Pollination and Comparative Reproductive Success in a Population of *Viola pedata* L. (Violaceae) with Bicolor and Concolor Morphs

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Introduction

Bird's foot or Pansy violet (*Viola pedata*) is a spring flowering herb of North American forest and woodlands. It is the only Viola species in North American, thus far, known to have a self-incompatibility mechanism. Flower self-pollinated by hand fail to set fruit[4]. Agents of cross pollination appear to vary throughout its natural distribution and include hawk moths[3], butterflies and bumblebees[1]. Extensive populations occur in woodland reserves here in Missouri.

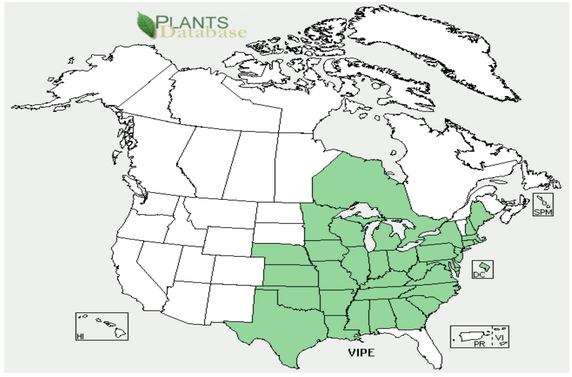

Figure 1: Map shows in shaded regions the distribution of the *V. pedata* in the United States[7].

As in many Viola species, flowers of *V. pedata* occur as two or more color morphs. In most cases, the population subdivides between a morph in which all petals are lilac in color (concolor) and a melanistic form in which the two superior petals are velvety deep purple (bicolor). As neither form can self-pollinate, do they have an equal opportunity to cross pollinate in the wild? How does cross pollination occur in sites in Missouri?

Collection and Analysis of Flower Samples

Using jeweler's tags, we marked the flower buds of 37 concolor and 35 bicolor. When the petals withered, we collected and fixed them in 90% ethanol: 10%glacial acetic acid transferring to 70% ethanol for storage after 4 hours. The pistils were softened and stained with the Decolorized Aniline blue, squashed under glass cover slips and observed under Epifluorescence microscopy[5]. We determined whether pistils contained pollen tubes and how far they grew into the pistil (stigma, style, ovary, ovule penetration).

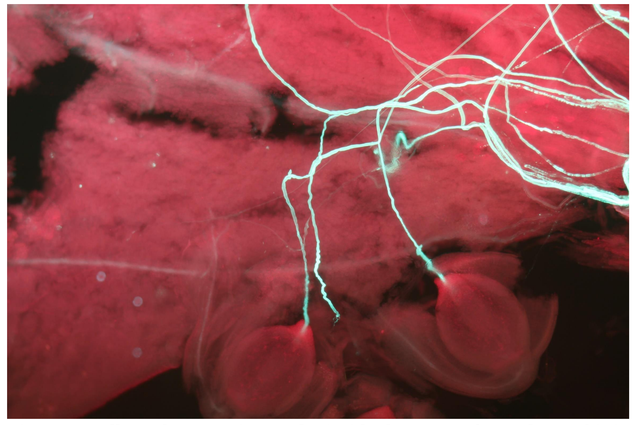

Figure 2: Pollen tubes entering ovules inside the ovary of *V. pedata*. This is from a bicolor flower.

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. These specimens were analyzed for their pollen loads by placing each insect in a glass slide, washing pollen off with drops of ethyl acetate, and then staining the pollen residue with Calberla's fluid[6]. The pinned insect were referenced with its pollen slide. Pollen grains of *V. pedata*, were considered present when greater than 25 grains appeared on a slide.

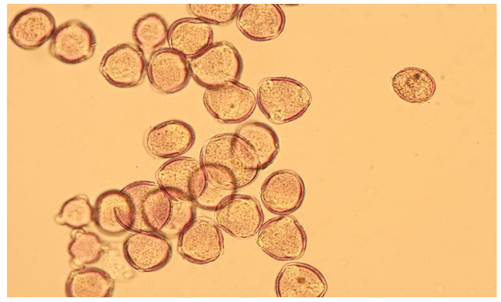

Figure 5: Pollen load from one of the *A. carlinii* showing a number of the *V. pedata* pollen.

Results

The only insects collected in *V. pedata* were females of *Andrena carlini* (Andrenidae). The bees have two ways of foraging on the flower. Bees foraging on the concolor flower landed on the lip petal, inserting their heads down the floral tube (presumably to collect nectar in the spur). In contrast, bees foraging on the bicolor flower landed either on the lip petal or they landed on the two melanistic petals and forage upside down. Pollen load analyses showed that they all carried the host's flower's pollen, but pollen analyses suggest they were all generalist foragers, collecting pollen and/or nectar from co-blooming species of woodland herbs

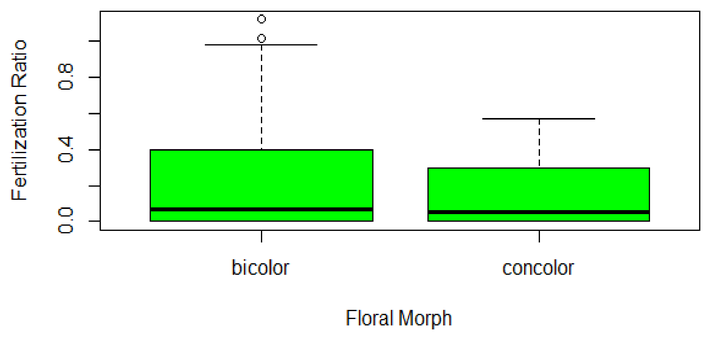

Figure 4. This graph shows that the fertilization ratio of the bicolor morph is significantly higher than the concolor morph

Of the total number of ripe fruits (capsules) collected 6 contained no seeds and 43 contained seeds, but a total of 76% of seeds were undersized and lacked embryos. The collected data on the squashed pistil showed a mean of 37 ovules, a standard deviation of 13, and a range from 10 to 62 ovules in an ovary. This suggests that the conversation rate of ovules into viable seeds is 0.012 seeds per ovule.

Discussion

Single season of field activity shows that differences in pistil pollination between two color morphs are significant. Pollen tube analysis and seed set counts indicate a low conversion rate of ovules to seeds even though all the flowers were pollinated. This suggests that the self-incompatibility system eliminates pollen tubes produced when pollinators visit more than one flower on the same plant (geitonogamy) and/or bees bring pollen to two different genotypes sharing one of more of the S-alleles.

Figure 3: The two color morphs of *V. pedata; Bicolor (left) and Concolor (right).*

The same bee species visits both morphs, but its approach to the bicolor morph occurred in two different ways. Beattie found the same horizontal and inverted foraging pattern in a variety of bees and flies on a wide variety of *Viola* in England[4]. Beattie noted that insects of different species pollinate the species of Viola family during their respective flowering season in England. In contrast, primary pollinators of *V. pedata* in North America may vary in diversity according to location and season. *Andrena Carlini* is known to be a generalist forager of spring wide flowers throughout its range. So the mix pollen loads on our females were predictable.

Further Studies

Future studies on this species should consider answering the following questions: Does the primary pollinator at Cuiver River change over several seasons? 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? Compare more than one population of *V. pedata* in the St. Louis area. Do they all have the same pollinators?

Acknowledgments

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