



HAND-POLLINATION OF

# *Cardamine californica*

IMPROVES SEED SET

Kazuki Ariyoshi,  
Emily Magnaghi,  
and Mark Frey

## ABSTRACT

Hand-pollinating milkmaids (*Cardamine californica* (Nutt.) Greene var. *integrifolia* (Nutt.) Rollins [Brassicaceae]) increased seed set from 8% to 85%. In the Presidio of San Francisco, a district of the Golden Gate National Recreation Area in California, our restoration efforts using milkmaids were hampered by low seed set of our locally adapted plants. Plants in 2 populations were successfully cross-pollinated by hand and seeds ripened about 53 d later and were harvested. Although exact seed germination rates are not available, we noted that germination of hand-pollinated seeds was at least 40% in the nursery. This technique may be appropriate for other species in which natural reproduction is insufficient. We also discuss the role of natural pollinators and the physical changes that occur in the flowers during pollen maturation.

Ariyoshi K, Magnaghi E, Frey M. 2006. Hand-pollination of *Cardamine californica* improves seed set. *Native Plants Journal* 7(3):249–252.

## KEY WORDS

milkmaids, seed production, propagule, pollinator, reproduction, Brassicaceae

## NOMENCLATURE

USDA NRCS (2006)

*Figure 1.* Milkmaids (*Cardamine californica* (Nutt.) Greene var. *integrifolia* (Nutt.) Rollins [Brassicaceae]) Photo by Mark Frey

**M**ilkmaids (*Cardamine californica* (Nutt.) Greene var. *integrifolia* (Nutt.) Rollins [Brassicaceae]) are a common native species in California and Oregon. This perennial herb grows below 500 m (1640 ft) in habitats such as open meadows, hill slopes, and canyons (Hickman 1993). Plants can reproduce from tuber-like rhizomes (Hickman 1993) and generally grow in clusters. At our location (described below), we have noted that milkmaids are one of the earliest spring wildflowers, blooming January through May. The flower closes its petals in late afternoon as the sun goes down and nods its pedicel before a rain, protecting the pollen (Fauver and others 1998). Synonyms include *Dentaria integrifolia*, *D. californica* var. *integrifolia*, *Cardamine paucisecta*, *C. integrifolia*, and *C. californica* prol. *integrifolia*.

Milkmaids can grow to a height of 20 to 70 cm (8 to 27 in) and develop both rhizomal and cauline leaves. Rhizomal leaves number 1 to 3 per plant (occasionally more), are always larger, make up the majority of the photosynthetic surface, and are usually more fleshy (Deitling 1936). Leaves on the rhizome generally have 3 leaflets, which are simple, entire, and elliptic to round, tapering at the base. Cauline leaves have 3 lobes or leaflets and are short-petioled, linear to oblong in shape, and generally entire. There are also upper cauline leaves, which are linear to oblong, entire, and sessile. The inflorescence is a raceme of white to light pink flowers with 9 to 14 mm (0.3 to 0.5 in) long petals. In fruit, the pedicels are ascending 10 to 30 mm (0.4 to 1.2 in) and the dehiscent siliques are erect, 20 to 50 mm (0.8 to 2 in) long and 1.5 to 2 mm (.05 to .08 in) wide (Hickman 1993).

Although common in California, milkmaids are uncommon in the Presidio of San Francisco. Only 1 of the 7 known Presidio populations has more than 50 individuals. This species has been collected for propagation, and

nursery records indicate low seed set. In 2002 we collected only 25 seeds. In an attempt to increase seed yields, a hand-pollination experiment was conducted in the field. We hypothesized that hand-pollination might: 1) reveal if milkmaids lack the capability to effectively produce seeds; and 2) increase the amount of seeds produced.

## SITE DESCRIPTION

Seven populations of milkmaids grow on the 603 ha (1490 ac) Presidio of San Francisco (Presidio), California. The Presidio is located within the Golden Gate National Recreation Area, at the northern edge of San Francisco, on the south side of the Golden Gate Bridge. The area's mediterranean-like climate is moderated by the Pacific Ocean, creating average temperatures of 14 °C (56 °F). An eastward onshore wind is predominant. The Presidio has many remnant and restored habitats, such as serpentine grassland, coastal scrub, live oak woodland, coastal salt marsh, coastal foredune, and riparian forest. Dominant vegetation assemblages also include plantation forests of trees not native to the Presidio. These plantations are populated by Monterey cypress (*Cupressus macrocarpa* Hartw. ex Gord. [Cupressaceae]), Monterey pine (*Pinus radiata* D. Don [Pinaceae]), and blue gum eucalyptus (*Eucalyptus globulus* Labill. [Myrtaceae]).

The Presidio was an active military base from 1776 to 1989. In 1994, with its transfer from the US Army to the National Park Service, the Presidio was designated for public use and habitat restoration commenced. The Presidio Native Plant Nursery is run by the non-profit Golden Gate National Parks Conservancy. The nursery has a 60 000 plant capacity and grows 200 native species, including milkmaids, to be planted at many habitat restoration sites. Ecological considerations dictate that the nursery collect all propagules from within the Presidio, collecting no

more than 5% of annual seed set from each population of any native species.

All Presidio populations of milkmaids are isolated from one another. Two populations were studied, located in areas of the park called Log Cabin/Doyle Drive South Corridor (Log Cabin) and Inspiration Point Natural Areas (Inspiration Point).

The Log Cabin population is located along a gravel road that extends from a parking lot and follows the edge of a seasonal wetland. This population is divided and partially surrounded by California blackberry (*Rubus ursinus* Cham. & Schlecht. [Rosaceae]) and Himalayan blackberry (*R. armeniacus* Focke [Rosaceae]), both shrubs are up to 2 m (8 ft) tall. Other associated plants include various nonnative species, such as common sheep sorrel (*Rumex acetosella* L. [Polygonaceae]), poison hemlock (*Conium maculatum* L. [Apiaceae]), common velvetgrass (*Holcus lanatus* L. [Poaceae]), English plantain (*Plantago lanceolata* L. [Plantaginaceae]), and shortpod mustard (*Hirschfeldia incana* L. Lagrèze-Fossat [Brassicaceae]) and native species, such as western blue-eyed grass (*Sisyrinchium bellum* S. Wats. [Iridaceae]), fringed willowherb (*Epilobium ciliatum* Raf. [Onagraceae]), and slough sedge (*Carex obnupta* Bailey [Cyperaceae]).

The Inspiration Point population is located in a serpentine grassland near the top of a dry, north-facing slope. The grassland contains *Holcus lanatus* L. [Poaceae], *Rumex acetosella* L. [Polygonaceae], and many native species including stemless morning-glory (*Calyptegia subacaulis* Hook. & Arn. [Convolvulaceae]), and California meadow barley (*Hordeum brachyantherum* Nevski [Poaceae]). The open exposure of the site appears to limit soil moisture, although the California meadow barley prefers to grow in areas with year-round moisture, possibly indicating an underground water source.

## METHODS

The Log Cabin population was pollinated with pollen collected from a population on the serpentine coastal bluffs. The Inspiration Point population was pollinated with pollen from the Log Cabin population. Subsamples of the Log Cabin and Inspiration Point populations were not hand-pollinated. Both hand-pollinated and non-hand-pollinated populations at Log Cabin and Inspiration Point were weeded to increase visibility of tags and flowers, to make monitoring easier, and to facilitate harvest of fruits.

For pollen collection, anthers were monitored every 3 d for maturity starting in early March. This species is protandrous. Stamens mature in 3 to 5 d of the flower opening and stigmas mature several days later. An immature anther is swollen, glossy yellow, with no visible pollen grains. The anther is thin and dull yellow once it has opened to release pollen. The stigma is receptive when it is a glossy whitish-green. When pollinated, the stigma turns yellow. We used forceps to cut the base of the filament on a mature anther. Anthers were placed in a sealed, black container (to

contrast with the plant parts) for immediate transport to another population. When a receptive stigma was found, forceps were used to pick up the filament from the container and to rub the stamens and stigma together lightly until the stigma turned yellow. The style was left intact to allow for natural pollination. To facilitate monitoring, a label with a unique number was attached to each pedicel, and flagging tape with a unique number was tied around the bottom of each stem.

After successful pollination, the ovary lengthens, transforming into a dehiscent silique in 2 to 3 d. The fruit is initially reddish purple. Within a month, approximately 10 seeds become visible through the capsule wall as the fruit color changes to light green. The fruit is finally ripe and ready to dehisce when it turns yellow.

The 2 locules of the silique are separated by a septum. In each locule, 6 to 12 seeds are attached in a line along the septum, on alternating sides. The silique is flattened in the same plane as the septum. The valves are elastic and open suddenly, ejecting the seeds some distance (Deitling 1936).

## RESULTS

The number of fruit-producing flowers for the Log Cabin and Inspiration Point hand-pollinated, weeded populations averaged 85% (Table 1), much higher than those weeded but not hand-pollinated (8%). Personal observations of non-weeded, not hand-pollinated plants on the coastal bluffs indicate that the natural pollination success rate was much lower (3.5%; data not shown). For hand-pollinated flowers, the average ripening duration was 50 d, and the highest number of pollinated fruits became ripe and dispersed seeds around the third week of May. Using the average ripening duration, naturally pollinated flowers were likely pollinated between late March and early April, coincident with our hand-pollination of treated plants. We collected 18 seedpods, representing 56% of the successfully hand-pollinated fruits. Although accurate records are unavailable, germination in the nursery was at least 40%.

TABLE 1

*Results of hand-pollinating milkmaids.*

Population	Pollen source	Number of plants	Number of flowers	Number of seedpods	Success rate (%)	Days to ripen <sup>z</sup>
<b>HAND-POLLINATED</b>						
Log Cabin	Coastal bluffs	8	29	25	86	53
Inspiration Point	Log Cabin	2	6	5	83	48
<b>NOT HAND-POLLINATED</b>						
Log Cabin		8	290	22	8	not applicable
Inspiration Point		1	12	1	8	not applicable

<sup>z</sup> Average days from hand-pollination to harvest at maturity

## DISCUSSION

Milkmaids can reproduce well in the Presidio if hand-pollinated, but hand-pollination is time and labor intensive. As blooming is not simultaneous, frequent visits are necessary. Hand-pollination, however, may be useful for species such as milkmaids that have low propagule demands and that exhibit limited natural reproduction.

We found it helpful to weed around the target population immediately following hand-pollination to successfully monitor fruit development and harvest seedpods. Weeding probably reduced competition as well and may have stimulated vegetative sprouting and reproductive success.

It is unclear what factors are causing low seed set in milkmaids at the Presidio. In the future, a simple cross-pollination experiment focusing on plants within only one of the Presidio's populations may help answer this question. If pollen transfer within a population is successful, low seed set is likely the result of a missing pollinator or competition with annual grasses that often overtop the plants. If pollen transfer within a population is unsuccessful, there may be genetic constraints and cross-pollinating between populations is recommended. This may be accomplished by either transplanting individuals or direct seeding and would need to be monitored carefully.

If pollinators are identified as the limiting factor, it is critical to identify milkmaids' native pollinators outside the Presidio. In late March, we observed bee flies (Bombyliidae; Proctor 1973; Bugbios 2003) hovering above and probing the center of milkmaids' flowers at the Log Cabin site. We do not know if this species is a pollinator or if other pollinators are necessary. If appropriate pollinators are present, further research is needed into why they are not able to pollinate milkmaids within the Presidio. Significant fragmentation of milkmaid populations in the Presidio, the small

sizes of existing populations, and the presence of many landmarks between those populations may also hamper pollination. Expanding existing populations to improve connectivity may enhance the ability of native pollinators.

## ACKNOWLEDGMENTS

The authors thank the Golden Gate National Parks Conservancy and the Presidio Trust for providing staff time for the authors to work on this project. Thank you to the editor and two reviewers for extensive and helpful comments on an early draft.

## REFERENCES

- Bugbios. 2003. Bee flies. URL: <http://www.insects.org> (accessed 21 Mar 2003).
- Deitling LE. 1936. A taxonomic and distributional study of the Pacific Coast species of *Cardamine* and *Dentaria* [PhD dissertation]. Palo Alto (CA): Stanford University. 122 p.
- Fauver T, Kemp M, Breck H. 1998. Wildflower walks and roads of the Sierra Gold County. Grassvalley (CA): Comstock Bonanza Press. 122 p.
- Hickman JC, editor. 1993. The Jepson manual: higher plants of California. Berkeley (CA): University of California Press. p 407–409.
- Proctor M. 1973. The pollination of flowers. London: Collins. p 77–80.
- [USDA NRCS] USDA Natural Resources Conservation Service. 2006. The PLANTS database, version 3.5. URL: <http://plants.usda.gov> (accessed 2 Aug 2006). Baton Rouge (LA): National Plant Data Center.

## AUTHOR INFORMATION

**Kazuki Ariyoshi**  
Propagule Collection Intern  
(former)

**Emily Magnaghi**  
Master's Degree Candidate  
San Francisco State University  
Propagule Collection Coordinator  
(former)  
EMagnaghi@CalAcademy.org

Presidio Native Plant Nursery  
Golden Gate National Parks  
Conservancy  
Bldg 201, Fort Mason, 3rd floor  
San Francisco, CA 94123

**Mark Frey**  
Ecologist  
The Presidio Trust  
1750 Lincoln Blvd  
PO Box 29052  
San Francisco, CA 94129  
MFrey@presidiotrust.gov