Testing pollinator syndromes and cross compatibility in evening primroses (Onagraceae)

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Introduction

Angiosperms, or flowering plants, appeal to specific pollinators they wish to attract by exhibiting certain floral colors and scents, and by opening when the pollinator is active (Dodd *et al.* 1999). Evolutionary adaptations increase the chances that pollination by a particular pollinator will occur, thus increasing the likelihood that pollen from the same species will contact the plant's stigma to produce a viable seed.

Cross compatibility of the male and female gamete is also important for reproductive success. *Oenothera hartwegii* subsp. *filifolia* and *O. gayleana* are suspected to be self incompatible members of the evening primrose family, Onagraceae. The mechanism for their self-incompatibility is thought to be a suite of alleles (SI alleles) preventing pollen tube formation (Emerson 1938). If so, a cross between siblings or between plants in the same population may exhibit reduced reproductive success when compared with crosses made between populations, because they have a higher likelihood of having the same SI alleles due to the principle of isolation by distance (Rousset 1997). Both successful pollination and the formation of a viable seed is crucial for reproduction. Studying these processes can provide valuable insight into the evolutionary relationship between different plants and pollinators.

Hypotheses

1. Flowers exhibiting hawkmoth pollination syndrome characteristics will be primarily visited by hawk moths, flowers exhibiting bee pollination syndrome characteristics will be primarily exhibited by bees, and flowers with an intermediate suite of characteristics will be visited by both.
2. *Oenothera gayleana* and *O. hartwegii filifolia* are self-incompatible species. Crosses of least relation (between population) will produce the greatest seed set, followed by crosses of possible relation (within population), and lastly crosses of definite relation (siblings of the same maternal line).

Table 1: Pollination syndrome and floral traits of five *Oenothera* species

Pollination Sydrome	Species	Photo	Anthesis	Floral Size	Floral Color	Nectar	Length of Time Open
Hawkmoth	*O. cespitosa* subsp. *marginata*		PM	Large	White	Yes	~15 hours
Intermediate	*O. lavandulifolia*		PM	Large	Yellow	Yes	~15 hours
Intermediate	*O. toumeyi*		PM	Large	Yellow	Yes	~24 hours
Bee	*O. gayleana*		AM	Small	Yellow	No	~12 hours
Bee	*O. tubicula*		AM	Small	Yellow	No	~12 hours

References

- Dodd, M. E., Silvertown, J., & Chase, M. W. (1999). Evolution, 53(3), 732-744 Friedman, W. 2009. The meaning of Darwin's "abominable mystery." Am. J. Botany 96 (1): 5-21
- Emerson, Sterling. "The genetics of self-incompatibility in Oenothera organensis." *Genetics* 23.2 (1938): 190.
- Rousset, François. "Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance." *Genetics* 145.4 (1997): 1219-1228.

Acknowledgements

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Methods: Pollinator Observations

- Pollination observation videos were taken using a Go Pro camera set up in the field during the summer of 2014. Cameras were placed approximately one foot away from newly open flowers during three observational periods: morning(5:00 to 11:00), afternoon(11:00 to 17:00)and night (17:00 to 20:00).
- Videos were scored and the following data were recorded: time of day, a sketch of the plant of interest, number of visits and identification of the floral visitors. Visitation rates per hour were calculated for each species.

Figure 1. Hawkmoth pollination syndrome. *Hyles lineata* visiting O. *harringtonii*. Photo Credit: Skogen

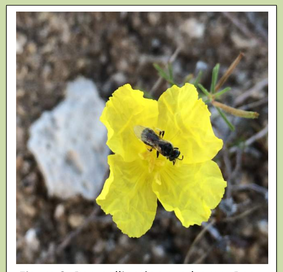

Figure 2. Bee pollination syndrome. Bee visiting O. *gayleana*. Photo Credit: Jogesh

Results: Pollinator Observations

- Our pollinator observation data are in support of the hypothesis that the dominant pollinator can be predicted by floral traits.
- As predicted, *O. cespitosa* subsp. *marginata* was visited primarily by hawkmoths, *O. tubicula* and *O. gayleana* were primarily visited by bees, and *O. toumeyi* was visited by both hawkmoths and bees (Figure 3).
- In contrast to expectations, *O. lavandulifolia,* thought to also visited by both hawkmoths and bees, was dominantly visited by hawkmoths only in the study sites investigated (Figure 3).

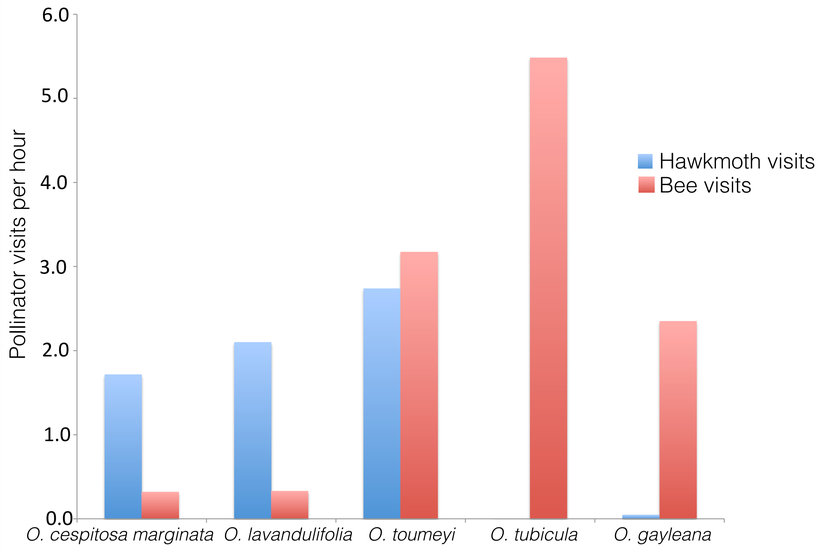

Figure 3: Bee and hawkmoth pollinator visitation rates for five *Oenothera* species.

Methods: Cross Compatibility

- Hand pollination treatments were performed at anthesis on *O. gayleana* and *O. hartwegii* subsp. *filifolia* grown from wild-collected seeds from 4 different populations in growth chamber conditions. The five treatments were: autogamous, geitonogamous, sibling, within population, and between population.
- The identities of the maternal and paternal parent plants and the date of the cross were recorded on a jeweler's tag which was then attached to the developing fruit.
- Fruits were collected eight weeks after crosses were conducted or as soon as they detached.
- The following data were collected on a total of 240 fruits from three populations (120 per species, 40 per population): fruit weight (g), length (mm), width (mm), and seed count.
- Data was analyzed using the program R version 3.2.1 to identify relationships between seed count and species, cross type, and original population of the maternal plant. Differences were tested with a negative binomial generalized linear model.

Results: Cross Compatibility

- Both species appear to be completely self-incompatible as no seeds were produced in either of the two self cross treatments, autogamous and geitonogamous (Figure 4A and B).
- No significant differences were observed between the three outcross treatments (sibling, within population and between population) in either species (Figure 4A).
- *Oenothera gayleana* did not differ among the three populations (Figure 4B) while in *O. hartwegii* subsp. *filifolia* population YB produced significantly more seeds than the other two populations (Figure 4B).

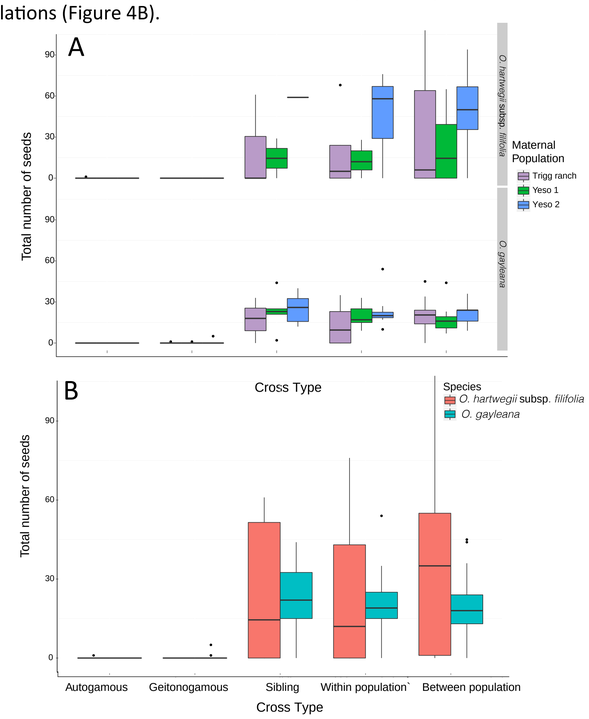

Figure 4. Total number of seeds of *O. hartwegii* subsp. *filifolia* and *O. gayleana* resulting from hand pollinations for each of the five treatments. A. By population, B. All populations pooled.

Differences between cross types: Chi-sq = 658.78, df = 4, p<0.001**; species: Chi-sq = 180.31, df = 1, p = 0.51 ns; maternal population: Chi-sq = 186.77, df = 2, p = 0.03*

Discussion

- Our findings demonstrate that pollinator syndromes are capable of accurately predicting dominant pollinators in wild populations of several species of *Oenothera*.
- As is common in many members of Onagraceae, *O. gayleana* and *O. hartwegii* subsp. *filifolia* were determined to be self incompatible but that there was no difference in seed production among different outcrosses types in the populations investigated.
- Future work should assess whether the patterns found here are found throughout the range of each species.