A first look at the reproductive biology of *Astragalus applegatei*: Oregon's most imperiled plant


(Melissa Carr)

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INTRODUCTION

- Applegate's milkvetch, *Astragalus applegatei,* is a caulescent perennial legume (Fabaceae) and is a narrow endemic of moist alkali meadows in southern Klamath County, Oregon (Fig. 1 & 2, ***1***).
- It was thought to be extinct until it was rediscovered in 1983, was listed as Endangered by the U.S. Fish and Wildlife Service in 1993, and is also an Oregon State Endangered species (***2***).
- Currently, *A. applegatei* is known to exist at only three major sites and two minor sites within the Upper Klamath Basin (Fig. 3).
- Factors threatening this species include habitat loss, competition from invasive species, herbivory by insects and larvae, and grazing by cattle. Additionally, it can only grow in soils that have been inoculated with a native arbuscular mycorrhiza in the genus *Glomus* (***3***).
- Small population size may also threaten the species' survival. There is often a relationship between population size and reproductive success in rare and endangered plants, particularly if the species requires allogamy (outcrossing) for reproduction.
- Autogamy (self-pollination) is a common mating system in the *Astragalus* genus, facilitated by simultaneous ripening of anthers and stigmas (**1**).
- It is assumed that *A. applegatei* can self-pollinate, but to date there has been no research to determine the frequency and success of autogamy of the species.


Figure 1. Moist alkali meadow habitat at Ewauna Flat Preserve, Klamath Falls, OR.


Figure 2. *A. applegatei* individual. Plants have multiple sprawling stems 30 – 90 cm long with 3.5-12 cm long leaves with 7-11 leaflets. Racemes typically bear 5-20 pea-like flowers with small white to light pink to lavender flowers.

RESEARCH QUESTIONS

1. Does self-pollination result in fruit and seed set in *A. applegatei,* and if so, how does it compare to fruit and seed set by outcrossed individuals?
2. Does fruit and seed set vary across different subpopulations?

Our study provides a first look at the reproductive biology of this critically endangered plant species.

METHODS

We performed our experiment in one subpopulation in 2014 (Ewauna Flat Preserve) and three subpopulations in 2015 (Ewauna Flat Preserve, Klamath Regional Airport, and the Miller Island Unit of the Klamath Wildlife Refuge). All subpopulations were located near or within the city of Klamath Falls in Klamath County, Oregon (Fig. 3).

❖Experimental Design:

- **Ewauna Flat Preserve (EFP)** (2014 & 2015): 3 parallel 50 m. transects within core population, randomly selected 10 plants at 5 m. intervals along each transect.
- **Klamath Regional Airport (Airport)** (2015): randomly selected 20 individuals along a 200 m. transect within fenced airport, 10 individuals along a 50 m transects outside of fenced airport.
- **Miller Island (Miller)** (2015): located 15 individuals within small subpopulation.

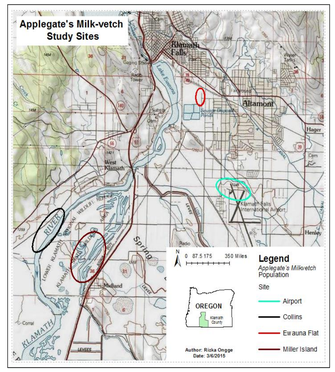

Figure 3. Current confirmed subpopulations of *A. applegatei*. An additional small subpopulation may exist on private land near the California border. The Crater Lake Klamath Regional Airport (Airport), The Nature Conservancy's Ewauna Flat Preserve (Ewauna Flat), and the Miller Island Unit of the Klamath Wildlife Refuge (Miller Island) subpopulations were used in this study.

❖Pollination Experiment:


Figure 4. Rizka encloses an *A. applegatei* stem with a fine mesh bag to exclude insect pollinators in May, 2015. A second stem, which served as an open-pollinated control, was selected in July and enclosed in a mesh bag once legumes began to mature, in order to capture mature seeds as the fruits dehisced.

- Selected 2 stems on each plant with no opened flowers.
- Randomly selected 5 racemes on each stem and counted the number of flower buds on each inflorescence.
- Covered one stem with a fine mesh bag with a removable zip-tie to exclude insect pollinators (Fig 4). The second stem served as an open-pollinated control.
- As fruits matured (mid-July to mid-September), we harvested stems and brought them back to the lab and counted the number of legumes and viable and aborted seeds per stem.

LITERATURE CITED & ACKNOWLEDGEMENTS

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RESULTS

❖ Although there was not a large difference in mean number of flowers produced by self- and open-pollinated racemes (Table 1), pollinator exclusion greatly reduced the number of legumes and viable seeds produced in our experiment across all subpopulations (Fig. 5 & 6).

Table 1. Number of flowers per raceme, estimated by counting the number of flowers on 5 randomly selected racemes per stem. Data represent mean (and SE).

Site	Treatment	
	self	open
Ewauna Flat (2014)	8.4 (0.4)	6.8 (0.5)
Ewauna Flat (2015)	6.8 (0.3)	8.6 (0.4)
Airport	6.6 (0.5)	6.3 (0.8)
Miller Island	8.6 (0.5)	10.1 (1.1)

❖ Fruit and viable seed production varied by location. Individuals at Ewauna Flat Preserve produced the greatest number of legumes and viable seeds per stem, while individuals at the smallest subpopulation, Miller Island Wildlife Refuge, produced the least number of legumes and viable seeds per stem (Fig. 5 & 6).

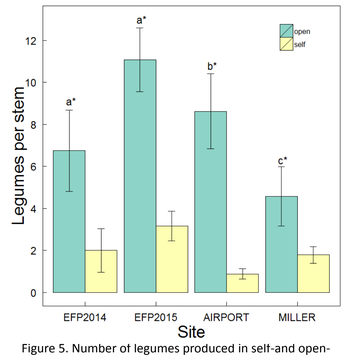

Figure 5. Number of legumes produced in self-and open-pollinated stems at Ewauna Flat in 2014 and 2015, and the Airport and Miller Island in 2015. Data represent mean +/- SE. Letters indicated significant location differences, and * represents significant differences between treatments within a site. P<0.05.

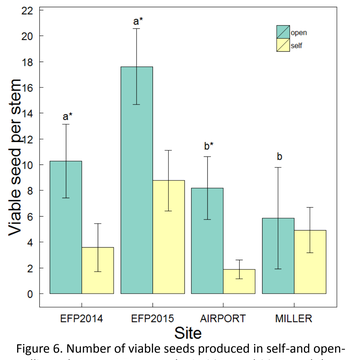

Figure 6. Number of viable seeds produced in self-and open-pollinated stems at Ewauna Flat in 2014 and 2015, and the Airport and Miller Island in 2015. Data represent mean +/- SE. Letters indicated significant location differences, and * represents significant differences between treatments within a site. P<0.05.

CONCLUSIONS

- While we cannot assume that all flowers in the open pollinated control were visited by pollinators, our research provides strong evidence that fruit and seed production in *A. applegatei* is dramatically reduced when pollinators are excluded.
- As we expected, the smallest subpopulation, Miller Island, had the lowest fruit and seed production. This may be due to pollen limitation or inbreeding depression.
- Interestingly, the largest subpopulation (Airport) did not have the highest fruit and seed production. Perhaps airport management or other abiotic factors such as water availability is limiting reproductive success at this location.
- More research should be done on the types of pollinators that visit *A. applegatei*, and future conservation efforts should focus on increasing out-crossing in this imperiled species.