Post-Mating Reproductive Isolation and Hybrid Pollen Inviability Between Two Subspecies of *Clarkia xantiana* (Onagraceae)

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Introduction

Speciation is a term used to describe the origin of new species of organisms, which usually occurs when two populations of the same species diverge and become reproductively isolated (Coyne and Orr 2004). Two types of changes within these diverging populations can limit the formation of hybrids and cause speciation to happen: phenotypic and/or genetic. Phenotypic changes may prevent mating from happening (pre-mating isolation) and genetic changes may render hybrids inviable (post-mating isolation). For my study, I examined the post-mating isolation between two diverging populations of one species.

Clarkia xantiana (Onagraceae), an endemic, native herb of California has two subspecies *C. x. xantiana* and *C. x. parviflora*. *Xantiana* is primarily outcrossing and *parviflora* is primarily selfing (Moeller 2005). In sympatry, they rarely form hybrids in nature but can be forced to produce hybrids in the lab. My study focused on the fertility of pollen grains (male fertility) in the parents and hybrids of these two subspecies to assess post-mating isolation.

Figure 1. Photographs of both subspecies of *Clarkia xantiana*

Methods

- Created two parental lines and two hybrid line cross types
  - *Parviflora* line was created by selfing, cross type "P"
  - *Xantiana* line was created by outcrossing two *xantiana*, cross type "X"
  - Hybrid lines of P and X created by reciprocally crossing by *xantiana* and *parviflora* lines
    - "Hp" is used when *parviflora* is the maternal parent
    - "Hx" is used when *xantiana* is the maternal parent
- Collected one sample of four anthers from one flower per plant in a vial filled with 75 µL of an Aniline Blue Lactophenol stain solution
- Pipetted two 25 µL subsamples of each sample onto one labeled slide, sealed with cover slip and nail polish
- Counted viable and inviable pollen from each subsample (Figure 2)

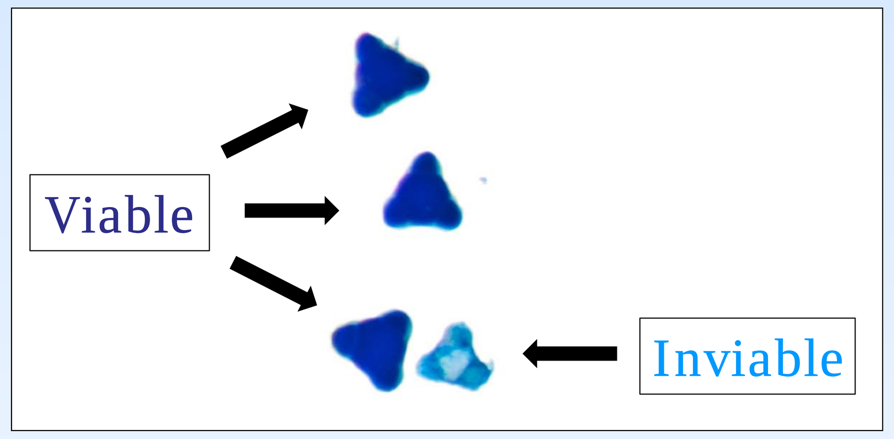

Figure 2. Viable and inviable pollen grains stained with an Aniline Blue/Lactophenol solution. Viable pollen are stained dark blue and inviable pollen are stained light blue.

Results

- 19,906 pollen grains assayed from 89 different samples
- Cross type did have an effect on the number of pollen grains collected per sample of four anthers (Figure 3). Parviflora had significantly lower pollen counts per sample than *xantiana* and both types of hybrids, which were similar.
- Cross type also had an effect on the percent of viable pollen per sample (Figure 4). *Parviflora* had a significantly lower percent of viable pollen with a mean of 37.32% viable. Both hybrids and *xantiana* all had significantly similar means between 52.73% and 60.68% viable pollen.

Results

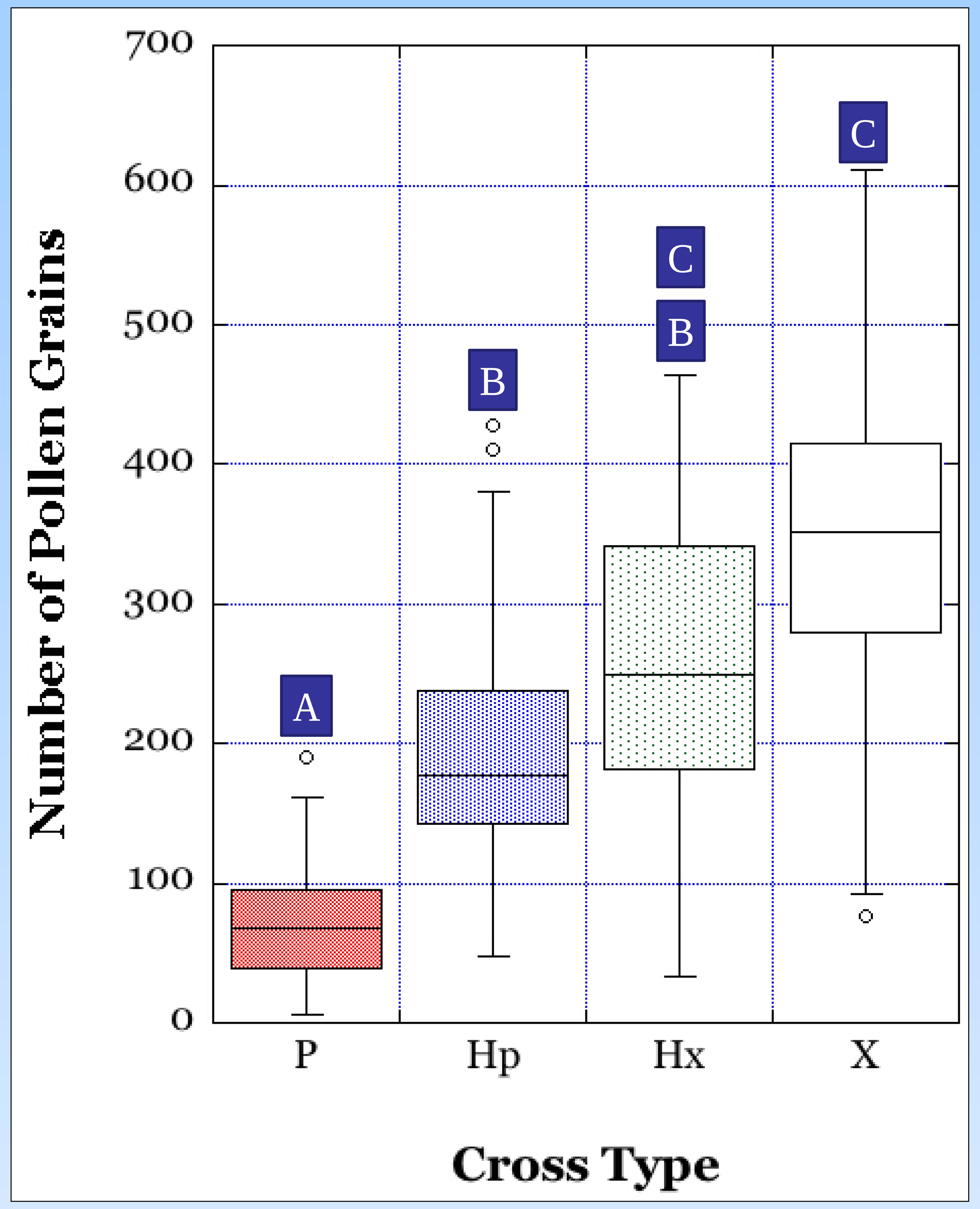

Figure 3. Number of pollen grains collected per sample by cross type. Box plots that share corresponding letters are not significantly different (Tukey Test).

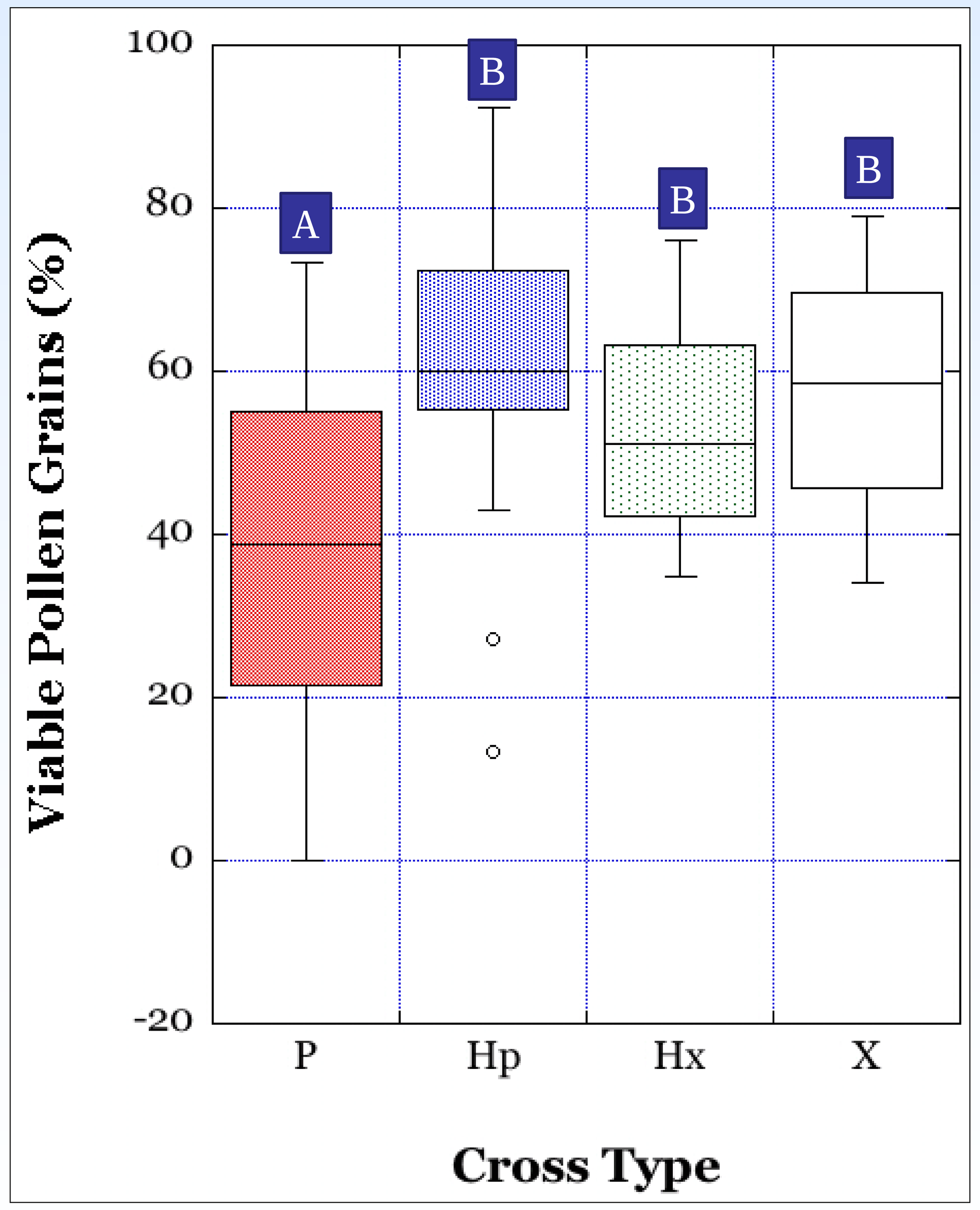

Figure 4. Percent of viable pollen per sample by cross type. Box plots that share corresponding letters are not significantly different (Tukey Test).

Discussion

These results suggest that *parviflora* produces significantly lower amounts of pollen per anther. *Parviflora* primarily selfs so it needs less pollen to reproduce because it has a higher probability of one pollen grain reaching an ovule. *Xantiana* outcrosses and has a lower probability of one pollen grain reaching an ovule, so it needs more pollen. Both hybrids are intermediate in pollen counts per sample, with Hp being similar to *parviflora* and Hx being similar to *xantiana*. This suggests that the genes controlling pollen number within *parviflora* and *xantiana* might have an additive effect in hybrids.

In addition to *parviflora* having significantly lower pollen counts than *xantiana* and both hybrid crosses, the pollen is also significantly less viable. Inbreeding usually causes problems in species like inbreeding depression (Charlesworth and Charlesworth, 1987). However, selfers like *parviflora*, who have been selfing for thousands of years, have rid their species of many of those problems. But it is possible that, although *parviflora* has been successful and surviving as a species, it may not be propagating at its highest potential. *Parviflora* may be unable to rid itself of recessive deleterious mutations that hinder it from creating more viable pollen (Glemin 2003). *Xantiana*, on the other hand, is an outcrosser and is much more capable of riding itself of any deleterious mutations and, thus, has more viable pollen. In the hybrid crosses, *parviflora* outcrosses with *xantiana*, which may mask the recessive deleterious mutations of *parviflora*.

References

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