SEX AND THE LONELY *ATRIPLEX*

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In principle, natural selection should have endowed species with the ability to assess their normal surroundings and respond to changes that enhance, or at least do not diminish, their fitness (Emlen et al. 1998). Hence, the chameleon changes colors to match its background to avoid being eaten, or a sweet pea's tendrils wrap around supporting structures. Buffalo gourds assess the level of phosphorus in determining their floral sex ratios (Pendleton 2000), and animals use photoperiod to precisely time the onset and termination of breeding activities for successful reproduction under appropriate environmental conditions (see Nelson 2004 for references). In addition to assessing physical aspects of the environment, animals can also assess biotic aspects, such as the density of conspecific individuals, as evidenced by the different morphs of locusts that can be induced by changes in density, with the best dispersing morphs being produced when densities are highest (Wilson et al. 2002). Parasitic wasps respond to the relative size of prey to determine the sex ratio of their offspring, and thereby maximize the number of grandchildren (Charnov 1982). Plants, too, can assess their physical environments. For example, arctic plants turn their flowers to track the sun, a response that increases the rate of pollen tube growth (Galen and Stanton 2003); and many aquatic plants are heterophyllous. Minorsky (2003) argued, "Heterophylly may increase the fitness of aquatic plants by decreasing leaf damage from mechanical forces or herbivores by decreasing water loss or by enhancing photosynthesis." Can plants also assess the density of conspecifics? More precisely, do plants alter their floral structure and the sex ratio of their progeny in response to their immediate surroundings?

Fisher (1930) showed that if males and females of dioecious species increase in fitness at the same rate with increasing size or age and if the sex ratios are not equal, then mothers that overproduce the rarer sex will have more grandchildren, on average, than mothers who produce an equal number of sons and daughters. The effects of adjusting the progeny sex ratio need not be small. Consider an annual dioecious plant population of only 2 females: female A produces 40 sons and 20 daughters; female B produces 10 sons and 30 daughters. According to Fisher (1930), female A (which overproduces males) should have the higher fitness—0.6, compared to female B's fitness of 0.4. Fitness is calculated as 0.5[(number of sons / total number of males) + (number of daughters / total number of females)].

There is ample evidence that plants often have skewed sex ratios. For example, Freeman et al. (1976) showed that sex ratios (males: females) of *Thalictrum fenderli* ranged from 0.24 to 6.77. Similarly, Pickering and Hill (2002) reported that the sex ratios of *Aciphylla simpliciflora* varied from 1.45 to 8.53 males per female along an elevational gradient (see also Freeman et al. 1997 and references therein, Decker and Pilson 2000). To assess the current population sex ratio, females of dioecious plants need some means of determining the relative density of males and females. Assessing the abundance of other females would indeed be difficult, but density of males can be assessed by determining the time elapsed before pollination of stigmas.

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In an earlier experiment, Miglia and Freeman (1995) showed that spinach (*Spinaceae oleracea* L. var. *americana*) flowers pollinated on the day of anthesis produced a slightly female-biased sex ratio, whereas those whose pollination was delayed for 2 weeks produced a significantly male-biased sex ratio. Moreover, unlike animal-pollinated flowers, the stigmas of wind-pollinated spinach flowers grew in size until they were pollinated, either by cross-pollination or self-pollination by the production of anthers. Miglia and Freeman (1995) also showed that the male-biased sex ratio observed was not due to certation (competition between and X- and Y-bearing pollen tubes [Correns 1928]), but rather to the age of the flower, as stigmas pollinated at either the tip or base produced a male-biased sex ratio in flowers for which pollination was delayed. These results showed that the distance traveled by a pollen tube to reach an ovule did not influence the outcome in terms of progeny sex ratio. Here we report a similar experiment using 2 species of *Atriplex*, which, like spinach, are members of the Chenopodiaceae (goosefoot family). However, these species differ from spinach in that they are noncultivars, occurring naturally in the western United States. We wanted to know if the same results occur in species that are native rather than cultivated.

The experiment was carried out at the USDA Forest Service Shrub Sciences Laboratory in Provo, Utah, and at a site nearby. The 2 species used were *Atriplex powellii* (Powell's saltbush) and *A. tridentata* (Basin saltbush), each native to several states in the western United States including Utah. *Atriplex powellii* is an herbaceous annual, whereas *A. tridentata* is a perennial shrub (Hall and Clements 1923, Welsh et al. 2003). Both species are wind-pollinated. Seeds were collected from *A. powellii* plants 15 km north of Woodside, Emery County, Utah and from *A. tridentata* plants 6 km southwest of Ephraim, Sanpete County, Utah, on 2 May 1996.

Seeds were germinated and seedlings grown in pots placed outdoors in random order. The pots were situated in waterproof containers so that the plants could be bottom-watered by partially filling the containers with water. As the plants neared flowering, they were transferred to a greenhouse that housed fans used for cross-pollination of plants. Fifteen females of both species were randomly assigned to 1 of 3 treatments: immediate pollination, delayed pollination (stigmas open and receptive for 2 weeks prior to pollination), and never pollinated. Thirty-two stigmas (2–3 stigmas per plant) were measured using digital calipers (±0.01 mm) the day they first emerged and again after 10 days. Pollination occurred in a separate room of the culture facility. Pollination was accomplished by using fans to blow pollen from male inflorescences (collected from at least 3 males) to stigmas of female plants (prior hand-pollinations failed to produce fruits).

Progeny studies were only conducted on *A. powellii*. Fruits from *A. powellii* plants were allowed to mature and then were harvested and stored at 4°C for 6 weeks (to mimic a dormancy period). Seeds were then germinated and planted to determine the progeny sex ratio. Germination occurred over a 2-year period, with new recruits being added weekly. No difference in the sex ratio was found between early and late-germinated seeds, so only the final data were used here. Seedlings were allowed to grow to maturity in a greenhouse. Plants were checked daily during the flowering period to census the number of males, females, and hermaphrodites. After anthesis, plants for which sex was difficult to determine, due to the extremely small flower size characteristic of *A. powellii*, were pressed and the flowers microscopically examined to unambiguously determine sex.

Stigmas that were immediately pollinated withered within a day, indicating that they were indeed receptive. Stigmas within the delayed pollination groups increased in length. For *A. powellii* they increased from 0.54 mm to 1.13 mm ten days later (an increase of 209%). For *A. tridentata* the increase was from 0.76 mm to 1.49 mm (196%). A paired t test showed that increase in length was significant for both species (paired t test, *A. powellii*: $n = 32$, $t = 7.20$, $P < 0.001$; paired t test, *A. tridentata*: $t = 5.44$, $P < 0.001$).

Surprisingly, plants deliberately not pollinated (i.e., the "never-pollinated" treatment) set fruit and produced both male and female progeny, indicating that these progeny were not parthenogenetically produced. We subsequently assumed that pollen was inadvertently moved via the air-handling system of the culture facility, that the number of pollen grains per stigma was sparse, and that pollination of these plants was delayed. These plants were

then classified as a "sparsely pollinated, delayed" treatment.

All progeny were classified into 4 categories: female, male, monoecious, and nonflowering (Table 1). Although the parental females were all outcrossed (being unable to self), they did produce monoecious offspring. When monoecious individuals and those that did not flower were included in a contingency table analysis (along with males and females), then the proportions in terms of sex did not differ significantly among the pollination treatments ($\chi^2 = 7.44$, $P > 0.25$). However, when the analysis was restricted to males and females only, the proportions did differ with more males in the delayed and the sparsely pollinated treatments (Fig. 1).

Here, and in an earlier experiment, Miglia and Freeman (1995) found that the stigmas of wind-pollinated chenopod plants continue to grow until they are pollinated. This is logical from an evolutionary perspective because increasing the size of the stigma increases the surface area for pollen capture in wind-pollinated plants. The data also suggested that pollination must terminate the expression of genes for stigma growth, though the mechanism for such regulation has yet to be elucidated. Finally, the data suggested that female wind-pollinated plants may sense the density of pollen and bias the sex ratio of their offspring as a result.

Chenopods are commonly found throughout the deserts of the world (McArthur and Sanderson 1984), and under such harsh conditions their population density can vary markedly in both time and space. For desert species, like *Atriplex*, the ability to adjust both stigma size and sex ratio of progeny may be adaptive because plants are widely spaced, at least at the edges of populations, and neighboring plants may not flower every year. Thus, pollen availability may actually limit seed set. In fact, McArthur et al. (1978) found that seed set of *Atriplex canescens* increases with the number of pollen grains deposited per stigma, up to

TABLE 1. The number of progeny in each of the 4 categories of *Atriplex*.

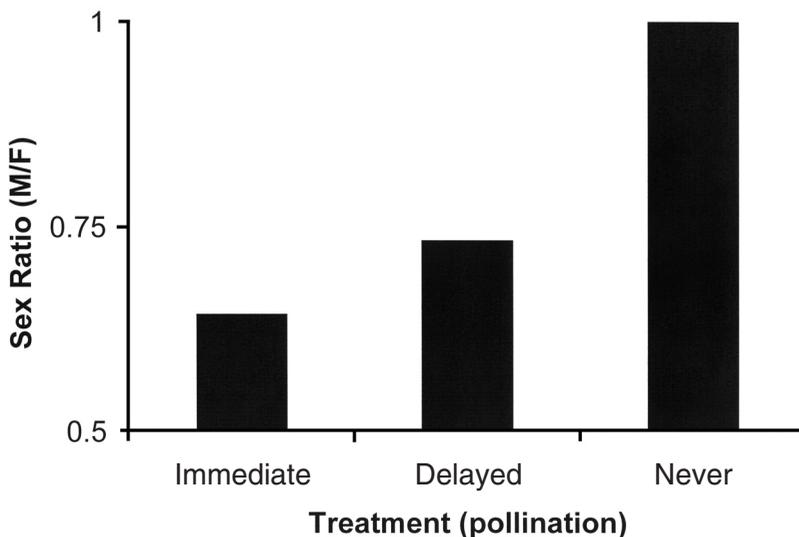| Pollination treatment	Female	Male	Monoecious	Nonflowering
Immediate	28	18	17	11
Delayed	30	22	11	14
Sparsely pollinated, delayed	45	45	26	11

Fig. 1. The ratio of male to female progeny in *Atriplex* by pollination treatment.

about 50 pollen grains per stigma. No increase in seed set was observed beyond this plateau.

For plants downwind of large population patches, 50 pollen grains per stigma was reached for plants separated by 15–18 m. Plants separated by a greater distance may be pollen limited. For plants in sparse populations (not downwind of large patches), plants must be ≤3 m apart to achieve the threshold of 50 pollen grains per stigma, indicating that pollen can be limiting even in areas of relatively high plant density. A 2nd study examined pollen flow around males found on steep slopes versus those on broad alluvial plains at the bases of slopes. Pollen flow was examined along the cardinal axes. Along the direction of the prevailing winds, plants separated by 30 m would still receive 50 pollen grains per stigma on both the slope and the alluvium. However, pollen limitation along the other axes could occur at distances <10 m (Freeman et al. 1993).

Freeman et al. (1997) showed that sexual lability is favored in populations in which there are strong resource gradients that differentially influence male and female fitness. They also showed that in the majority of these species there is a spatial segregation of plants based on sex (Freeman et al. 1976). Male plants are typically found in relatively harsh environments, whereas females predominate in more favorable habitats. Here, we have shown that the sex ratio is manipulated across a generation; the proportion of males to females increased as the time to pollination increased. Because plant density is likely to be lower in harsh sites compared to more favorable sites, it is possible that a portion of the skewed sex ratio may be attributed to the effect we have seen here, and that not all of the effect may be due to sexual lability.

How plants modify the sex ratio of their offspring is unknown. Based on our results and those of Miglia and Freeman (1995), time elapsed before pollination appears to be a critical factor. The adaptive value of manipulating sex ratios of progeny has been obvious for nearly 3 quarters of a century and has been demonstrated in animals (Werren and Buekeboom 1998). Plants tend to be viewed as static entities because they are sessile. However, being sessile in a changing environment requires a considerable level of plasticity, as well as the ability to sense the local environment and respond appropriately. Here we have shown

that females of *A. powellii* and *A. tridentata* respond to the time it takes for them to be pollinated. Their responses include increased stigma length and, in the case of *A. powellii,* a progressive modification of the sex ratio of progeny to increase fitness.

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