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Reproductive ecology and the persistence of an endangered plant

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Abstract. *Amsinckia grandiflora* (large-flowered fiddleneck) is an extremely rare California annual wildflower, known only from three populations. We conducted field and greenhouse experiments to compare the rare heterostyle with a cryptic self-incompatibility system (*A. grandiflora*) to a common, self-compatible, homostylous, sympatric congener (*A. tessellata*). Inter-species comparisons of adult plants suggested that in the greenhouse, *A. grandiflora* balances low floral seed set (seeds per flower) with increased floral output (flowers/plant) and a greater number of flowers per inflorescence. Seed set from active self-, intra- and inter-morph pollinations was high in *A. grandiflora*, indicating that the cryptic self-incompatibility system does not prevent seed set in the species. In the field, *A. grandiflora* floral output was only slightly greater than for *A. tessellata*, and did not fully balance lower floral seed set. *Amsinckia tessellata* average seed weight was lower than that of *A. grandiflora*, which, along with the lower number of flowers produced, indicated lower maternal investment per nutlet than for *A. grandiflora*. Under conditions of unlimited resources, it appears that *A. grandiflora* fitness is not intrinsically limited when compared to its weedy relative *A. tessellata*. The differences in nutlet output between *A. grandiflora* and *A. tessellata* under field conditions are more likely due to differential responses to extrinsic factors such as competition and pollinator availability.

Introduction

Comparing the ecology of rare species with sympatric, closely related common species provides us with the opportunity to examine factors that contribute to rarity (Kunin and Gaston 1993; Pantone et al. 1995; Bevill and Louda 1999). Rare species are of interest because of their high risk of extinction, and a great deal of effort has been directed towards developing a scientific framework within which to understand the patterns and causes of rarity (Rabinowitz 1981; Cody 1986; Fiedler and Ahouse 1992; Gaston 1994). Determining whether the cause of rarity is intrinsic (related to the biology of the species) or extrinsic to the species (related to environmental factors) can also aid in assessing population viability and in developing management plans to reduce the likelihood of extinction (Pavlik et al. 1993a). In addition, comparing the ecology of older and younger related plant species can also help us understand the ways in which displacement of species may occur over time (Fernald 1924; Lewis and Raven 1958).

Amsinckia grandiflora (Gray) Kleeb. ex Greene (Boraginaceae) is one of four

rare heterostylous species within the genus *Amsinckia* that have highly restricted distributions and from which the more weedy homostylous congeners are thought to have evolved (Ray and Chisaki 1957a, b; Shoen et al. 1997). The historic distribution of *A. grandiflora* (first recorded in the late 1800s) extended 72 km northward from its current, restricted location in the Livermore-Tracy area of California, USA (Figure 1). Potential extrinsic limits to the populations include the invasion of non-native species (U.S. FWS 1997), livestock grazing, fire suppression, and land conversion. Possible intrinsic limitations on *A. grandiflora* could result from its breeding system, or from genetic homogeneity as a result of low population density (Stebbins 1942; Hamilton and Mitchell-Olds 1994). Genetic variability in sexually reproducing organisms can lead to intrinsic limitations on fecundity. Recombination can produce extremely unfit individuals, and inbreeding depression is a risk for both self-compatible and self-incompatible plants. While self-incompatible plants may suffer less inbreeding depression than their self-fertilizing brethren (Johnston and Schoen 1996), partially self-incompatible plants that produce only small amounts of seed when pollinator visitation is absent can become inbreeding-depressed quite quickly in a series of unfavorable years (Cane and Tepedino 2001). Rare plants are less likely to have preferential pollinators and hence are more vulnerable to stigmatic clogging from extraspecific pollen (Waser 1978) and interspecific disease transmission through the pollination vector (Marr 1997).

Ornduff (1976) specifically proposed heterostyly as the main factor contributing to *A. grandiflora*'s low seed set. Pantone et al. (1995) found that dynamic fitness-component compensation (i.e., fewer seeds per flower compensated by more flowers

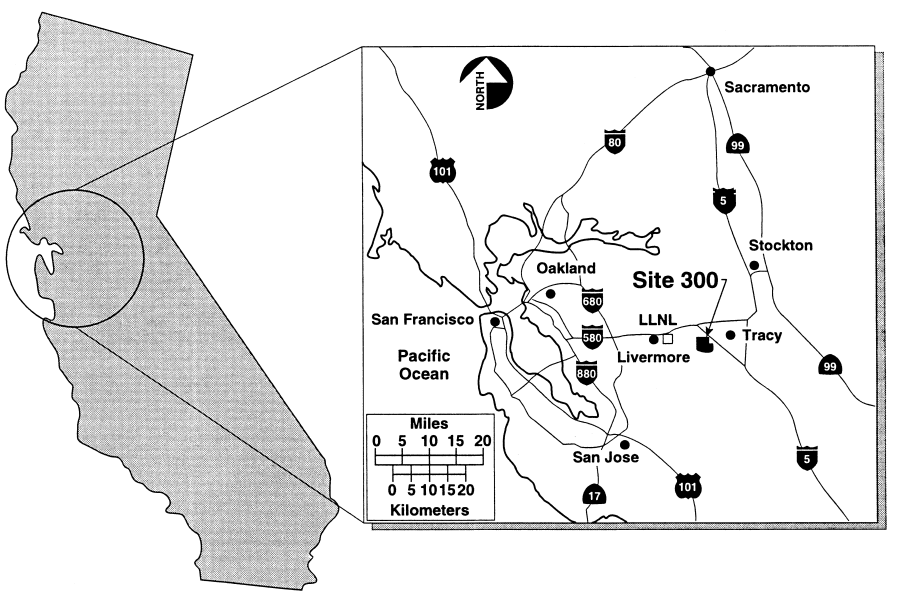

Figure 1. Location of Site 300 in California, USA.

per inflorescence) did not occur within either *A. grandiflora* or the widespread homostyle *A. menziesii* var. *intermedia* (Fischer & C. Meyer) Ganders in an outdoor common garden experiment in an exotic environment more than 800 km from the native location in Texas, USA. They also suggested that increased floral production by *A. grandiflora* could not compensate for its low seed set per flower (relative to *A. menziesii* var. *intermedia*), thus indicating an intrinsic limit to fecundity.

While the range of *A. menziesii* var. *intermedia* overlaps that of *A. grandiflora, A. menziesii* var. *intermedia* plants do not occur within the remaining known populations of *A. grandiflora*. Plants of *A. tessellata* A. Gray, an autogamous homostyle, however, are sympatric with *A. grandiflora* populations. *Amsinckia tessellata* is classified as a 'successful' species since it is widespread and quite common. *Amsinckia tessellata* is more closely related to *A. grandiflora* than *A. menziesii* var. *intermedia* (Ray and Chisaki 1957b), and may have similar ecological requirements. By comparing these two species, we can examine reproductive capacity and relate this to patterns of rarity in *A. grandiflora*. If *A. grandiflora* is intrinsically limited by its reproductive capacity or characteristics of its breeding system, it may be inferior to *A. tessellata* in (1) fecundity (seed output/plant), and/or (2) germination rates or seedling survivorship. If the two species are equal in their ability to produce viable seed under controlled conditions, the rarity of *A. grandiflora* may not be attributable to intrinsic factors related to heterostyly. The goal of this study was to assess whether reproductive capacity is a significant contributing factor to the decline of *A. grandiflora* by comparing reproductive ecology between it and a more common species. To achieve this end, we (1) conducted greenhouse trials to compare the nutlet output and germinability of the two congeners, including nutlet output of *A. grandiflora* under different pollination methods, and (2) compared field survivorship and reproductive performance of these two species.

Materials and methods

The study species

Amsinckia grandiflora and *A. tessellata* are both members of the California winter annual grasslands. As such, both species germinate with the onset of fall or winter rains, grow vegetatively throughout the winter, flower in the early spring, set seed and die prior to the summer drought (Heady 1988). *Amsinckia grandiflora* has a very small seed bank that is directly linked to population size (Pavlik 1995). Precise information concerning seed banks of *A. tessellata* is not available, although it is likely that only a small percentage of seed carry over from year to year as well (Evans and Young 1989). *Amsinckia grandiflora* is currently known from only three natural populations containing individuals numbering from fewer than 30 to several thousand. These populations occur in grasslands which border on blue oak woodland and coastal sage scrub on steep, well-drained, north-facing slopes of low (300 m) elevations. All three populations are located in the coastal range of California,

about 80 km east of San Francisco on or near Site 300 (Figure 1). Site 300 is a Lawrence Livermore National Laboratory high-explosive testing facility, operated by the University of California for the US Department of Energy. The Drop Tower population of *A. grandiflora*, which contains up to 1900 individuals, and the Draney Canyon population, which contains around 30 individuals (and may have been extirpated in the years 1997–2000), are both located at Site 300. The Carnegie Canyon population, containing several thousand individuals, is located adjacent to Site 300.

Amsinckia grandiflora exhibits a form of heterostyly known as distyly, in which pin and thrum flower forms (or 'morphs') are produced (Ganders 1976, 1979), with one morph per plant. Pin flowers have a stigma exserted and the anthers within the corolla tube. Thrum flowers have the opposing morphology, with anthers exserted and the stigma within the corolla tube. This morphology promotes a type of outcrossing known as phenotypic disassortative mating (i.e., pins pollinate thrums, and thrums pollinate pins). Many distylous species are not self-compatible and may be 'morph' incompatible (pollination by a different plant of the same floral morph is unsuccessful). Distyly appears to be controlled by a pair of alleles. Pin plants have the genotype recessive *ss* and thrum plants have the genotype *Ss* or *SS*. Under conditions of complete disassortative mating (no self- or intra-morph pollination), the *SS* genotype does not occur, and the phenotypic pin and thrum ratio in the progeny is 1:1. However, the occurrence of either self- or intra-morph thrum pollinations could result in the presence of the *SS* genotype. Distyly in *A. grandiflora* appears to be only partially disassortative (Weller and Ornduff 1977), with significant seed production from intra-morph crosses.

Amsinckia tessellata is a common weedy annual that has spread throughout much of the western United States from its phytogeographic center in the coastal ranges of California (Ray and Chisaki 1957a). *Amsinckia tessellata* is a homostylous (the anthers and stigma are opposite each other), self-compatible species exhibiting a high degree of assortative mating. Ray and Chisaki (1957b) presented evidence that *A. grandiflora* gave rise to *A. douglasiana*, which then gave rise to *A. tessellata*. *Amsinckia tessellata* occurs sympatrically with *A. grandiflora* in the field. Although Shoen et al. (1997) did not include *A. tessellata* in their cpDNA phylogeny of the *Amsinckia* genus, they found support for the close relationship between *A. grandiflora* and *A. douglasiana*.

Germination trials

Germination trials were conducted to compare germinability of *A. grandiflora* nutlets to that of *A. tessellata* nutlets. Table 1 outlines the germination trial conditions. In 1995, because the initial germination percentage was very low over the initial 10 days, ungerminated nutlets were allowed to air-dry for an additional 10 days, then moistened and observed for 15 days to determine the occurrence of additional germination.

Table 1. Summary of germination trial methods and results.

Trial	Species	Nutlet source	Nutlet storage[a]	Temperature/light conditions	Number of days	Number of replicates/ container type	Germination nutlets/ replicate	(%	± 1 se)
1: (27 November–7 December 1993)	*Amsinckia grandiflora*	DA88	4 °C, 5.5 years	22 °C, dark	3	3/petri plates	20	75.0	± 2.9[c]
	A. tessellata	DA88	4 °C, 5.5 years	22 °C, dark	3	3/petri plates	20	73.3	± 10.1[c]
2a: (23 January–2 February 1995)	*A. grandiflora*	CC94	4 °C, 9 months	5 °C, day	10	3 1 petri plates	10	43.3	± 8.8[d]
	A. grandiflora	EX94	4 °C, 9 months	5 °C, day	10	6/petri plates	10	13.3	± 8.0[e]
	A. tessellata	GR94	4 °C, 9 months	5 °C, day	10	6/petri plates	10	55.0	± 5.6[d]
2b: (13–28 February 1995) treated[b]	*A. grandiflora*	CC94	Air-dried 22 °C, 10 days	5 °C, day	15	3/petri plates	4–7	13.3	± 3.3[d]
	A. grandiflora	EX94	Air-dried 22 °C, 10 days	5 °C, day	15	6/petri plates	5–10	32.8	± 8.1[f]
	A. tessellata	GR94	Air-dried 22 °C, 10 days	5 °C, day	15	6/petri plates	2–6	6.6	± 3.3[d]
2-Total: (23 January–28 February 1995)	*A. grandiflora*	CC94	2a and 2b	5 °C, day	35	3/petri plates	10	56.6	± 6.6[d]
	A. grandiflora	EX94	2a and 2b	5 °C, day	35	6/petri plates	10	46.1	± 10.4[d]
	A. tessellata	GR94	2a and 2b	5 °C, day	35	6/petri plates	10	61.6	± 4.8[d]

[a] DA88 – nutlets collected from a common garden study conducted at the University of California, Davis, in 1988; EX93 – nutlets collected from an experimental population at Site 300 in 1993; EX94 – nutlets collected from an experimental population at Site 300 in 1994; CC94 – nutlets collected from the Carnegie Canyon natural population in 1994; GR9 – nutlets collected from greenhouse-grown plants in 1994. [b] Nutlets from germination trial 2a were allowed to dry for 10 days at room temperature before being remoistened. [c] Species not different within trial, $P = 0.98$. [d] Populations not different within trial, $P > 0.05$. [e] Population different from the other two in the trial, $P = 0.004$. [f] Population different from the other two in the trial, $P = 0.015$.

Greenhouse studies

Each seedling from germination trial 1 was established in a 15.25-cm pot filled with soil from Site 300. Seedlings were maintained in an ambient temperature, pollinator-sealed greenhouse (at Lawrence Livermore National Laboratory) and irrigated with tap water. Beginning in March 1994, all plants received weekly supplements of 50% strength Hoagland's solution.

To test the self-incompatibility system in *A. grandiflora*, 40 uniform-size plants were randomly selected (20 pins and 20 thrums) in early March 1994. Plants were divided into five blocks (arranged in an east-to-west orientation). Flowers on each plant received one of four pollination treatments: (1) no manipulation ('passive self-pollination', abbreviated ps), (2) manual self-pollination ('active self-pollination', abbreviated as), (3) manual intra-morph pollination (abbreviated intra), and (4) manual inter-morph pollination (abbreviated inter). This resulted in a total of eight treatments (four treatments for each flower morph) and 75% of the flowers on each plant were actively pollinated. Pollinations were conducted on newly opened flowers on 22–31 March 1994. This resulted in at least three flowers per treatment per plant, with totals between 56 and 64 individual flowers per treatment. Resulting nutlets were collected, air-dried, weighed, and then stored at 4 °C.

Nutlets from the *A. grandiflora* self-incompatibility test were germinated during December 1994 (8 months after collection). Nutlet numbers ranged from 67 to 131 per treatment (at least five maternal plants/treatment) and were grouped into three or four replicate petri dishes with approximately 20 nutlets per dish. Germination under ambient greenhouse temperature was recorded for 24 days.

Four additional seedlings of *A. grandiflora* (all ps flowers), along with four seedlings of *A. tessellata* (also all ps) were used to compare growth and reproductive attributes. Once flowers had senesced, the total number of inflorescences per plant (inflorescence output) and total number of flowers per plant (floral output) were determined. Any nutlets were collected, air-dried and weighed. Above- and below-ground biomass was separated, oven-dried at 60 °C, and weighed.

In February 1995, 15 seedlings each of *A. grandiflora* EX94 and *A. tessellata* GR94 from germination trial 2 were each transplanted into 15.25-cm pots containing standard greenhouse potting soil. Methods were otherwise the same as in the 1994 trial, except that seven randomly selected *A. grandiflora* plants had all flowers pollinated with the inter treatment, while the remaining eight plants experienced the ps treatment. All *A. tessellata* plants experienced the ps treatment. Only above-ground biomass was collected in the 1995 trial.

Inter-species comparisons in the field

In October 1994, plots were established near the native Drop Tower population (location DT) and on an ecological reserve adjacent to Site 300 owned by the California Department of Fish and Game (location CDFG). Each of these locations has been used for experimental populations in the past, chosen because of their similarity to the native site in community, slope, aspect, and soil characteristics.

Each plot was seeded with nine *A. grandiflora* nutlets from the EX94 source, sown in three rows of three, equidistantly spaced at 10 cm. With the onset of winter rains, each germinating *A. grandiflora* was marked, along with a paired *A. tessellata* germinating from the existing seed bank. A total of 53 species pairs were marked: 29 at DT and 24 at CDFG. Survivorship and inflorescence output were recorded throughout the growing season. After each individual flower senesced, the fully filled, green nutlets were counted. Nutlets were collected from five *A. tessellata* (one CDFG and four DT) and 10 *A. grandiflora* plants (four CDFG and six DT).

Data analysis

Percentage data were arcsine square-root transformed prior to statistical analysis (Krebs 1989). A χ^2 analysis was performed on the distribution of morph type in cross progeny in order to compare observed and expected frequencies (Zar 1984). χ^2 analysis was also used on the raw field survivorship data to assess differences between species and sites. Analysis of variance (ANOVA) statistical analyses were conducted using the SAS general linear model (GLM) procedure, and linear regressions were conducted using the SAS regression (REG) procedure (SAS 1990). Mean separation between treatments was conducted using Dunnet's test. Regression line slope differences were tested by homogeneity of regression (Tabachnik and Fidell 1996). Results were considered significant if the α value was less than or equal to 0.05, although nearly significant results (P less than 0.1 but greater than 0.05) are also presented.

Results

Germination trials

Table 1 summarizes the results of the germination trials. During trial 1, after only 3 days, *A. grandiflora* germination averaged 75.0% and *A. tessellata* germination averaged 73.3%. The germination of the two species was not significantly different in this trial ($P = 0.98$). As part of a separate study, in 26 plates, each containing 20 *A. grandiflora* nutlets from the Davis nutlet source, germination averaged 77.8% with a standard error of 2.7% within the first 3 days (unpublished data).

Trial 2 examined the germination of 9-month-old seed sources which had been stored for several months at 4 °C, and germinated under cool conditions. Germination for all three seed sources occurred slowly and sporadically over the first 10-day period. Nutlets collected from the *A. grandiflora* experimental population (EX94) had significantly less germination ($P = 0.004$) compared with nutlets from both the natural population (CC94) and greenhouse-grown *A. tessellata* (GR94).

However, after allowing the nutlets to air-dry for 10 days, the nutlets from the experimental *A. grandiflora* population experienced significantly more germination during the second germination period compared with the greenhouse-produced *A. tessellata* nutlets ($P = 0.015$). While not significantly different from *A. tessellata* (P

Table 2. Summary of greenhouse pollination statistics.

Source	df	MS	F	*p*
Dependent variable: no. of seeds per flower				
Model, overall	79	7.35	9.41	0.0001
Error	402	0.78		
Corrected total	481			
Morph × block × treatment × date	200	3.39	4.37	0.0001
Pins				
Block × treatment × date	58	0.87	1.38	0.0534
Block × treatment	16	3.42	5.38	0.0001
Treatment × date	20	1.03	1.62	0.0567
Thrums				
Block × treatment × date	63	0.72	0.80	0.8471
Block × treatment	16	1.36	1.50	0.1078
Treatment × date	20	1.13	1.24	0.2293
By floral morph and treatment				
Pins – as (active self)				
Date	5	2.75	2.91	0.0281
Block	4	4.37	4.63	0.0046
Date × block	12	0.56	0.71	0.7670
Pins – intra (intra-morph)				
Date	5	0.42	0.51	0.7668
Block	4	5.37	6.44	0.0005
Date × block	12	2.17	2.60	0.0138
Pins – inter (inter-morph)				
Date	5	1.02	1.22	0.3186
Block	4	3.93	5.14	0.0021
Date × block	12	0.93	1.34	0.2318
Thrums – as (active self)				
Date	5	1.49	0.81	0.6667
Block	4	0.84	0.64	0.6392
Date × block	17	1.08	1.12	0.3691
Thrums – intra (intra-morph)				
Date	5	2.27	2.52	0.0456
Block	4	1.74	1.94	0.1243
Date × block	17	0.47	0.52	0.9075
Thrums – inter (inter-morph)				
Date	5	0.68	0.48	0.7916
Block	4	2.70	1.89	0.1348
Date × block	17	1.25	0.87	0.5971
Thrums – ps (passive self)				
Date	5	0.07	0.84	0.5281
Block	4	0.15	1.77	0.1558
Date × block	17	0.11	1.29	0.2490

= 0.45), the natural *A. grandiflora* population also experienced a substantial amount of germination during this period. All germination during this second period occurred extremely slowly and sporadically. Although the total amount of germination for all three nutlet sources was not significantly different, *A. tessellata* nutlets had the highest average percent germination, followed by *A. grandiflora* nutlets

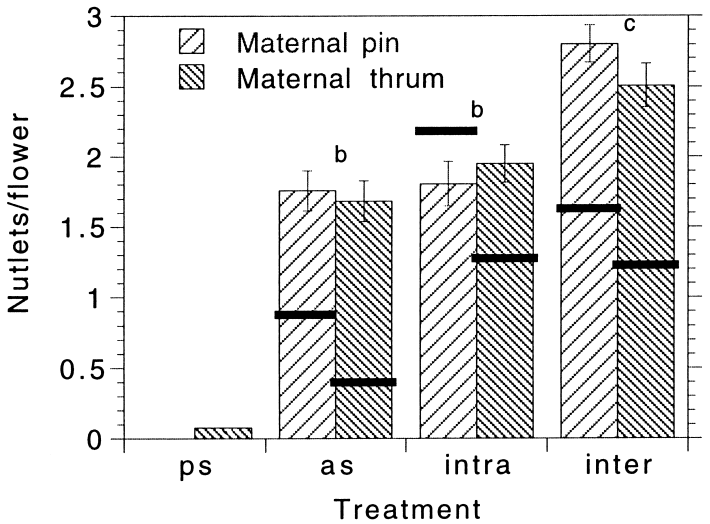

Figure 2. Average number of seeds per flower in *A. grandiflora* from artificial pollinations in the greenhouse in spring 1994. ps – passive self-pollination; as – active self-pollination; intra – intra-morph pollination; inter – inter-morph pollination. Treatments with the same letter are not significantly different at $P = 0.05$. Solid bar represents results of Weller and Ornduff (1977), assuming their self-pollination treatment is equivalent to our active self-pollination treatment. Error bars are one standard error.

Table 3. Floral morphs of progeny.

Pollination[a]	No. of pin progeny	No. of thrum progeny	Expected segregation	χ^2 (CV = 3.0)	df
P × P as	12	0	1:0	0	1
P × P intra	4	0	1:0	0	1
P × T inter	3	0	1:1	3.00	1
T × T as	3	4	1:3	1.19	1
T × T intra	0	2	1:3	0.67	1
T × P inter	3	3	1:1	0	1

[a] Seed parent × pollen parent: as – active self; intra – intra-morph pollination; inter – inter-morph pollination.

from the natural population, with nutlets from the *A. grandiflora* experimental population having the lowest average percent germination.

Greenhouse studies: A. grandiflora *self-incompatibility system*

Inter-morph pollinations of *A. grandiflora* in the greenhouse produced the highest floral seed set (>2.5 nutlets/flower), regardless of the floral morph of the parental plant (Figure 2). The intra-morph and active self-pollinations also produced a significant floral seed set (>1.5 nutlets/flower). Passive self-pollinations produced essentially no nutlets. There was a statistically significant four-way interaction between morph, block, treatment and pollination date (Table 2). When broken down by morph, pins experienced significant block by treatment interactions. Block

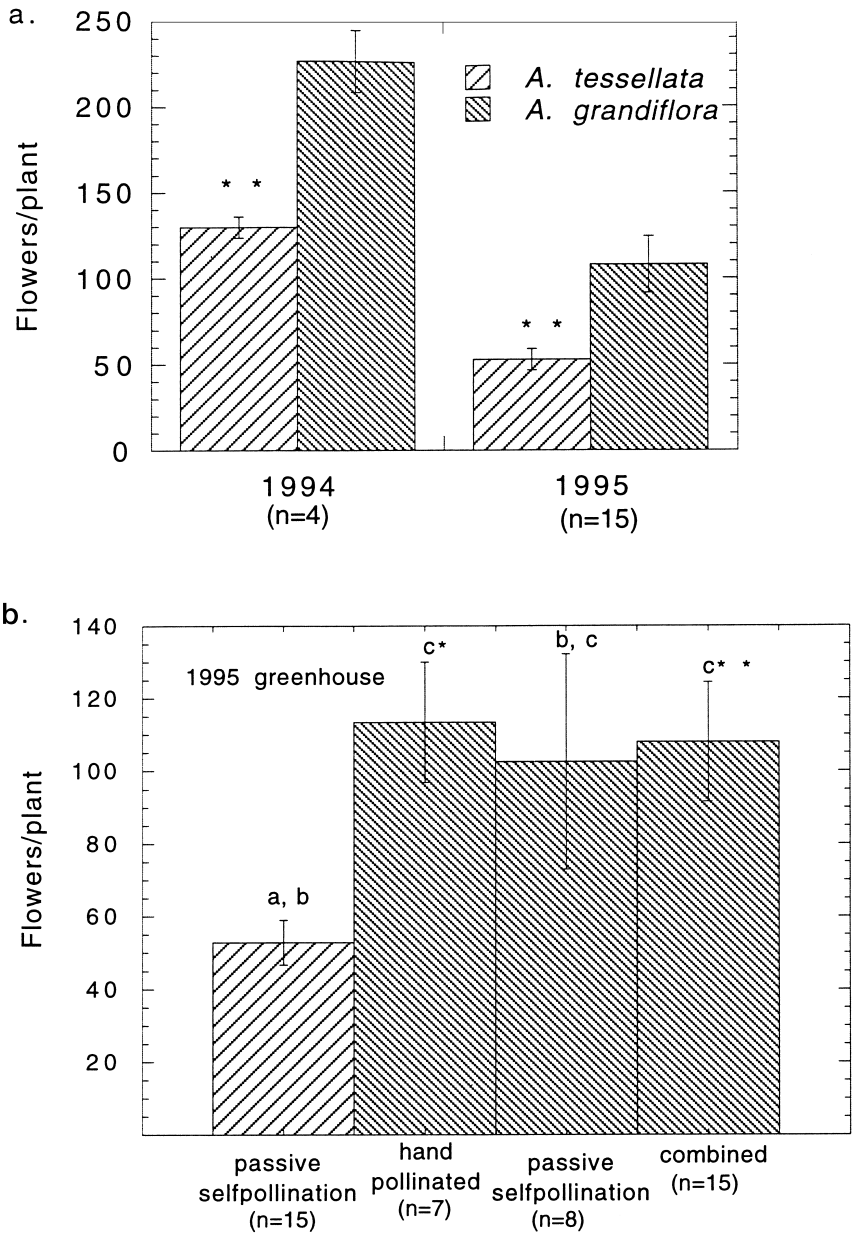

Figure 3. Number of flowers per plant by greenhouse-grown *A. tessellata* and *A. grandiflora*. *Significantly different at $P = 0.05$. **Significantly different at $P = 0.005$. Error bars are one standard error.

effects were significant for all pin pollination treatments. The only significant effect on thrum plants, when treated separately, was the treatment effect.

Although no difference in floral seed set was detected between pins (average 1.59 nutlets/flower) and thrums (average 1.52 nutlets/flower, $P = 0.58$), there was a significant difference in nutlet weight (average nutlet weight in mg/nutlet). Nutlet weight of pins at 4.99 mg/nutlet was approximately 1.2 mg/nutlet greater than thrum nutlet weight ($P = 0.05$, see Table 5 for details).

The floral morph of the progeny plants was consistent with that expected for the type of cross (Table 3). The number of progeny tested was very low, therefore this result should not be used to draw any conclusions about the operation of the *s*-allele system in *A. grandiflora*. However, the 100% pin progeny from pin × pin crosses suggests a lack of contamination during manual pollinations.

Greenhouse studies: interspecies comparisons

In both 1994 and 1995, floral output of greenhouse-grown *A. grandiflora* was twice that of *A. tessellata* (Figure 3a, $P = 0.0023$ for 1994 and $P = 0.0041$ for 1995),

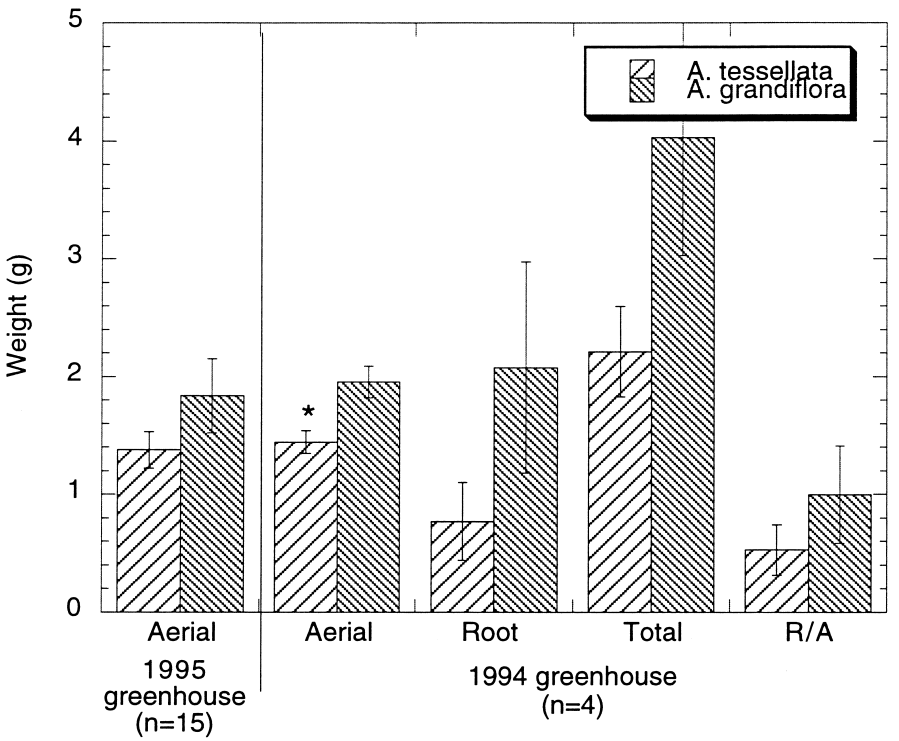

Figure 4. Distribution of dry biomass of greenhouse-grown *A. tessellata* and *A. grandiflora* plants. Shoot biomass in 1995, shoot and root biomass grown in 1994, *R/A* = root:shoot biomass ratio. *Significantly different at $P = 0.05$. Error bars are one standard error.

although plants were of similar size with respect to above-ground (shoot) biomass (Figure 4, $P = 0.2$ for both years). Plants in 1994 were grown from nutlets obtained from the DA88 source, whereas the plants in 1995 were grown from nutlets collected from either the GR94 (*A. tessellata*) or EX94 (*A. grandiflora*) source.

Passively self-pollinated (ps) *A. grandiflora* plants had statistically similar floral output to passively self-pollinated (ps) *A. tessellata*. But since ps *A. grandiflora* plants produced essentially no seed, and ps *A. tessellata* plants produced substantial seed, a comparison of floral output between an active pollination of *A. grandiflora* and the ps of *A. tessellata* may be more appropriate. When comparing the active treatment of *A. grandiflora* plants to the ps treatment of *A. tessellata*, floral output was two times greater for *A. grandiflora* (Figure 3b, $P = 0.005$).

The destructive analysis of a small number of plants in 1994 (Figure 4) suggested that under conditions of unlimited water and soil nutrients, *A. grandiflora* apparently allocated its resources into additional shoot and root biomass, which in turn produced more inflorescences and flowers, with *A. grandiflora* showing more variation compared to *A. tessellata*. Even though the mean root weight of *A. grandiflora* (1.8 g) was more than twice that of *A. tessellata* (0.61 g), the difference was not statistically significant ($P = 0.22$). The shoot weight of 1.9 g for *A.*

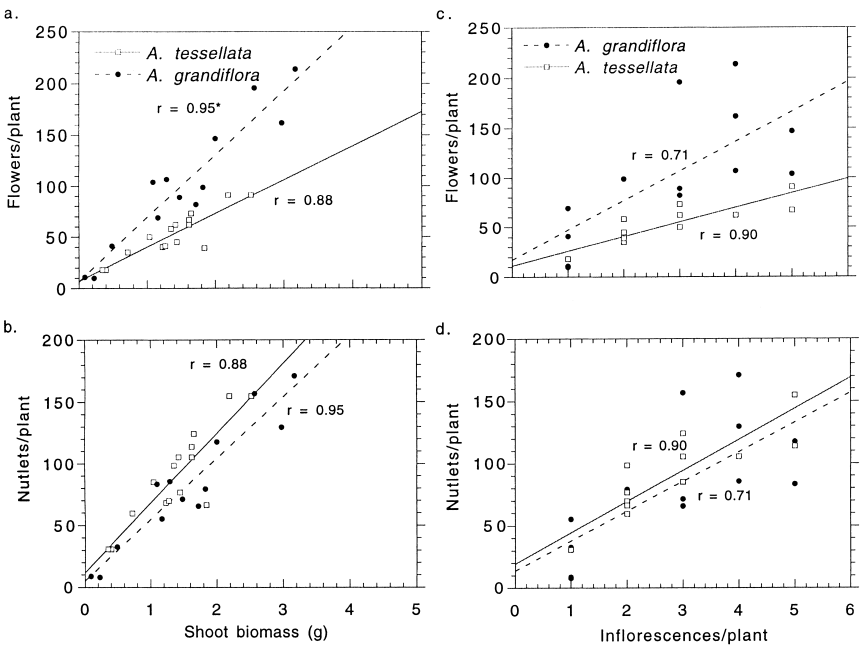

Figure 5. Reproductive attributes of 1995 greenhouse-grown *A. tessellata* and *A. grandiflora* plants ($n = 15$). (a) Number of flowers per plant by shoot biomass. (b) Estimated number of seeds per plant by shoot biomass. (c) Number of flowers per plant by number of inflorescences per plant. (d) Estimated number of seeds per plant by number of inflorescences per plant. All slopes of lines are significantly different from zero at $P < 0.0001$. *Slopes different from each other at $P = 0.0037$.

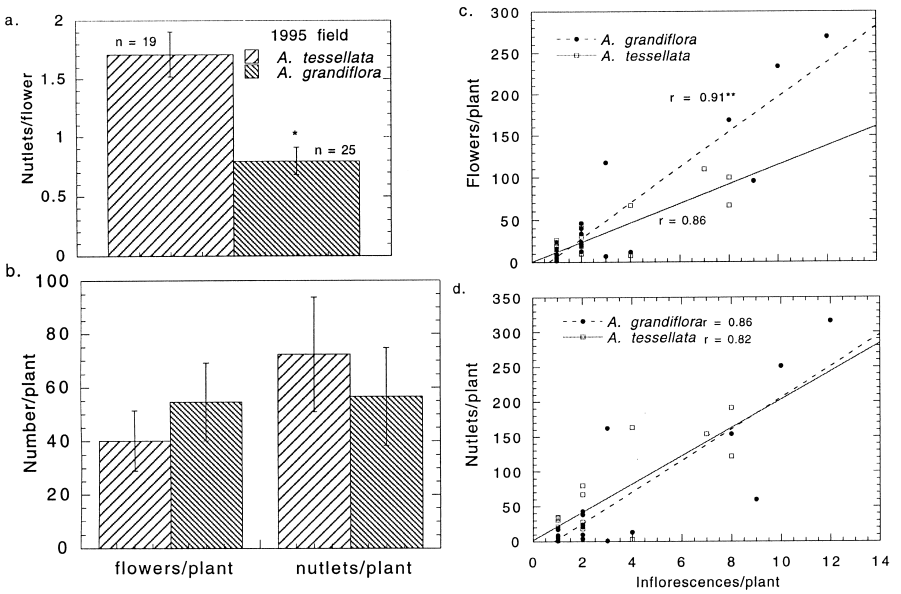

Figure 6. Reproductive attributes of 1995 field-grown *A. tessellata* and *A. grandiflora* plants ($n = 53$). (a) Number of seeds per flower. (b) Number of flowers per plant and number of nutlets per plant. (c) Number of flowers per plant by number of inflorescences per plant. (d) Number of nutlets per plant by number of inflorescences per plant. *Significantly different at $P = 0.005$. All slopes of lines are significantly different from zero at $P < 0.0001$. **Slopes are nearly significantly different from each other at $P = 0.01$.

grandiflora was significantly higher than the 1.4 g for *A. tessellata* ($P = 0.021$) in 1994, but the shoot weights of the two species in 1995 were not significantly different ($P = 0.097$).

For an equivalent amount of aerial biomass, *A. grandiflora* floral output was also twice that of *A. tessellata* in the greenhouse in 1995 (Figure 5a, $P = 0.0037$). Using the floral seed set observed in the field for each species (1.7 nutlets/flower for *A. tessellata*, and 0.8 for *A. grandiflora*, Figure 6a), seed output for the greenhouse-grown plants was estimated. Estimated seed output per unit of aerial biomass was similar for *A. grandiflora* and *A. tessellata* (Figure 5b, $P = 0.49$). When inflorescence number is used as a covariate instead of aerial biomass (Figure 5c and d), *A. grandiflora* appears, again, to have greater floral output per inflorescence than *A.*

Table 4. Comparative survivorship.

Site[a]	No. of marked pairs	Mortality (%)	
		Amsinckia tessellata	*A. grandiflora*
CDFG	24	75	50
DT	29	55	55

[a] CDFG – California Department of Fish and Game Reserve; DT – Drop Tower Site on Site 300.

Table 5. Field and greenhouse nutlet weight (mg/nutlet).

Year[b]	*Amsinckia grandiflora*[a]			*A. tessellata*[d,e]
	Pins[c,e]	Thrums[c,e]	Combined[d]	
Field				
1993 (1)	4.15 ± 0.140 (21)[f‡]	3.39 ± 0.297 (11)[g‡]	4.00 ± 0.829 (48)[h]	2.58 ± 0.072 (7)[i‡]
1994 (2)	4.79 ± 0.345 (10)[f]	3.82 ± 0.109 (10)[g]	4.31 ± 0.209 (20)	ND
1995 (3)	4.73 ± 0.217 (6)[f‡]	3.89 ± 0.325 (3)[g]	4.50 ± 0.203 (10)[h]	3.41 ± 0.347 (5)[i‡]
Greenhouse				
1994 (4)	4.99 ± 0.076 (129)[f+]	3.85 ± 0.079 (130)[g‡]	4.42 ± 0.065 (259)[h]	3.13 ± 0.038 (14)[i‡]

[a]Results are presented ± one standard error. Numbers in parentheses indicate number of plants, except in the case of 1993 *A. tessellata* and 1994 greenhouse results, where it indicates number of nutlet subsamples. Combined *A. grandiflora* plants do not equal the sum of pin and thrum plants due to inclusion of plants that had senesced prior to floral morph determination. [b]Nutlets collected from (1) EX93 *A. grandiflora* experimental population with naturally occurring *A. tessellata*, (2) EX94 *A. grandiflora* experimental population with naturally occurring *A. tessellata*, (3) combined *A. grandiflora* experimental plots at DT and CDFG locations with naturally occurring *A. tessellata*, and (4) *A. grandiflora* plants in greenhouse pollination study and *A. tessellata* greenhouse grown plants. [c]Different letters indicate significant difference between pins and thrums at $P = 0.05$. [d]Different letters indicate significant difference between combined *A. grandiflora* and *A. tessellata* at $P = 0.05$. [e](‡, +) Different symbols indicate significant difference between *A. tessellata* and a floral morph of *A. grandiflora* at $P = 0.05$. ND – not determined.

tessellata ($P = 0.1$), and similar seed output ($P = 0.89$). However, while the species type and aerial biomass have a significant combined effect on floral output, species type and inflorescence number have a less powerful combined effect.

Field inter-species comparisons

Mortality for both species was very high at both sites (Table 4). Mortality of *A. grandiflora* and *A. tessellata* seedlings was equal at the DT site (55%). The difference between *A. tessellata* mortality (75%) and *A. grandiflora* mortality (50%) at the CDFG site was not significant ($\chi^2 = 0.63$, $CV_{\alpha,0.05} = 3.84$). Most of the mortality occurred within the first month of the growing season. The cause and timing of mortality did not appear to differ between the two species.

Under field conditions, floral seed set in both *A. grandiflora* and *A. tessellata* was less than the maximum of four nutlets per flower (Figure 6a). (Because there was no statistical difference in reproductive attributes between the two sites, the data were combined.) *Amsinckia tessellata* produced 1.7 nutlets/flower, which was a little more than twice that of *A. grandiflora* (0.8 nutlets/flower, $P = 0.005$). Floral output was greater in *A. grandiflora*, but this difference was not statistically significant (Figure 6b, $P = 0.31$). Seed output in *A. tessellata* was greater than in *A. grandiflora* (Figure 6b), but again this difference was not significant ($P = 0.86$). Floral output per inflorescence was greater in *A. grandiflora* (Figure 6c, $P = 0.0058$), resulting in a similar seed output per inflorescence between the two species (Figure 6d, $P = 0.65$). This was similar to the results observed in the greenhouse.

Nutlet weight: field and greenhouse

Nutlet weight for field-grown *A. tessellata* plants was consistently below that of the average combined nutlet weight of pin and thrum *A. grandiflora* plants, by 1.1–1.4 mg/nutlet (Table 5, $P = 0.05$). Nutlets produced by *A. grandiflora* pin plants weighed substantially more than *A. tessellata* nutlets, by as much as 1.6 mg/nutlet. Nutlets from thrum *A. grandiflora* plants also generally weighed more than *A. tessellata* nutlets, although by only 0.5–0.8 mg/nutlet. These differences in nutlet weight under field conditions in 1993 and 1995 were similar to those observed under greenhouse conditions in 1994. Variations in nutlet weight among years appeared to be the same between species: nutlet weight of both species was lower in 1993 when compared to 1995.

Summary

Our pollination study shows that while intra- and inter-morph crosses are both effective in producing seed in *A. grandiflora*, pollinators are needed in order for this species to produce seed. Germination and survivorship were similar between *A. grandiflora* and *A. tessellata*. In the greenhouse, nutlet output per unit biomass and nutlet output per inflorescence was similar between the two species. Floral output was higher in *A. grandiflora* than *A. tessellata* in the greenhouse, but the two species had similar floral output in the field. *Amsinckia tessellata* had more than twice as many nutlets per flower than *A. grandiflora* in the field. Nutlet weight was higher in *A. grandiflora* in both the greenhouse and field.

Discussion

Our pollination study results indicate that *A. grandiflora* is not completely self-incompatible, supporting results found by other researchers (Ray and Chisaki 1957a; Ganders 1979; Weller and Ornduff 1977, 1989, 1991). Although differential pollen-tube growth is a likely cause of *A. grandiflora*'s cryptic self-incompatibility (Weller and Ornduff 1989), our data suggest that in the absence of pollen competition, significant nutlet production can occur from illegitimate (intra-morph) pollen. While the incompatibility may be cryptic, the incompatibility itself is probably not responsible for low seed production. Our greenhouse-grown and inter-morph hand-pollinated *A. grandiflora* plants produced a floral seed set (>2.5 nutlets/flower) that was much higher than that observed by Ornduff (1976), and was comparable to that of the field-grown *A. tessellata* observed by Ornduff (1976) in 1967 (3.02 nutlets/flower) and 1971 (2.49 nutlets/flower). It is possible that conditions in our greenhouse were somehow more conductive to *A. grandiflora* growth. Analyzing each floral morph separately revealed a block by treatment interaction in the pin morph data. Each treatment on the pin plants was affected by the block. All three blocks located along the eastern greenhouse windows generally produced a higher

floral seed set than the two blocks located in the interior of the greenhouse. This suggests that floral seed set, particularly for pin plants, is sensitive to environmental conditions, such as light. Differences in greenhouse conditions can also explain the differing degree of self-compatibility seen in our study compared to Ornduff's, as self-compatibility has been found to be under partial environmental influence in other plants (Reinartz and Les 1994).

Deviations from disassortative mating by intra-morph pollination in the field could account for deviations from the expected morph ratio of 1:1, as has been observed, particularly when population sizes are small (Ornduff 1976; Pavlik 1994). Our somewhat lower than expected number of thrum progeny from thrum × thrum pollinations has occurred in other studies (Ornduff 1976; Weller and Ornduff 1989). Also, Ornduff (1976) found that the morph ratios of pollen deposited on pins in the field were disassortative (for example, thrums preferentially received pin pollen). Under-representation of thrum progeny has been observed in other species as well (Casper 1985; Kohn and Barrett 1992; Mal and Lovett-Doust 1997).

Our inter-species comparisons under greenhouse conditions indicate that *A. grandiflora* may balance low floral seed set with increased floral output. This increased floral output does not appear to be a function of simply larger plants, but a result of more flowers per inflorescence for *A. grandiflora* than *A. tessellata* for similar-sized plants. However, this conclusion depends upon our floral seed set results, which differ from those of other researchers. Field floral seed set for *A. tessellata* in 1995 was barely twice that of *A. grandiflora* (1.7 nutlets/flower for *A. tessellata* vs. 0.8 for *A. grandiflora*). Pantone et al. (1995) found *A. menziesii* var. *intermedia* floral seed set to be 2.6 nutlets/flower vs. 0.6 for *A. grandiflora*, whereas Ornduff (1976) reported a field floral seed set for *A. tessellata* of between 2.5 and 3.0 and for *A. grandiflora* of between 0.7 and 1.5. Had we observed a similar floral seed set distribution in our experiment, seed output of *A. grandiflora* could not have equalled that of *A. tessellata*. Moreover, although *A. grandiflora* floral output was greater than that of *A. tessellata* in the field, this difference was not statistically significant. The observed greater seed output for *A. tessellata* compared with *A. grandiflora* was also not statistically significant.

While our results suggest that seed output in *A. grandiflora* can be comparable to that of *A. tessellata*, it is questionable as to whether or not *A. grandiflora* can consistently compensate for low floral seed set by increasing floral output. Increased flower production has been observed in other rare species compared to widespread congeners (MacDonald et al. 1987; Ng and Corlett 2000). Year-to-year variation could result in significantly greater *A. tessellata* nutlet output compared with *A. grandiflora*. Other studies of rare plants have found their reproductive output more variable than that of more common species (Fiedler 1987; Byers and Meagher 1997). In poor years when population sizes are small, there is less ability to attract pollinators, less mate availability, fewer plants flowering synchronously and the potential for bottlenecks and consequent inbreeding depression is high.

The low germination rates of seeds collected in the current year vs. seeds in storage suggest that *A. grandiflora* and *A. tessellata* may have an after-ripening requirement which imposes summer dormancy and delays germination until au-

tumn. Such an after-ripening requirement has been observed in other winter annuals (Young et al. 1968; Baskin and Baskin 1976). Although our germination trials were not extensive enough to determine the type of dormancy and the exact physical requirements (i.e., light, temperature and precipitation) for germination initiation during nutlet maturation, in general we observed similar germination responses in *A. grandiflora* and *A. tessellata*. There did appear to be a difference in germination response from nutlets collected from the different populations of *A. grandiflora*. Germination was greater for the CC94 nutlets during the first 10 days. Germination during the second 15 days was greatest for nutlets from EX94. This resulted in roughly equal germination for the two groups by the end of the trial. We cannot say whether this is due to different environmental conditions experienced by the maternal plants between the two populations, or to a genetic difference between the very large, native population and the small, experimental population. Nutlets used to find the smaller experimental population can be traced to the Drop Tower. If this germination response is genetically based, it would appear that the Drop Tower population may have developed the capacity to delay germination until after several rain events. Such diversified germination may be a risk-spreading strategy to ensure that some seedling establishment occurs even if early germinating cohorts are lost due to a return of unfavorable conditions (Haig and Westoby 1988a; Silvertown and Lovett-Doust 1993).

The average weight of nutlets collected from both field- and greenhouse-grown plants of the common *A. tessellata* species was consistently lower than that of the rare *A. grandiflora*. The advantages of smaller seeds have been described in terms of resource allocation (Harper and Ogden 1970; Haig and Westoby 1988a) and dispersal ability (Venable and Lawlor 1980; Aizen and Paterson 1990; Oakwood et al. 1993); the costs are increased intra-specific competition (Thompson et al. 1999) and less viability in stochastic environments (Venable and Brown 1988). Our data show that germination was not negatively affected by *A. tessellata*'s lower nutlet weight. Field seedling mortality had the potential to be greater in *A. tessellata* compared to *A. grandiflora*. Larger seed size has been correlated with greater survivorship in many studies (see Haig and Westoby (1988a) for review). *Amsinckia tessellata* nutlet weight was more variable between years than *A. grandiflora*, although germination within each year was similar. Both species produce seed that would be classified as small- to medium-sized, and even though one is larger than the other, the ecological significance of this difference in size may be limited. *Amsinckia tessellata* appears to be more plastic in the investment of maternal resources into each nutlet. *Amsinckia grandiflora* nutlet weight, on the other hand, is relatively less plastic. Both allocation strategies have advantages in stochastic environments: the smaller, but perhaps less viable in varying growing conditions, seeds of *A. tessellata* can be more numerous even under poor environmental conditions, and, *A. grandiflora* produces high quality, but tolerant of more variable growing conditions, seeds every year but fewer of them in poor years.

As population size increases, pollinator attraction increases and the number of plants visited by each pollinator is likely to be larger (Haig and Westoby 1998b). The Draney Canyon population with its thirty plants may not have been large

enough to attract pollinators. And then, once one plant was found, the likelihood that another would be visited was small. Larger populations such as the Carnegie Canyon and Drop Tower populations are more likely to experience the type of pollinator visitation necessary for population maintenance. Pollinator activity is extremely variable between years (Parker 1997) and is affected by weather conditions (Piper et al. 1986). For annual plants that require pollinator visitation to reproduce, a poor pollinator year can have a large effect, particularly when seed banks are small. In *A. grandiflora*, composition of visiting pollinator species may even have an effect on morph ratios in pollen deposited on stigmas (Ornduff 1976). While it is under debate whether pollinator populations are currently declining overall (Thomson 2001), climate change due to global warming will certainly have an effect on pollinator activity and may impact *A. grandiflora* long-term survival.

Under conditions of unlimited nutrients, light, water, and pollinators, it is unlikely that *A. grandiflora* fitness is intrinsically limited by its reproductive capacity in general and by heterostyly in particular when compared with the weedy relative, *A. tessellata*. Under field conditions, it appears that the reproductive ecology of *A. grandiflora*, which is dependent upon pollinators, may result in lower seed output than *A. tessellata*. However, this may be a result of an interaction with the extrinsic factor of competition with exotic plants. Although we carefully paired the two species in an attempt to minimize the confounding impact of the community, it is possible that *A. grandiflora* is affected to a greater extent by the presence of exotic annual grasses than *A. tessellata*. It has been shown that *A. grandiflora* competes poorly with exotic grasses (Pavlik et al. 1993b) and performs better in a matrix of native perennial bunch grasses (Carlsen et al. 2000). The higher root:shoot ratio of *A. grandiflora* may provide a competitive edge in the relatively undisturbed perennial bunch grass community, where substantial root competition may occur (Wilson and Tilman 1993). Carlsen et al. (2000) showed that competition for light between *A. grandiflora* and exotic grasses resulted in a reduction in the number of inflorescences and number of flowers. While *A. grandiflora* can produce amounts of nutlets comparable to *A. tessellata* under conditions of unlimited resources, *A. grandiflora* must invest resources into additional floral structures in order to do so. Fewer number of flowers produced, along with lower nutlet weight, means that maternal investment for an equal number of progeny nutlets is lower for *A. tessellata* than for *A. grandiflora*. This makes *A. grandiflora* more vulnerable to resource limitation where resources cannot be expended into making those additional flowers, suggesting that competition for resources with exotic grasses may impact the reproductive ability of *A. grandiflora* to a greater extent than that of *A. tessellata*. This suggestion is reinforced by the differences found between flower number per plant in the greenhouse and in the field between the two species. *Amsinckia grandiflora* averaged between 110 and 230 flowers per plant in the greenhouse, but only averaged 55 flowers per plant under field conditions. *Amsinckia tessellata* averaged between 50 and 130 flowers per plant in the greenhouse and 40 in the field. *Amsinckia grandiflora* flower number was reduced more dramatically from greenhouse to field conditions than *A. tessellata* flower number. Again, under field conditions, *A. grandiflora* and *A. tessellata* produce a similar number of

flowers, but as *A. grandiflora* produces fewer nutlets per flower than *A. tessellata*, therefore *A. grandiflora* produces fewer total nutlets than *A. tessellata* under these conditions of presumably limited resources.

Evolutionarily speaking, the fact that *A. grandiflora* does not simply increase floral seed set when presented with unlimited resources is probably a consequence of its reliance on pollinators. Rather than allocate excess resources to increase the number of seeds produced per flower, *A. grandiflora* must instead increase the number of flowers produced to attract pollinators (Haig and Westoby 1998b). The highly selfing *A. tessellata* is able to reduce the number of flowers produced under resource limitation through self rather than pollinator reliance. In an exotic environment more than 800 km from the native location of *A. grandiflora* and in absence of other competitors, *A. grandiflora* competed effectively with *A. menziesii* with respect to biomass accumulation, but was not effective in converting those resources into seeds (D.J. Pantone (1996) unpublished data). This indicates the importance of natural pollinators to the reproductive success of *A. grandiflora*. Pollinator reliance not only means that *A. grandiflora* seed set is directly related to pollinator activity, but also that *A. grandiflora* cannot successfully divert resources away from pollinator attraction.

An obvious management practice for populations of rare heterostylous species would be to encourage equal pin:thrum ratios in order to ensure reproductive viability (Pantone et al. 1995). However, because our results suggest that the overall seed production of *A. grandiflora* can approach that of *A. tessellata* under conditions of unlimited water, light, soil nutrients, and maximum pollination (even from illegitimate pollen), it is possibly more important to manage field populations to maximize the presence of pollinators rather than to optimize the pin:thrum ratio.

Although we compared two different sets of intrinsic factors to assess the relative effects of each on reproductive output, the fact that the response to extrinsic factors may not have been the same between species did not allow us to control for extrinsic effects between species in the field. A controlled field competition experiment comparing the impact of exotic annual grasses on both *A. grandiflora* and *A. tessellata* would be a next step to further examine the characteristics of *A. grandiflora* that contribute to its rarity.

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