

Comparative Rates of Reproductive Success in the Two Color Morphs of Bird's foot Violet (*Viola pedata*) in Two Missouri Populations

Dowen Jocson¹, Ren Zong-Xin^{1,2}, Justin Zweck¹, Retha Meier¹, Peter Bernhardt¹

¹Saint Louis University, ²Kunming Institute of Botany

Introduction

- Bird's foot violet (*Viola pedata*) is a spring flowering herb of North American forest and woodlands, and the only one known to have a self-incompatibility mechanism⁴.
- Pollinators vary throughout its natural distribution and include hawk moths³, butterflies and bumblebees¹.
- Extensive populations occur in woodland reserves in Missouri.



Figure 1: The two color morphs of *V. pedata*; Bicolor (left) and Concolor (right) taken at Shaw Nature Reserve, Gray Summit, MO.

- *V. pedata* occur mostly as two color morphs; lilac in color (concolor) and a melanistic form with two superiors, velvety, deep purple petals (bicolor)
- Given that neither form can self-pollinate, do they have an equal opportunity to cross pollinate in the wild? How does cross pollination differ across sites in Missouri?

Collection and Analyses of Pistils vs. Ripe Capsules

- Using jeweler's tags, a number of flower buds were marked for bicolor ('b') and concolor ('c') morphs. (23 c and 9 b in Shaw Nature Reserve; 18 c and 23 b in Cuivre River).
- Withered flowers were collected and fixed in 3:1 ethanol to glacial acetic acid transferring to 70% ethanol for storage after 4 hours.
- A second set of tagged flowers were allowed to let each ovary ripen on its stalk protected by a marked bonbonerie bag (B for bicolor or C for concolor) harvesting and counting seeds four weeks later.
- The pistils were softened and stained with Decolorized Aniline blue, squashed under glass cover slips and observed under Epifluorescence microscopy⁶.
- Determined whether pistils contained pollen tubes and how far they grew into the pistil (stigma, style, ovary, ovule penetration).

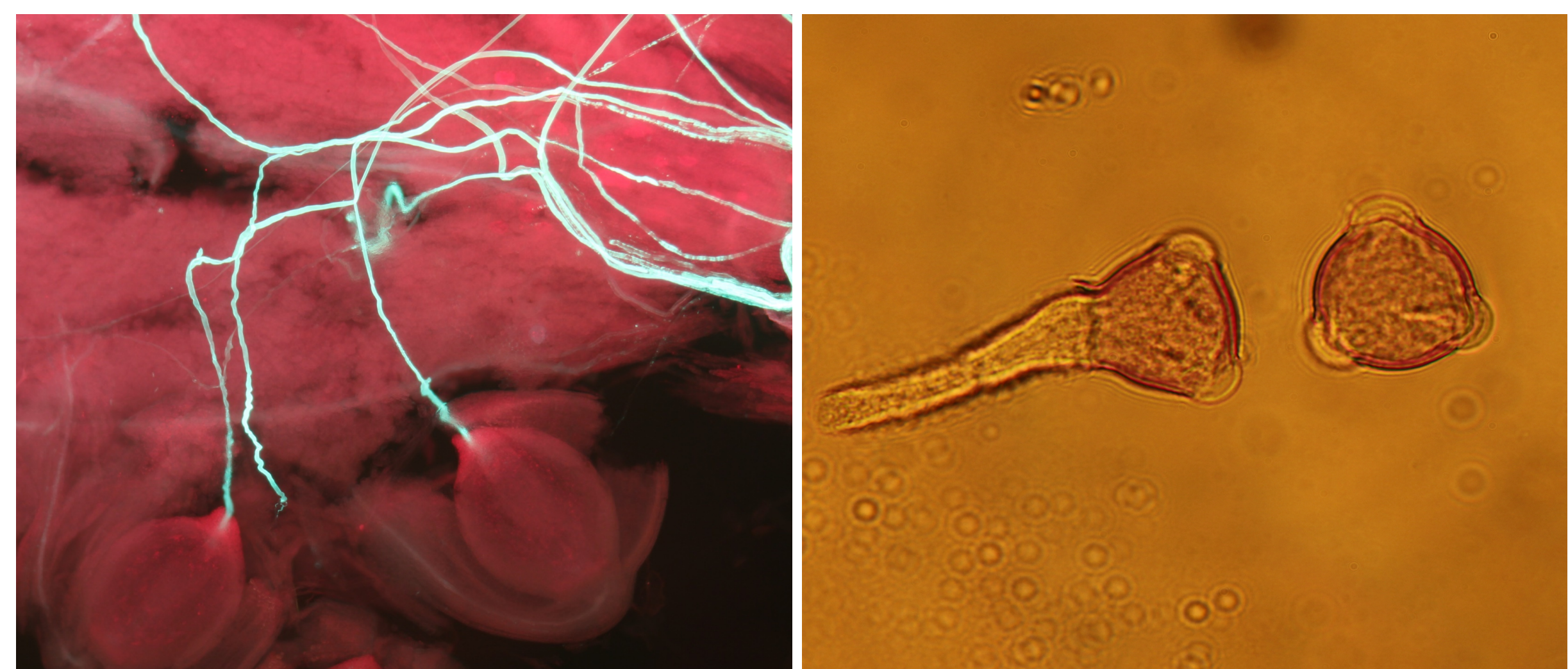


Figure 2: (Left) Pollen tubes entering ovules inside the ovary of *V. pedata*. This is from a bicolor flower. (Right) Pollen grains in pollen wash from an *A. carlinii* bee. The left pollen is starting to germinate.

Methods for Pollinator Activity and Pollen Analyses

- Flowers were observed for insect activity between 10 AM until 1 PM.
- Insects were caught with butterfly nets, euthanized in glass jars with fumes of ethyl acetate.
- Analyzed their pollen loads by placing each insect on a glass slide, washing pollen off with drops of ethyl acetate, and then staining the pollen residue with Calberla's fluid⁵.
- Pollen grains of *V. pedata*, were considered present when greater than 25 grains appeared on a slide. This was repeated for each site.

Species	Cuivre River	Shaw
<i>Andrena carlinii</i>	14/9	6/4
<i>Andrena cressonii</i>	0/0	2/2
<i>Andrena narsonii</i>	2/1	1/1
<i>Andrena pruni</i>	1/1	0/0
<i>Anthropora ursina</i>	1/1	3/1
<i>Augochlorella aurata</i>	0/0	1/0
Diptera (<i>Conopidae</i>)	0/0	1/0
<i>Lasioglossum bruneri</i>	0/0	1/1
<i>Lasioglossum cressoni</i>	0/0	1/0

Figure 3: Listed above are the species of pollinators. The numbers represent the number of individuals caught/number of individuals carrying the *V. pedata* pollen.

Results

- Using the statistical program R (v. 3.0.2), analysis of variance was carried out for the flower squashes.
- Showed significant differences in the number of pollen tubes in the style ($P=0.0383$) and pollen tubes entering the ovaries ($P=0.0419$) between the sites, and in the number of ovules ($P=0.0346$) between the color morphs.
- It also showed significant differences in the number of seeds produced between the two color morphs ($P=0.0483$) and between the two sites ($P=1.21e-10$). **Fig. 4** visually summarizes the results.

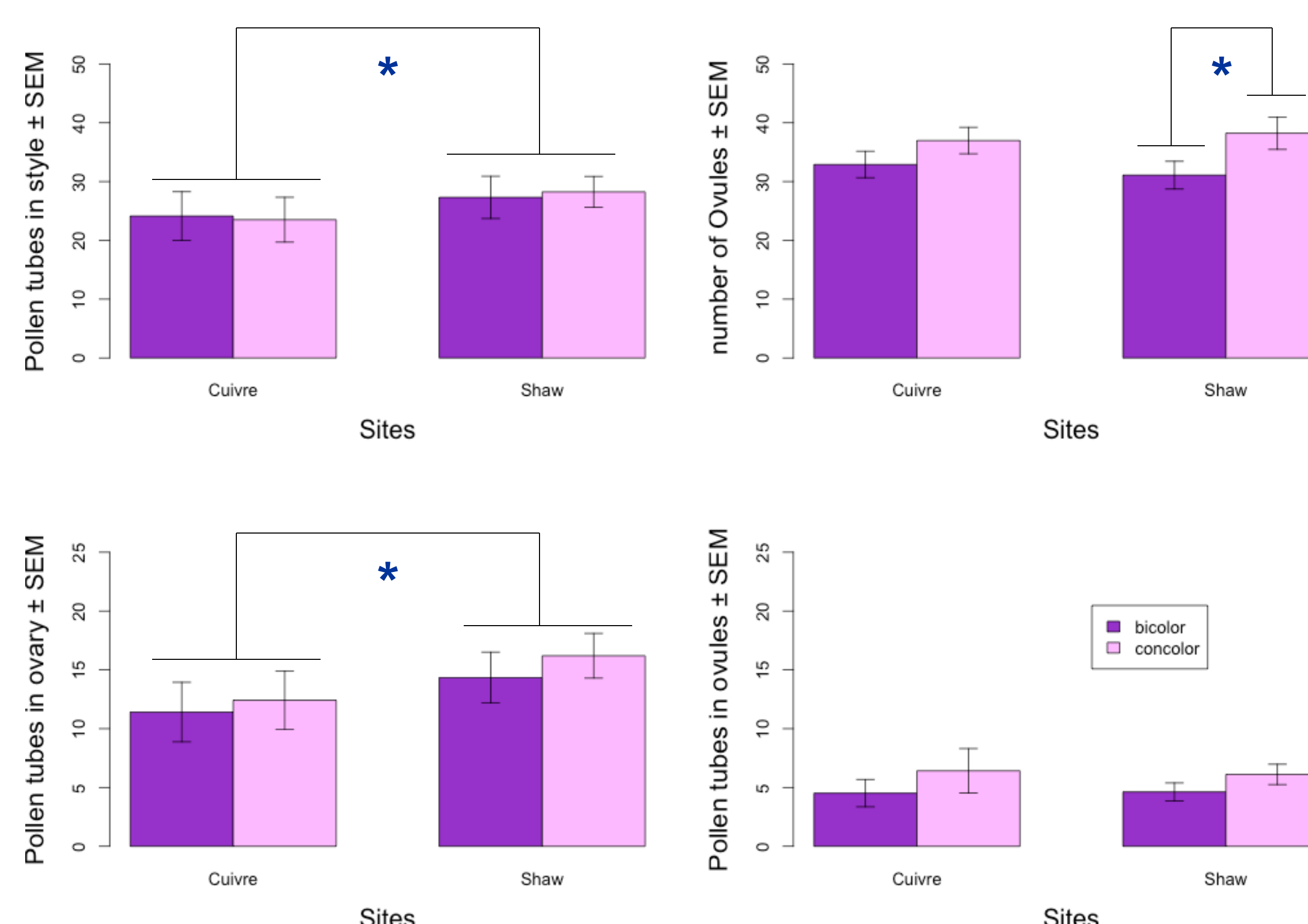


Figure 4. Average number of ovules and of pollen tubes in style, ovary, and ovules as a function of color morph and site ($P<0.05$ (**)).

- 11% of the bicolor and 4% of the concolor contained no seeds from the total number of ripe fruits from Shaw Nature Reserve.
- In contrast, 65% of the bicolor and 67% of the concolor contained no seeds from the fruits collected from Cuivre River.

Discussion

- Even though there were no significant differences in the number of ovules penetrated by pollen tubes (fertilization) between the sites there was still a very significant difference in the number of seed set.
- Cuivre River population consists of just about 60 individuals (in contrast to over 1000 in Shaw)
- As in *Xerophyllum tenax*⁷, pollination rates in *V. pedata* at Cuivre River are far higher than the actual conversion of ovules into seeds.
- Some S alleles must be shared between siblings and parents so bees repeatedly deposit pollen sperm that pistils always reject at pre- or post-zygotic levels.
- The number of zygotes produced in Cuivre River is then decreased due to the self-incompatibility mechanism.

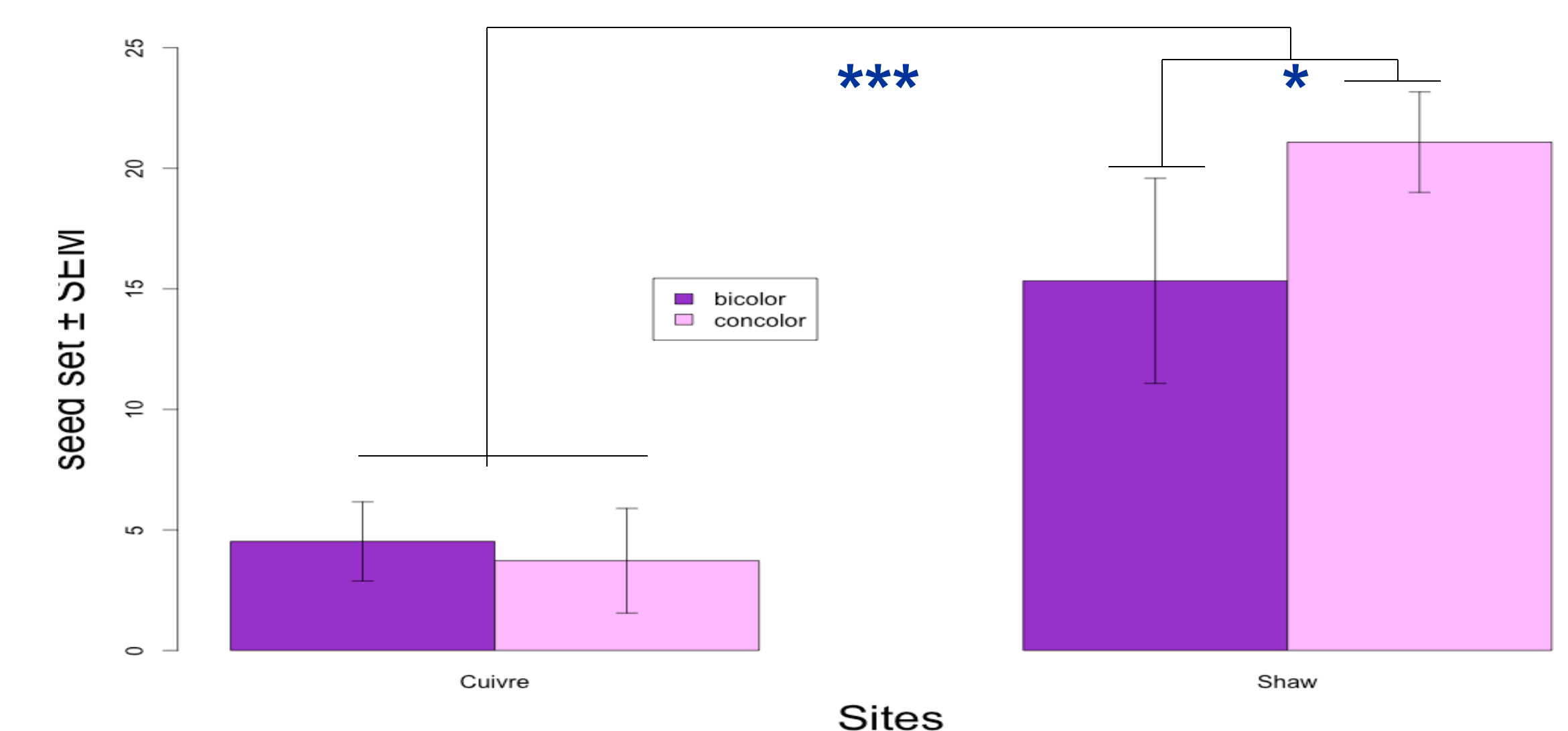


Figure 5: Seed set as a function of color morph and site. (****= $P<0.001$, **= $P<0.5$).

- Andrew Beattie noted that insects of different species pollinate the species of *Viola* family during their respective flowering season in England⁴.
- Primary pollinators of *V. pedata* in North America may vary in diversity according to location and season.
- *Andrena Carlinii* is known to be a generalist forager of spring wild flowers throughout its range.

Further Studies

Future studies on this species should consider answering the following questions: Do pollinators forage selectively preferring one color morph and visit the second only when the nectar and/or pollen are depleted from the preferred morph? Why does the frequency of color morphs differ between sites?

Acknowledgments

We would like to thank the following people: Bruce Schuette (naturalist, Cuivre River Natural Area), Ginger Allington (Missouri Botanical Garden), and Gerardo Camilo (Dept. of Biology, St. Louis University)

References

1. Beattie, A. J. (1971). Pollination mechanisms in *Viola*. *New Phytologist*, 70(2), 343-360.
2. Beattie, A. J. (1974). Floral Evolution in *Viola*. *Annals of the Missouri Botanical Garden*, 61: 781-793
3. Beattie, A. J. (1976). Plant dispersion, pollination and gene flow in *Viola*. *Oecologia*, 25(4), 291-300.
4. Beattie, A. J. and Culver, D.C. 1979. Neighborhood size in *Viola*. *Evolution*, 33:1226-1229.
5. Ogden, E. C. (1974). *Manual for Sampling Airborne Pollen*. New York: Hafner Press
6. Vance, N., Bernhardt, P. & Edens, R. 2004. Pollination and seed production in *Xerophyllum tenax* (Melanthiaceae) in the Cascade Range of central Oregon. *American Journal of Botany*, 91:2060-2068