixed-mating system, moths are implicated in self- and cross-pollination in this species. However, *A. ammophila* also attracts diurnal visitors not suggested by its floral morphology, including bumblebees and butterflies, and our study suggests that these taxa are also effective, albeit less frequent, pollinators. Similarly, Tillett (1967) described both nocturnal and diurnal visitors in *A. pogonantha* and *A. umbellata*, although he did not document the importance of these diurnal visitors as potential pollinators. In our study, bumblebees carried some *Abronia* pollen on their mouthparts, but lacked pollen in their scopae. Thus, the bees were likely to be using *A. ammophila* as a nectar source only. As the nectaries are deeper in the perianth tube than the stigma, the bumblebees have the potential to contact the stigmas and pollinate the flowers. In contrast, *A. macrocarpa* was visited by, but not pollinated by, bumblebees because of its longer perianth tube, which prevented bees from reaching the nectaries and therefore also the stigmas (Williamson *et al.* 1994). Williamson's and our studies underscore the importance of observing all floral visitors, and of differentiating between visitors and pollinators.

Despite the diversity of pollinators visiting *A. ammophila*, overall visitation rates were extremely low. Cool daytime temperatures (<19°C), precipitation and strong or gusty winds appear to have a negative impact on pollinator activity. Thus, fruit set in the population at large is probably mostly because of autogamy, or possibly agamospermy. Although our study did not distinguish between them, autogamy appears more likely than agamospermy because the floral morphology and functional phenology of *A. ammophila* makes automatic self-pollen deposition not only possible but highly likely. In addition, agamospermy has not been reported in Nyctaginaceae,

but self-compatibility and autogamy occur in a few taxa in this family (Spellenberg 1986; McMullen 1987; Hodges 1995; Kelso *et al.* 2003). Regardless of which breeding system it is, one ecological outcome is the same: *A. ammophila* is not dependent on pollinators for most of its seed set. If this species is indeed autogamous, then the timing of pollen dehiscence and viability, relative to that of stigma receptivity, suggests 'competing autogamy' *sensu* Lloyd and Schoen (1992) because there is no delay between the time in which the flower may be outcrossed and the time in which autogamous pollination may occur (Fig. 2).

Levin (1972) predicted that population bottlenecks, lack of mates or irregular or infrequent pollinator availability drive the evolution of autogamy. Nevertheless, several *Abronia* species, some of which are also subject to the reproductively limiting ecological constraints of small populations, maintain obligate outcrossing breeding systems. *Abronia macrocarpa*, *A. latifolia*, *A. maritima* and *A. umbellata*, the only species in the genus for which there are detailed accounts of breeding system, are obligate outcrossers (Tillett 1967; Williamson *et al.* 1996). Numerous other examples exist of obligate outcrossing rare plants (e.g. Tepedino *et al.* 1997 and references therein), including the narrow endemic *Solidago shortii* Torr. & Gray and *Asclepias meadii* Torrey (Buchele *et al.* 1992; Tecic *et al.* 1998). Thus, the reproductive challenges of rare species do not necessarily drive breeding systems towards autogamy.

Even in instances where a rare, auto-fertile species has arisen from an outcrossing, widespread progenitor, the breeding system change is not necessarily driven by scarcity of pollinators or mates. For example, *Stephanomeria exigua* 'Malheurensis', an auto-fertile rare species known from a single population in Oregon, is believed to have recently speciated sympatrically from a more common, outcrossing ancestor (Gottlieb 1973). In this case, auto-fertility, rather than arising because of rarity, is believed to have driven the speciation of a rare, neoendemic species by providing reproductive isolation. Possibly, a similar event has produced *A. ammophila*, if this species has been derived from an outcrossing ancestor. Work on the phylogenetic relationships in *Abronia* is currently underway and may further elucidate the evolution of *A. ammophila*'s reproductive mode.

*Abronia ammophila* appears to have little inbreeding depression in terms of viable seed production resulting from self-pollination (61.0%) versus cross-pollination by far neighbors (56.9%). This lack of depressed seed production observed in our study does not, however, preclude reduced fitness in subsequent life history stages (e.g. Dudash 1990) or differential fitness based on population size (Fischer & Matthies 1998). For example, in *Gentianella germanica*, a self-compatible rare species, population size was positively correlated with germination, long-term

survival and fecundity in future generations, and this correlation was attributed, at least in part, to genetic effects (Fischer & Matthies 1998). Measurement of seed set in smaller populations of *A. ammophila* would shed light on this possibility.

Pollinator-mediated outcrossing, however infrequent, allows for increased mixing and recombination of alleles, and may thus be important to the long-term adaptive potential and survival of *A. ammophila*. Thus, while autogamy may help buffer *A. ammophila* reproductively at times when insect pollinators are not abundant, pollinators may still be essential to the long-term persistence and health of the species.

As predicted by Tepedino *et al.* (1997), coflowering species may be highly significant to the reproductive success of rare plants such as *A. ammophila*. The bees, butterflies and moths visiting *A. ammophila* also visited *Phacelia hastata*, *Phlox multiflora* and other dune plants. These species may affect *A. ammophila* reproduction, whether by attracting pollinators to the area, by providing additional resources or competing for pollinator visits (Thomson 1982; Rathcke 1988; Sipes & Tepedino 1995).

Immediate, economical conservation actions can be undertaken to increase our understanding of *A. ammophila* and to facilitate its persistence. Long-term surveys of population size (seasonally and annually) would further our understanding of this rare plant. Transplant tests may be appropriate to determine whether *A. ammophila* is restricted by habitat specificity or whether it can be re-established in its historical habitats. At least two known historical populations (Lake Hotel and Fishing Bridge) have been extirpated in the past 25 years and re-establishment at these sites appears appropriate and may serve as a corridor for the movement of gametes among the larger NS and RP populations. More stringent protection of existing populations, together with additional demographic data, should tell us whether *A. ammophila* is adversely affected by park activities.

Finally, *A. ammophila* is a desirable subject for conservation attention, not only because of its own imperiled status, but also because it belongs to a clade that includes many rare and endemic species. *Abronia* species in general tend to be characterized by local endemism, indeed all appear to be limited to the western half of North America. Very few, if any, are known to span the entire range of the genus, and some (e.g. *A. macrocarpa*, *A. ammophila*, *A. bigelovii*) are rare enough to warrant protection under federal or state conservation laws. A number of these may be neo- or paleoendemics, or they may represent once-widespread species that have not fared well under human expansion and habitat destruction. It is also possible that they are neoendemics, isolated by recent speciation. Detailed phylogenetic studies should

reveal more about the biogeography of *A. ammophila* and its congeners, and elucidate possible reasons for the high incidence of rarity.

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