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Stephen D. Hendrix
University of Iowa

B Molano-Flores

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BRENDA MOLANO-FLORES[1] and STEPHEN D. HENDRIX

University of Iowa, Department of Biological Sciences, 420 CB,
Iowa City, Iowa 52242

ABSTRACT--We examined the mating system of candle anemone (*Anemone cylindrica*), a herbaceous prairie perennial. Four lines of evidence suggested that this species is autogamous: first, anthers shed pollen as stigmas matured, resulting in homogamy; second, only 7% of emasculated flowers received any pollen (usually one grain), and none set fruit; third, caged and open pollinated flowers had nearly 100% fruit set; and fourth, the pollen-ovule ratio was 371 ± 54.7 (±S.D.), within the known range of autogamous species. Understanding the mating system of prairie species is important to conservation efforts because autogamous species, such as *Anemone cylindrica*, are more likely to survive in a fragmented landscape than xenogamous species, which are dependent on pollinator service. However, small populations of autogamous and xenogamous species are equally vulnerable to destruction by stochastic events. Thus, autogamous as well as xenogamous species will benefit by protection at the habitat level.

Key words: Autogamy, reproductive biology, mating system, pollination, Iowa, *Anemone cylindrica*, candle anemone.

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[1] Current address: Illinois Natural History Survey-Center for Biodiversity, Midewin National Tallgrass Prairie, 30071 S. State Route 53, Wilmington, IL 60481.

The mating system of a plant species is an important aspect of its reproductive biology, potentially affecting responses to human induced changes in the landscape. For example, habitat fragmentation results in the isolation and reduction in size of plant populations (Petanidou et al. 1995) and for outcrossing species, decreases in fruit set and seed set are usually associated with small populations (Jennersten 1988, Kwak 1988, Byers and Meagher 1992, Hendrix 1994, Ågren 1996, Kyhl 1997). Such decreases in reproductive output may be because the pollinators are less abundant (Rathcke and Jules 1993, Olesen and Jain 1994) or because the efficiency of pollen transfer is reduced (Rathcke and Jules 1993, Aizen and Feinsinger 1994). In contrast to cross-pollinated species, self-pollinated, i.e., autogamous, species should be little affected by changes in plant-pollinator interactions. Thus, understanding the mating system and reproductive biology of species will be important in determining potential responses to habitat fragmentation and in developing sensible management plans.

In the genus *Anemone*, several species, including meadow anemone (*A. canadensis*), are xenogamous (Cruden 1977, Douglas and Cruden 1994) and evidence suggests that a decrease in population size or population density in *A. canadensis* reduces fruit set in some years (Molano-Flores and Hendrix in press). In our study, we ask the following two questions: first, what is the mating system of candle anemone (*Anemone cylindrica*)? and second, is this mating system similar to other *Anemone* species, e.g., *A. canadensis*, found in the Great Plains (Sutherland 1986, Runkel and Roosa 1989), one of the most fragmented ecosystems in North America (Sampson and Knopf 1994)? Also, we address concerns about the conservation of autogamous species in disturbed, e.g., fragmented, habitats.

METHODS

Study species

Anemone cylindrica Gray (Ranunculaceae), is a nonrhizomatous or short-rhizomatous perennial, 2.5 to 7 dm tall, found mostly in open prairies and pastures (Sutherland 1986). The flowering period of *A. cylindrica* in Iowa is from June to August. Flowering stems produce two to seven flowers in a cyme, or rarely a solitary flower. Flowers are about 2 cm across, with whitish sepals. The numerous pistils are arranged in a cylindrical head and stigmas have a reddish color at maturity (pers. observ.). Two sets of stamens, long and short, are arranged in concentric circles around the pistils. Flowers do not have nectaries. The fruit is a dense cylindrical spike 20 to 35 mm long. Achenes are covered by white woolly pubescence.

Study site

We conducted our study during 1993, 1994, and 1995 at Cayler Prairie, Dickinson County and Anderson Prairie, Emmet County, Iowa. Cayler Prairie is a 64 ha preserve with close to 300 native prairie species. The landscape of Cayler Prairie is typical of glacial end moraines, consisting of a complex of potholes and ridges. The plant communities represented on Cayler Prairie include dry and wet prairies, and marshes. The dry prairies are dominated by little bluestem (*Andropogon scoparius*) with mesic sites dominated by big bluestem (*Andropogon gerardii*) and Indian grass (*Sorghastrum nutans*). Sedges (*Carex* spp.), cattails (*Typha* spp.), and American sloughgrass (*Beckmannia syzigachne*) are common in the marsh areas.

Anderson Prairie is an 80 ha preserve with over 250 plant species. This preserve is located on the Altamont moraine. The landscape consists of dry, gravel ridges and wet swales. Anderson Prairie is bordered on the eastern edge by floodplain forest along the West Fork of the Des Moines River. Dominant grasses of the dry prairie are side-oats grama (*Bouteloua curtipendula*) and *Andropogon scoparius*. The mesic prairie is dominated by *Beckmannia syzigachne*, blue-joint (*Calamagrostis canadensis*), and *Carex* spp. The savannas have been degraded by grazing, but still contains numerous large bur oaks (*Quercus macrocarpa*).

We used Cayler Prairie and Anderson Prairie in different years for different portions of our study based upon availability of *A. cylindrica* populations. Cayler Prairie was the study site for flower phenology, plant mating system studies and pollen-ovule ratio analysis in 1993 and 1994. Anderson Prairie was the study site for pattern of pollen grain arrival to stigma and flower emasculation studies to examine pollen deposition and movement in 1995.

Flowering phenology

In late June, 1994, we tagged 20 plants before flower buds opened. The flowering phenology of each plant, including stigma receptivity and timing of anthesis, was followed for a period of seven days until sepals abscised.

Pattern of pollen arrival to stigmas

To establish the number of pollen grains that reach the stigmas of flowers of *Anemone cylindrica* over time, we tagged 90 plants before flowers opened in 1995. For a period of six days, we collected 15 flowers daily. Flowers collected on day one had been open one day; on day two, for two days, and so on. We stored flowers in a solution of 3:1 95% ethanol:acetic acid.

We divided the cylindrical head of the flowers of *A. cylindrica* into three sections: top, middle, and bottom, to estimate the number of pollen grains arriving per stigma per day. From each section, we haphazardly selected 15

pistils per flower and prepared them for UV light microscopy. We soaked pistils in 1N NaOH for 12 hrs to soften the tissue, stained them with 0.1% aniline blue in 0.1 M K_3PO_4 for 12 hrs (Martin 1959), and mounted them in glycerol. For each pistil, we scored the number of pollen grains and pollen tubes.

To determine if outcrossed pollen reached stigmas, we manually opened and emasculated 15 additional flowers before the sepals had begun to spread. We collected these flowers at day six and prepared pistils for UV light microscopy (see above).

Mating system

To test for xenogamy or autogamy, we performed the following series of open and caged treatments on 20 plants per treatment (one plant per cage) in June, 1994: 1) caged flowers to measure fruit formation by self fertilization, 2) open-pollinated flowers to measure fruit formation by both self or cross pollination, and 3) open-pollinated, emasculated flowers to measure fruit formation by cross pollen. The cages consisted of wooden frames covered with mosquito netting. We sprayed flowers of caged plants with manitol every other day to prevent the presence of thrips, which may move pollen grains (Cruden et al. 1990, Baker and Cruden 1991). Also, we applied Tanglefoot® at the base of the receptacle to prevent crawling insects from reaching the flowers of caged plants. At the end of fruit development, we collected infructescences to determine fruit set.

Pollen-ovule ratio

We estimated the pollen-ovule ratio for *Anemone cylindrica* by using ten unopened flowers collected from different plants in July of 1993. For each flower, we counted the number of ovules, number of anthers, and the number of pollen grains per anther. To count the number of pollen grains per anther, we dissected anthers on a slide and carefully removed the anther walls. We dyed pollen grains with cotton-blue in lacto-phenol and using a gridded slide we counted them. We followed the methods of Cruden (1977) to estimate the pollen-ovule ratio.

Statistical analyses

We used Kruskal-Wallis tests to examine differences in the number of pollen grains and pollen tubes per stigma per day based on pistil location (top, middle, and bottom) and to examine variation in total number of pollen grains and pollen tubes per stigma between days. Following the Kruskal-Wallis tests, we used Student-Newman-Keuls (SNK) post hoc test when sample sizes were equal, and Dunn post hoc test when sample sizes were unequal. To obtain the average number of pollen grains and pollen tubes per stigma per day, the

number of pollen grains and pollen tubes per stigma per location was pooled. We performed all tests by using SigmaStat version 1.01 (1992).

RESULTS

Flowering phenology

In the two to three days following flower opening, the partially expanded sepals of *A. cylindrica* closed each evening and open the following morning. By the third day, sepals were fully and permanently expanded. Stamens were bent over the pistils for the first few days before the sepals fully expand. Progressively, the stamens separated from the pistils and by the second day, the anthers of stamens with long filaments started dehiscing. Anthers progressively dehisced until the fifth day when all anthers, including those with short filaments, have dehisced. Styles were initially parallel to the axis of the head, but bent away from the long axis of the cylindrical head starting at the top on the first day, and continuing until the bottom styles were bent between the third and fourth days. Almost simultaneously with bending of the styles, the stigmas changed from a greenish to reddish coloration. Between the fourth and seventh days, sepals and the stamens shrivel and abscise, and all stigmas turned brown.

Pattern of pollen arrival to stigmas

There was an increase in the number of pollen grains per stigma per day, from the first to the fourth day after flowers opened (Fig. 1). However, during the fifth and sixth days, the number of pollen grains per stigma declined significantly (Kruskal-Wallis test: $H = 940$, $df = 5$, $P< 0.001$, Dunn test $P< 0.05$). In the stigmas examined, all pollen grains produced pollen tubes.

The median number of pollen grains per stigma per day showed significant variation between the top, middle, and bottom of the head (Kruskal-Wallis test: all H values >10.11, P values < 0.006, SNK all P values < 0.05, Fig. 2). After the first day when no pollen appeared on stigmas, stigmas located at the middle received more pollen grains than stigmas at the top or bottom.

For flowers that were emasculated and examined after six days, only 48 stigmas of 675 stigmas examined (7%) received pollen grains from another *Anemone cylindrica* flower (Fig. 3). Of those stigmas that received pollen, most received only one pollen grain. We found alien pollen grains on 8% of stigmas of *A. cylindrica* sampled (57 of 675 stigmas).
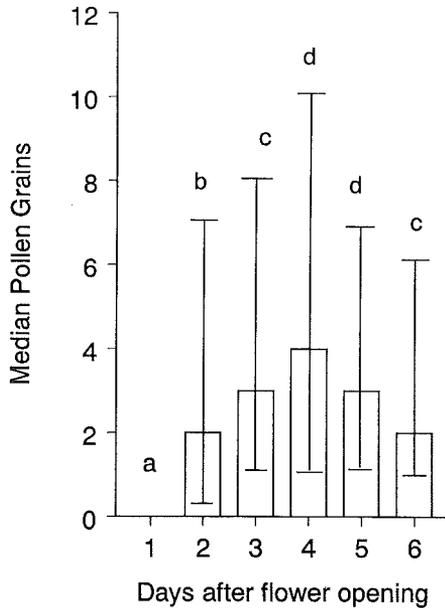
Figure 1. Median number of cumulative pollen grains per stigma (± 75% and 25% quartiles) on successive days after flowers of *Anemone cylindrica* opened. Histogram bars with different letters differed significantly ($P < 0.05$).
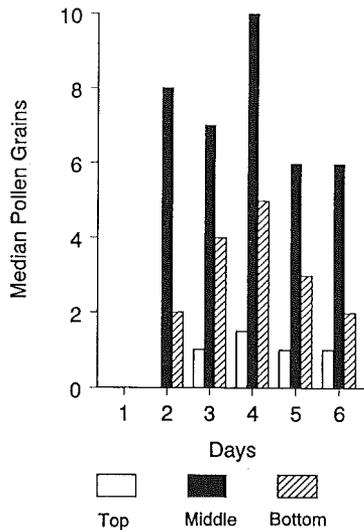

Figure 2. Median number of pollen grains per stigma per day at the top, middle, and bottom of the cylindrical receptacle of *Anemone cylindrica*.
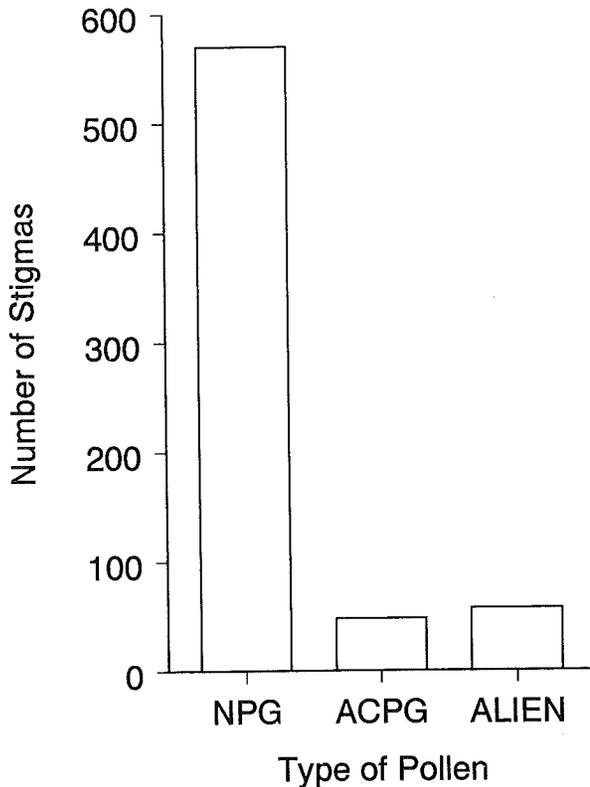
Figure 3. Frequency distribution of number of pollen grains on *Anemone cylindrica* stigmas in emasculated flowers. NPG = No pollen grains on stigmas, ACPG = *Anemone cylindrica* pollen grains, and ALIEN = Alien pollen grains.

Mating system

Fruit formation was nearly 100 percent in both caged and open pollinated flowers. Emasculated flowers did not set fruits.

Pollen-ovule ratio

Anemone cylindrica averaged 257 ± 40.6 ($\overline{X}$ ± S.D.) ovules and 44 ± 9.2 anthers per flower (n = 10). The average number of pollen grains per anther was 2,166 ± 305 (n = 10) yielding a pollen-ovule ratio for *A. cylindrica* of 371 ± 54.7.

DISCUSSION

Anemone cylindrica has many characteristics of an autogamous or self-pollinated species (Wyatt 1983, Richards 1986), including small flowers, homogamy, i.e., anthers shed pollen as the stigmas mature, lack of nectar and low levels of outcross pollen grains arriving to emasculated flowers. Also, the pollen-ovule ratio of _A. cylindrica_ was 371, within the range of 34 autogamous species (mean ± S.E., 18.1 ± 0.5 to 396.9 ± 13.7) reported by Cruden (1977) and Cruden and Lyon (1989). Compared to other members of the genus _Anemone_ with known pollen-ovule ratios and breeding systems, _A. cylindrica_ had the lowest pollen-ovule ratio, which supported the conclusion that _A. cylindrica_ is autogamous. Tall anemone (_A. virginiana_), had a pollen-ovule ratio of 934 (206 ovules, 119 anthers, 1616 pollen grain per anther) (Molano-Flores unpubl. data) and was considered facultatively xenogamous. Both _Anemone canadensis_ with a pollen-ovule ratio 9,895 (Douglas and Cruden 1994) and pasque flower (_A. patens_) with a pollen-ovule ratio 3,492 (Cruden 1977) were considered xenogamous species.

The extremely low levels of pollen arrival to the stigmas of emasculated flowers (Fig. 3) suggested that stigmas rarely receive outcross pollen. The fact that as much alien pollen as _A. cylindrica_ pollen appeared on emasculated flowers suggested haphazard visits by pollinators or movement of pollen by non-living agents. An unidentified solitary bee was observed visiting flowers, but such events were rare. Nonetheless, as Schemske and Lande (1985) suggested, even small amounts of cross-pollination in self-pollinated species can maintain genetic variation that may help the species respond to temporally and spatially varying selection pressures.

One finding of our study that did not have a clear explanation was that cumulative pollen grain deposition on _A. cylindrica_ stigmas increased during the first four days, but declined thereafter (Fig. 1). Although no more accumulation of pollen grains after the fourth day should be expected because stamens began to abscise at that time, pollen numbers should remain the same. Probably, factors such as rain or insects that feed on pollen, e.g., syrphid flies and beetles, contributed to the decline of pollen grains after the fourth day.

Anemone cylindrica is one of several autogamous species found in the fragmented prairie ecosystem that may not be subjected to plant-pollinator problems associated with habitat fragmentation (Jennersten 1988, Kwak 1988, Aizen and Feinsinger 1994, Petanidou et al. 1995, Kyhl 1997). However, their existence in a fragmented habitat is not assured. Autogamous species are known to have low genetic variability and many of them are homozygous at many loci (Richards 1986). Thus, changes in allele frequency in response to

environmental changes associated with fragmentation, e.g., herbicides, edge effects, etc., may be slower than in xenogamous species.

Determining the breeding system of species is essential before estimating the size of the area that needs to be protected as part of plant conservation plans (Ayensu 1981). Autogamous species may require less habitat than xenogamous species, because of the lack of pollinator interactions associated with cross-pollinated species. This may account for the relatively few endangered or threatened autogamous species that are protected. However, both autogamous and xenogamous species, can be affected by habitat destruction as in the case of presidio clarkia (*Clarkia franciscana*) (Onagraceae) and malheur wire-lettuce (*Stephanomeria malheurensis*) (Asteraceae) (Endangered Species Technical Bulletin 1993, Code of Federal Regulations: Wildlife and Fisheries Part 50 1995). Increasing evidence suggests that protecting individual species may not be the most effective conservation practice (Samways 1994, Wilcove 1994, Williams 1995). Thus, autogamous species, such as *A. cylindrica*, may benefit more by inclusion in plans aimed at protecting the habitat.

ACKNOWLEDGMENTS

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