

Influence of Different Pollinator Guilds on Inbreeding and Genetic Diversity

Introduction



Image 1. CLARKIA BREWERI



Image 2. CLARKIA CONCINNA

Questions

In many plant species, pollinators are the main agents for the dispersal of genetic material within and between populations. Pollinator guilds can have very different foraging behaviors that may ultimately impact inbreeding and genetic diversity levels within and between populations.^{1, 2} Pollinators vary in foraging ranges, number of flowers they visit in one feeding bout, and distance they travel between bouts, all factors which will determine the number and diversity of pollen they carry, and therefore genetic material they distribute.

Clarkia breweri and Clarkia concinna are rare plant species in the Onagraceae family that are endemic to California. These sister species differ in major pollinators, providing an ideal opportunity to study pollinator effects on genetic diversity and inbreeding levels.

Is there a correlation between pollinator guild and the levels of genetic diversity and inbreeding in a population? If there is a correlation, can we use genetic measures to predict the most likely pollinator guild of a population?

Methods

Collection of Plant Material

In California, 6 populations of *C. breweri* and 6 populations of *C. concinna* were chosen that vary in their predominate visitor groups. Researchers collected leaf material and seeds from 15 randomly chosen individuals within the population. They also conducted extensive observations to identify the primary pollinator of each population.

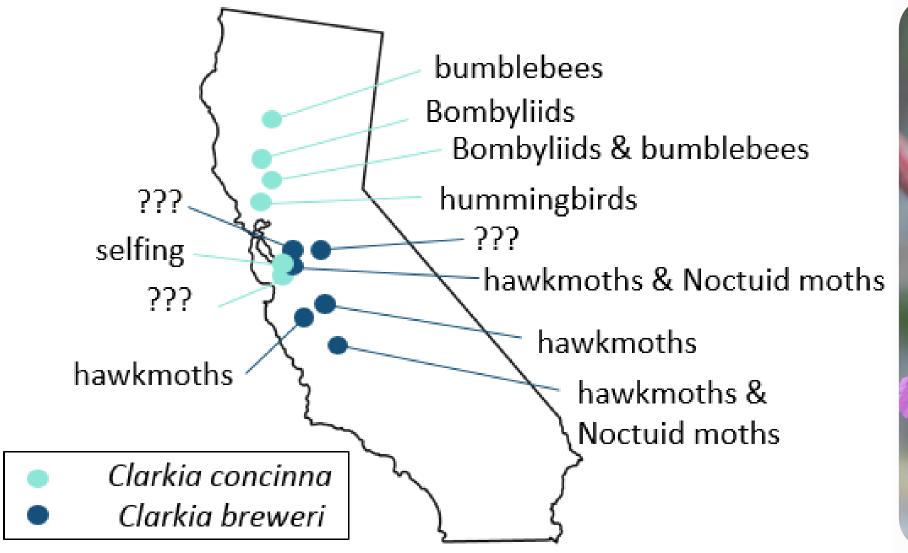




Figure 1. CLARKIA POPULATION SITES & PRIMARY POLLINATORS

Elizabeth Donaldson¹, Jeremie Fant^{2,3}

¹University of Northwestern—St. Paul, St Paul, MN ²Chicago Botanic Garden, Glencoe, IL ³Northwestern University, Evanston, IL



Collection of Maternal Line Data

We selected leaf material from 15 individuals in each of the 12 *Clarkia* populations. We extracted plant DNA and amplified **3 polymorphic trinucleotide microsatellite loci** using **PCR**.

Collection of Progeny and Paternal Line Data

We germinated seeds from 2 C. breweri populations and 2 C. concinna populations, chosen because they had the greatest variation in pollinator guilds. We amplified microsatellites from 12 seedlings from a single maternal line for each population.

Analysis of All Genetic Lines

We ran the maternal and progeny samples in a **DNA genotyper** to identify the different microsatellites in each population. By comparing the genotypes of the progeny to the maternal plant, we determined the number of paternal plants which contributed to reproduction. We used GenAlEx 6.503 and COLONY 2.0.6.4 to determine significant correlations in our data.

Results

Image 3. CLARKIA POLLINATION

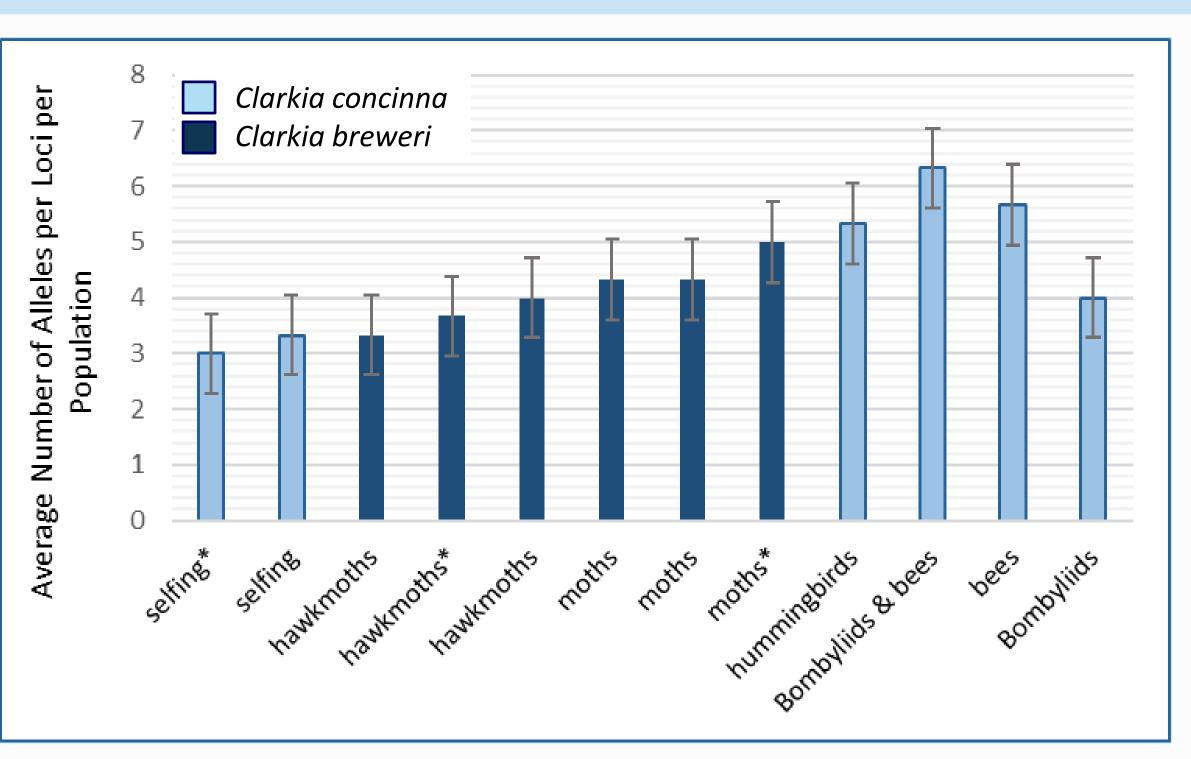
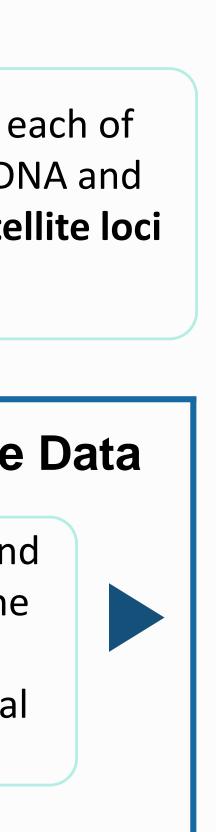


Figure 2. GENETIC DIVERSITY LEVELS IN POPULATIONS.

The genetic diversity of Clarkia populations correlated with pollinator guild type (Figure 3). Selfing populations had the lowest level of genetic diversity, while Bombyliid-and-bee-pollinated populations had the highest diversity.



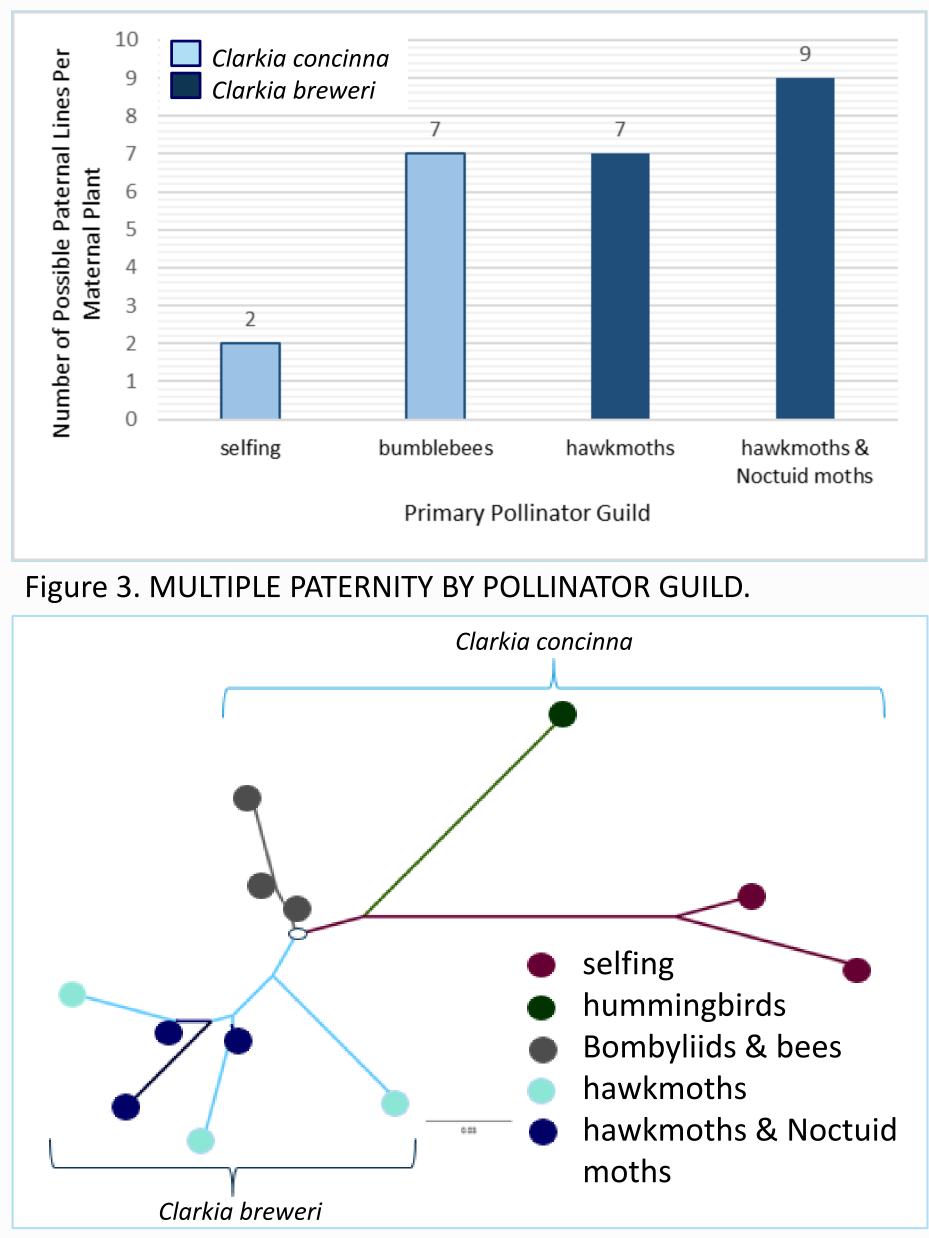


Figure 4. GENETIC RELATEDNESS OF POPULATIONS WITH SHARED POLLINATOR GUILDS.

Discussion

Results

Our results strongly confirm that pollinator guild impacts gene flow within and between populations. We do not see a direct correlation between a large foraging range and higher genetic diversity levels; however, factors such as small sample size, difference between species diversity, and limited number of primers may distort trends which might otherwise appear in the data, and should be considered in future experiments. Future research directions could include testing of more primers with a larger sample size, controlled experiments on multiple paternity, and confirmation of the proposed pollinator guilds for the unassigned populations.

Our research demonstrates correlation between pollinator guild and genetic diversity. This knowledge can contribute towards informed and intentional decisions in conservation and restoration efforts.

Acknowledgements

A sincere thank you to the awards which made this research possible-the National Science Foundation REU award DBI-1461007, NSF awards DEB-13442873, DEB-13442805, and DEB-13442792. Thank you to Anita Cisternas Fuentes for providing sample material and advice on *Clarkia* study, to Hilary Noble for her continuous help and support in the lab, to Dr. Andrea Kramer and Christopher Woolridge for their leadership of the REU program, and to Yocelyn Pina for additional data collection.

References

¹ Kramer, A.T., Fant, J.B., and Ashley, M.V. (2011). Influences of landscape and pollinators on population genetic structure: examples from three Penstemon (Plantaginaceae) species in the Great Basin. American Journal of Botany. doi: 10.3732/ajb.10000229 ² Rhodes, M.K., Fant, J.B., and Skogen, K.A. (2017). Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Molecular Ecology.* doi: 10.1111/mec.14115



Multiple paternity levels also varied with pollinator guild (Figure 3), with populations pollinated by both hawkmoths and Noctuid moths having the greatest number of potential fathers per maternal plant. Populations that shared the same pollinator guild showed the greatest genetic similarity for all parameters (Figure 4). By comparing genetic variables, we were able to make a prediction of the most likely pollinator guild for the 3 unassigned Clarkia populations. There was large differences in inbreeding coefficient, although we think this is driven by fluctuations is population sizes. Hence inbreeding was driven by bottleneck rather than directly due to preferred pollinator guild.

