

“Sex ratio and reproductive success
along elevational gradients of gynodioecious
populations of *Geranium richardsonii*”

Student: Susana Antecon
Mentor: Charles F. Williams

Part-time Independent Research and Methods in Field Ecology Course
Summer 2016

ABSTRACT

We studied the sex-ratio and reproductive success of gynodioecious populations of *Geranium richardsonii* along elevational gradients near Gothic, Colorado. Gynodioecious means that the species produces hermaphrodite and female plants. The hermaphrodites have male (anthers and pollen) and female (ovules and seeds) functioning parts, while females produce non-functional anthers. Genetic and ecological factors are known to influence sex ratio and reproductive success of gynodioecious species (Alonso, 2005). We hypothesized low female frequency at high elevations because wetter, cooler environmental conditions would favor the hermaphrodites, which have higher resource demands, but also have higher individual relative fitness compared to females. Also, we expected hermaphrodites to have larger pollen receipt compared to females due to self-pollination and allocation of resources to pollinator attraction (flower size), when pollinators are limiting. We measured the sex ratio of 31 populations and compared 4 of our populations with data collected in 1997. Reproductive success was assessed for 10 out of the 31 populations spanning an elevation range from 9400-11500 feet, by counting stigma pollen loads on hermaphrodite and female flowers. There was a significant negative correlation between the proportion of females in populations and elevation. However, there was no significant relationship between elevation and hermaphrodite or female pollination success. Relative female pollination success declined somewhat as population sex ratio increased, as predicted, but this relationship was not significant in the populations studied. Therefore, pollination success does not appear to be the main reason driving the increase in hermaphrodite frequency in higher elevation populations. Sex ratio has not changed over the past 20 years in four populations studied near Gothic, suggesting that individuals may be long-lived, or that selection maintains constant sex ratios over time.

INTRODUCTION

The species *Geranium richardsonii* found at RMBL is gynodioecious and sex ratio varies widely among populations (Williams et al. 2000). The flowering season extends from late June through early August (Williams et al. 2000). *Geranium richardsonii* plants are native to moderately disturbed habitats, either on open meadows or aspen understory. Gynodioecious species have two gender morphs: hermaphrodite and female (male-sterile individuals). The hermaphrodite flower has 10 purple anthers and 5 stigmas that allow the plant to self-fertilize. As a result, it produces both pollen and seeds, which can contribute to the next generation (Williams et al. 2000). On the other hand, the female flower has non-functional yellow anthers and it can only produce seeds. This prevents self-fertilization and the female flower is obligated to outcross; thus putting females at a reproductive disadvantage.

Both genders co-exist within a population due to habitat- and population-specific fitness trade-offs. The flower size of each gender morph varies. Female flowers of almost all gynodioecious species tend to be smaller than those of hermaphrodites (Williams et al. 2000, Shykoff 2003). This results in smaller pollen receipt for the female flower and larger pollinator attraction to hermaphrodites. According to Williams and colleagues (2000), hermaphrodites may be fertilized by self-pollen, either through autogamy (within-flower selfing) or geitonogamy (selfing between flowers on the same plant). This reproductive process decreases diversity and induces inbreeding depression. Since, female flowers depend on outcrossing to reproduce, they might receive less pollen, but usually enough to produce fruits and seeds (Williams et al. 2000).

Females tend to compensate for their lack of functioning male structures and increase their fitness by reallocating resources to seed and fruit production (Williams et al. 2000). The hermaphrodite morph has to allocate resources to both male and female reproductive structures (anthers and ovules/seeds), as well as larger attractive structures (petals). High levels of self-fertilization in hermaphrodites lead to low-quality seeds relative to their female counterpart due to inbreeding depression. However, hermaphrodites tend to gain higher pollinator visitation due to their larger floral size (Williams et al. 2000). Females may be advantaged from their reallocation of resources saved from pollen reproduction in male sterile individuals or from avoidance of inbreeding by females unable to self-pollinate (Shykoff et al. 2003). Females may also produce higher quality offspring if pistillate flowers provide a more intensely competitive sieve for pollen, allowing only the best genotypic combinations to give rise to zygotes (Shykoff et al. 2003).

Along an elevational gradient, environmental conditions are different. We expect different pollinator abundance, habitat diversity, and climate (water, temperature). According to Petry et al. (2016), differences between sexes in morphology and physiology can result in sex-specific responses to the environment (Petry et al. 2016). In a study on the species *Valeriana edulis*, it is argued that increasing elevation is associated with increasing water availability and female frequency. This is likely due to sex-specific water use efficiency and survival (Petry et al. 2016). This study has shown that males decrease in frequency with increasing elevation. Furthermore, increasing elevation is associated with decreasing temperature and increasing soil moisture (Petry et al. 2016). This strongly suggests that spatial climatic change affects the sexes differently, potentially creating an imbalance in the frequency of males and females and altering patterns of fertility and population dynamic (Petry et al. 2016). Therefore, these factors (pollinator abundance, habitat diversity, and climate) were expected to affect the relative reproductive success of hermaphrodites and female plants and the evolution of the sex-ratio in populations of *G. richardsonii*.

These competing factors (resource and inbreeding advantages of females and pollinator attraction/reproductive assurance advantages of hermaphrodites) were expected to change the sex-ratio of *G. richardsonii* along elevation gradients. Furthermore, the actual number of pollen grains in hermaphrodite flowers and resources to make seeds in both genders could also vary depending on the soil moisture, the sun exposure, and the community in general at different elevations. Therefore, we hypothesized that sex-ratios will vary along elevational gradients, (1) favoring hermaphrodites, which were expected to have large pollen receipt due to allocation of resources to pollinator attraction (flower size), if pollinators are limiting. (2) We expected to have lower female frequency at high elevations because environmental conditions would favor the hermaphrodites, which have higher resource demands, but also have higher individual relative fitness compared to females.

METHODS

We measured the sex-ratio for 31 populations (Figure 1) and the reproductive success for 10 populations of *G. richardsonii* at different elevations (low, medium, and high). For the low elevation, we measured plant populations around the Gothic town site, the Research Meadow, and Mt. Red Rock. For medium elevation, we worked on areas north-east of Gothic such as Virginia Mine Road and Copper Creek Trail. For high elevation, we studied *G. richardsonii* populations that were near Virginia Basin and Queen Basin.

To measure sex-ratio, we first found a geranium population and placed flags around it. The area of the population was estimated by counting paces and then converting them to meters. Then, we used flags, tape, and a clicker to record the number of hermaphrodites and female plants within the population. Each plot was divided into sections that were carefully assessed to ensure plants were not counted twice. The location was recorded using the iNaturalist mobile application, which also helped us calculate the elevation for each geranium population.

In order to measure the reproductive success, we collected around 30 flowers of each gender morph for 10 out of the 31 populations along the elevational gradient. Stigmas were collected in the morning. Flowers were collected near the pedicel and placed in an egg carton tray with separate spots in order to avoid pollen contamination. When plants were limited in a population, two flowers per plant were collected. When flowers were not limited, one flower per plant was collected. The samples collected had all or most of the petals gone, which indicated that the flower had been exposed long enough to pollinators to obtain the maximum pollen receipt. For the flowers collected, the sepals were exposed, the anthers had fallen off, and only the filaments and the stigmas were visible. We prepared two flowers per slide. We used a magnifying glass and forceps to spread the stigma lobes wide open. Using a pipette, we added two drops of fuchsin dye to every flower in order to highlight the geranium pollen (Figure 2) on the five stigma lobes. Then, we covered the slide, and let it dry for a couple of minutes. Once the slides were dried, we counted and recorded the number of pollen grains around each of the five stigma lobes using a light microscope.

In order to measure the significance of sex ratio as a function of elevation, we used an ANOVA to test the significance of the slope of the regression of population sex ratio (y) on population elevation (x). We also tested the significance of the difference of pollination success (number of pollen grains per flower, y) between hermaphrodite and female flowers (x) using t-tests. We examined the relationship between elevation (x) and pollination success (y) using regression and ANOVA. Furthermore, we compared our sex-ratio data from four Gothic populations with that of Charles F. Williams' data from the 1990s (Williams et al. 2000) using t-tests.

RESULTS

Sex ratios

We surveyed 31 populations in our study (Table 1). Most of the populations had both hermaphrodites and female plants. All the plants studied produced either hermaphrodites or females flowers. Female frequency varied from 0 to 83% among populations. We found a significant negative relationship between elevation and population sex-ratio (% female). ($F_{(1,29)}=5.04$, $r^2=0.148$, $p=0.033$; Figure 3). At low elevations (< 9700 ft.) the sex ratio (% female) was greater than 30%. Elevations greater than 9700 ft. show the proportion female was less than 30% (Figure 3). The lowest-elevation (9440ft.) populations show the highest female percentages to be between 51 and 83%. The highest elevation site (11150 ft.) had only 6% females.

By comparing the Gothic sites from 1997 and 2016, we observed that most populations maintained a very similar proportion of females ranging from 13% to 83%. Except Gothic-1B reduced its female percentage from 42% in 1990 to 36% in 2016 (Figure 4).

Reproductive success:

Overall, hermaphrodites get larger pollen receipt than female flowers (Figure 5). For 7 of the 10 populations, hermaphrodites have an advantage in pollen loads over females. There is no significant correlation ($p=0.401$) between relative female pollination success (female pollen receipt/hermaphrodite pollen receipt) and population sex ratio (Figure 6).

There is no significant difference ($p>0.05$) between relative female pollination success and elevation (Figure 7). Also, we found out that there is no significant difference in pollination success of hermaphrodites or females at different elevations ($p\geq 0.05$, Figure 8 and 9).

DISCUSSION

Sex ratio:

After measuring sex ratio for 31 populations around Gothic, CO, we found a significant negative correlation of sex ratio with elevation (Figure 3, $p=0.033$). As we expected, females become less frequent at higher elevations. Assuming that at high elevations environmental conditions are ideal, hermaphrodites are at an advantage because they have higher resource demands compared to females. Our results show that females tend to be less abundant at higher elevations. For instance, at low elevations (< 9700 ft.) the proportion of females was greater than 30%. Elevations greater than 9700 ft. show the proportion female was less than 30% (Figure 3). The lowest-elevation (9440ft.) populations show the highest female percentages to be between 51 and 83%. The highest elevation site (11150 ft.) had only 6% females.

We compared 4 of our populations around Gothic with 4 Gothic populations that were surveyed back in 1997. We found no significant change in sex ratio for any of the *Geranium* populations (figure 4). Perhaps, the explanation to why there is no change could be that the environment has not changed dramatically. Therefore, *Geranium* populations have been able to maintain a balance between the number of female and hermaphrodite plants within this populations. Alternatively, *Geranium* individuals may be long-lived and population change in sex ratio only occurs over long time periods. Given that we only compared four populations, it should be of interest to study *geranium* populations more in depth.

Pollination success:

Reproductive success (pollination success) was assessed by measuring stigmatic pollen loads. We measured 10 different populations around Gothic, Co. For each population, we collected 30 hermaphrodite and 30 female flowers. After comparing pollen receipt for all ten populations, we found that only one population (VB-6) favors female pollen receipt over hermaphrodites, but in most populations (7 out of 10) hermaphrodites have significantly more pollen than females. This suggests that females are at a reproductive disadvantage. Also, these results support our hypothesis that hermaphrodites have higher pollen loads relative to females (Figure 5).

After measuring the number of pollen grains as a function of elevation for each gender morph, we found no significant effect of elevation on pollen load ($p>0.05$). It seems that there is

a weak positive trend between pollen number and elevation. However, the number of pollen grains does not change significantly with elevation for neither of the gender types (Figures 8, 9). This strongly suggest that pollinators are not limited. It seems that all flowers regardless of floral size (hermaphrodite-large, female-small) are getting pollinated. Perhaps, this is a good pollinator year, and pollen is abundant. Furthermore, limited pollen does not appears to explain the change in sex ratio with elevation. Perhaps, differences in seed set and survival could be impacting sex ratio in *Geranium* populations.

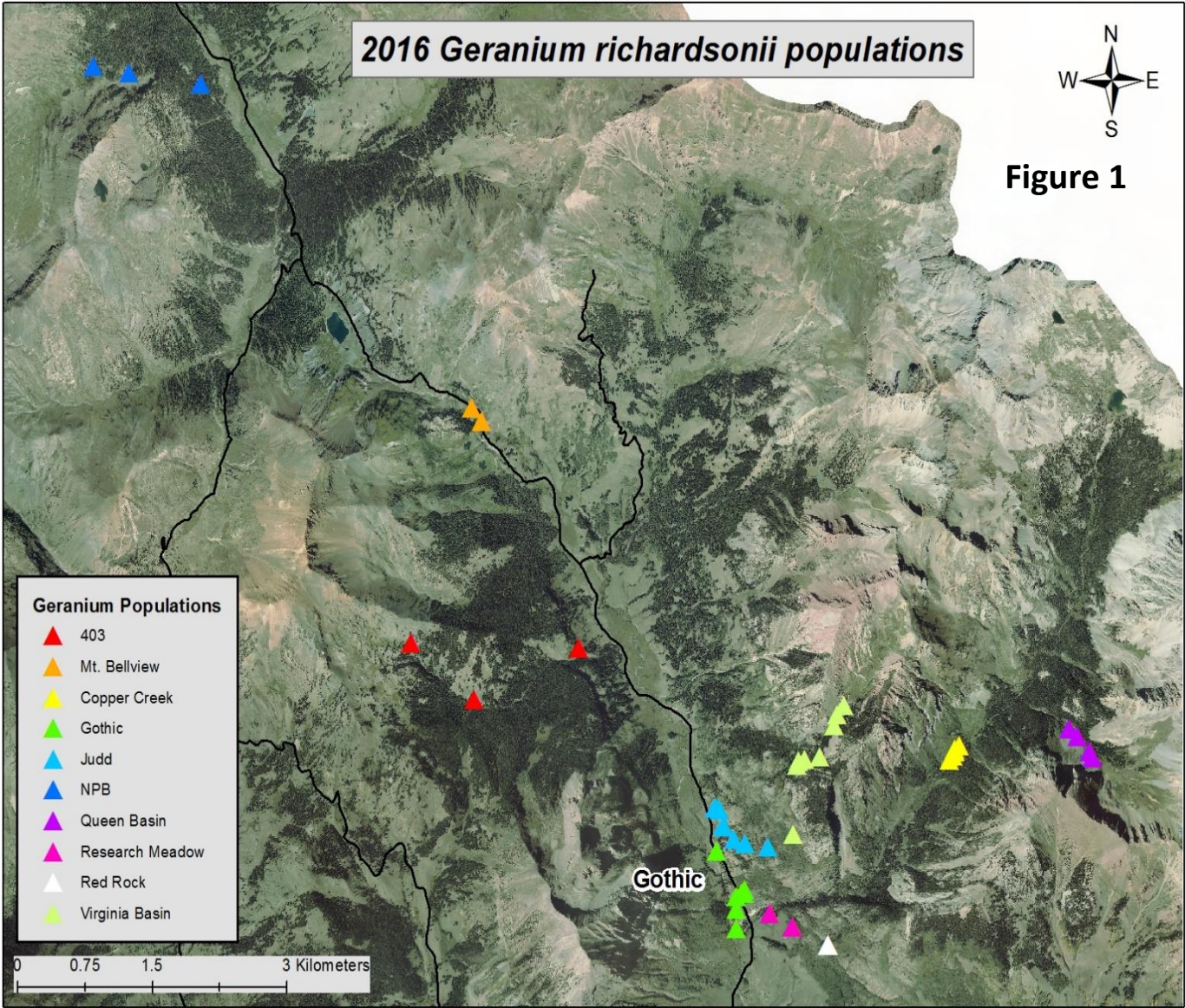
We also compared the relative female pollination success against elevation. There is a weak but non-significant relationship between relative female pollination success and elevation ($p>0.05$). This does not support our hypothesis that females would be at greater reproductive disadvantage at higher elevations. Again, it seems that this might be a good pollinator year so both females and hermaphrodites are getting pollen a high elevations. In years when pollinators are limiting, females may experience greater pollen limitation compared to hermaphrodites.

Literature Cited

- Alonso, Conchita. "Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*." *American Journal of Botany* 92.8 (2005): 1264-1269.
- Asikainen, Eija, and Pia Mutikainen. "Female frequency and relative fitness of females and hermaphrodites in gynodioecious *Geranium sylvaticum* (Geraniaceae)." *American Journal of Botany* 90.2 (2003): 226-234.
- Petry, W. K., J. D. Soule, A. M. Iler, A. Chicas-Mosier, D. W. Inouye, T. E. X. Miller, and K. A. Mooney. "Sex-specific Responses to Climate Change in Plants Alter Population Sex Ratio and Performance." *Science* 353.6294 (2016): 69-71. Print.
- Shykoff, Jacqui A., et al. "Effects of Male Sterility on Reproductive Traits in Gynodioecious Plants: A Meta-Analysis." *Oecologia* 135.1 (2003): 1–9. Web.
- Williams, Charles F., Margaret A. Kuchenreuther, and Allison Drew. "Floral Dimorphism, Pollination, and Self-Fertilization in Gynodioecious *Geranium Richardsonii* (Geraniaceae)." *American Journal of Botany* 87.5 (2000): 661. Print.

FIGURES AND TABLES

Figure 1. The map of all 31 *Geranium richardsonii* populations.



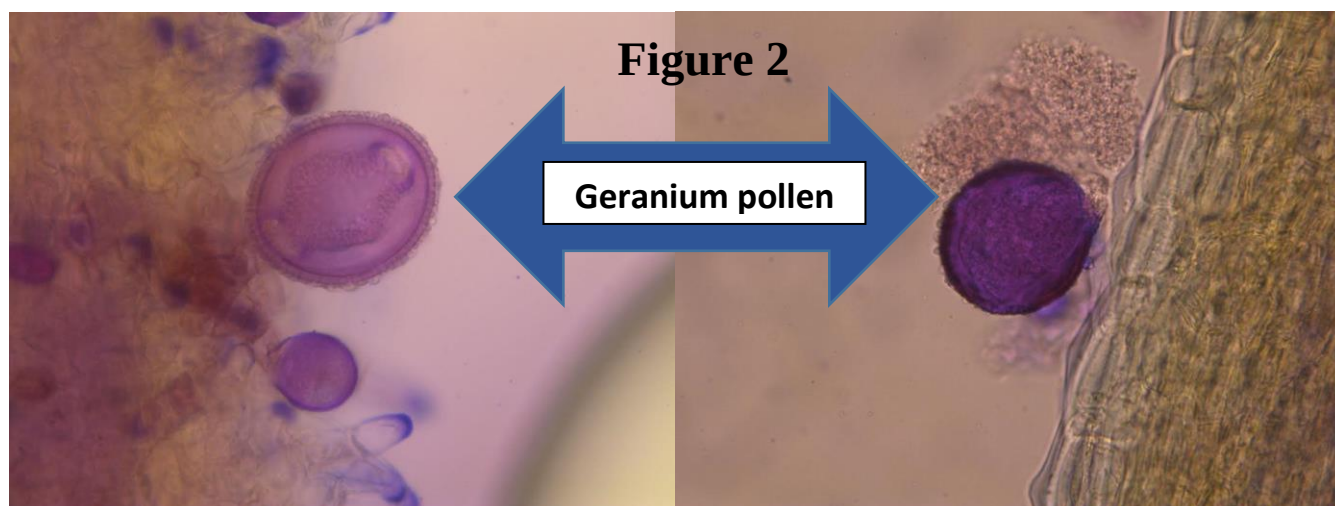


Table 1. Populations (n=31) surveyed around Gothic, Colorado. GPS coordinates, elevation, and % Female are shown. (*) means data from 1997.

Table 1 POP	Lat	Lon	Elevation (ft.)	%Female (1997*)
PH*/Gothic-1b	38.95965	-106.98948	9520	0.42*/0.36
ASP*/Gothic-1c	38.95998	-106.98913	9550	0.13*/0.13
VA*/Gothic-2	38.95822	-106.99017	9440	0.81*/0.83
LEV*/Gothic-3	38.95645	-106.99012	9440	0.49*/0.51
Gothic-4	38.95934	-106.99016	9480	0.04
bb	38.96344	-106.99281	9600	0.29
RM-1	38.95669	-106.98293	9640	0.28
RM-2	38.95787	-106.98584	9520	0.07
VB-1	38.96503	-106.98293	10200	0.00
VB-2	38.97132	-106.98258	10560	0.17
VB-3	38.97184	-106.98167	10650	0.15
VB-4	38.97212	-106.97969	10840	0.07

VB-5	38.97501	-106.97788	11040	0.03
VB-6	38.97599	-106.97752	11150	0.06
BV-1	39.00190	-107.02389	10100	0.07
BV-2	39.00308	-107.02519	10150	0.17
CC-1	38.97203	-106.96303	10120	0.06
CC-2	38.97287	-106.96236	10160	0.07
CC-3	38.97332	-106.96173	10160	0.09
CC-4	38.97334	-106.96174	10160	0.21
QB-1	38.97252	-106.94453	11270	0.16
QB-3	38.97502	-106.94758	11040	0.20
RR-1	38.95510	-106.97832	9840	0.01
NPB-1	39.03286	-107.07011	10780	0.00
NPB-2	39.03201	-107.06074	10600	0.00
NPB-3	39.03339	-107.07473	10900	0.00
Judd-1	38.96734	-106.99314	9640	0.65
Judd-2	38.96581	-106.99202	9670	0.04
Judd-3	38.96453	-106.99062	9740	0.00
Judd-4	38.96412	-106.98909	9800	0.19
Judd-5	38.96388	-106.98619	9880	0.00

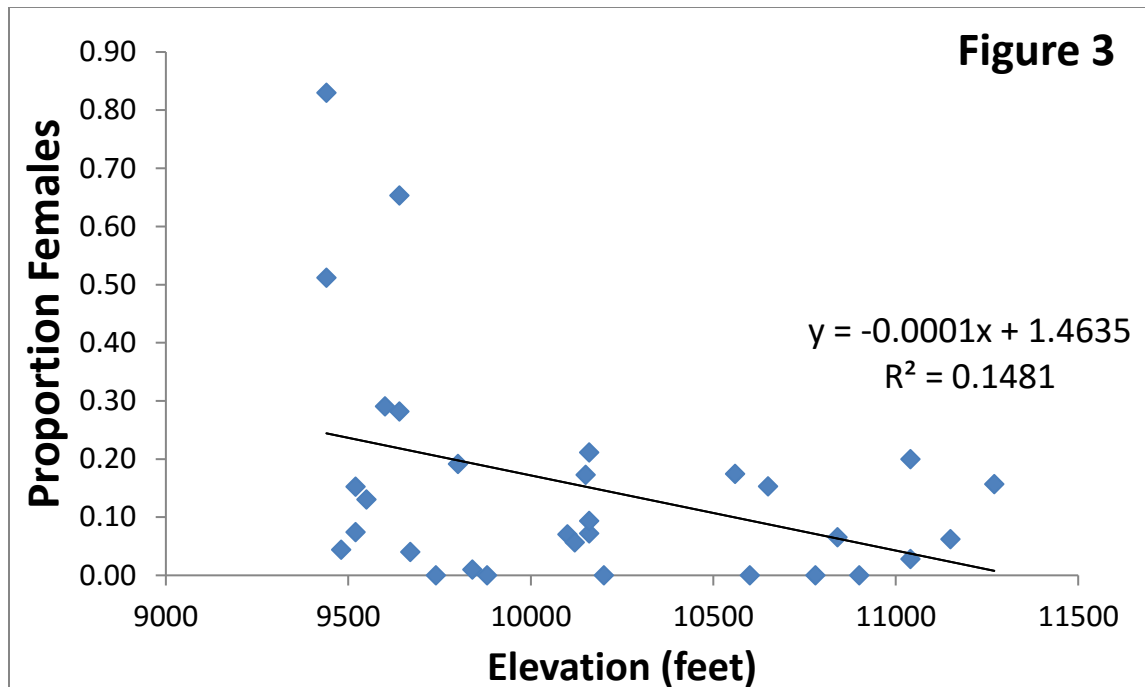


Figure 3. There is a significant negative relationship between elevation and the proportion of female plants in populations of *Geranium richardsonii* ($F_{(1,29)}=5.04$, $r^2=0.148$, $p=0.033$). Populations with very high female frequency (>30%) only occurred at the lowest elevations (<9700 ft.).

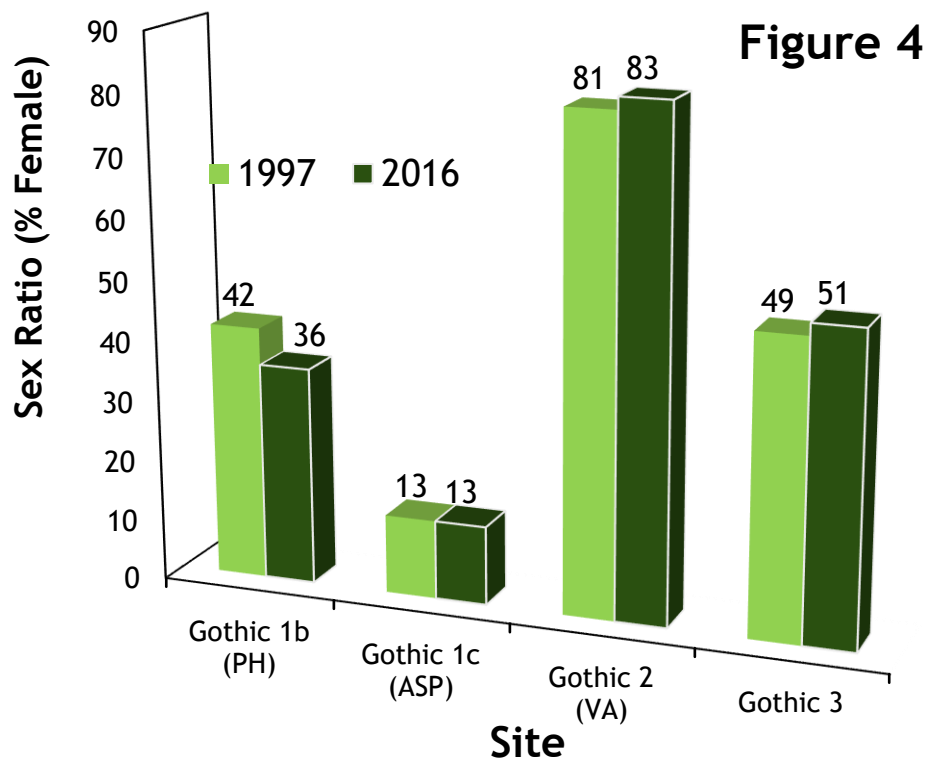


Figure 4. Sex ratio does not change over time for Gothic populations. The greatest change in % female was in Gothic 1b, which decreased by only 6%.

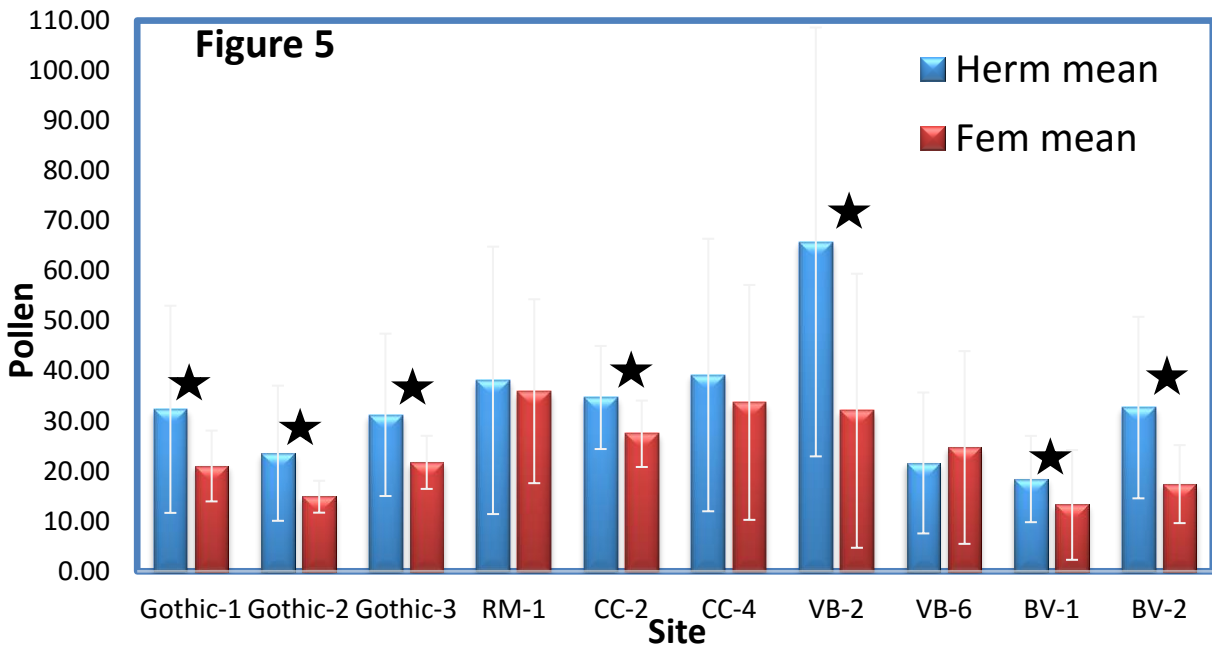


Figure 5. Comparison of stigmatic pollen loads on hermaphrodite and female plants in 10 populations of *Geranium richardsonii* near RMBL. In most populations, hermaphrodites have an advantage in pollen receipt over female flowers, except for the Virginia Basin (VB-6) population, where hermaphrodites have slightly less pollen load compare to female flowers. (Significant results are indicated by a white start).

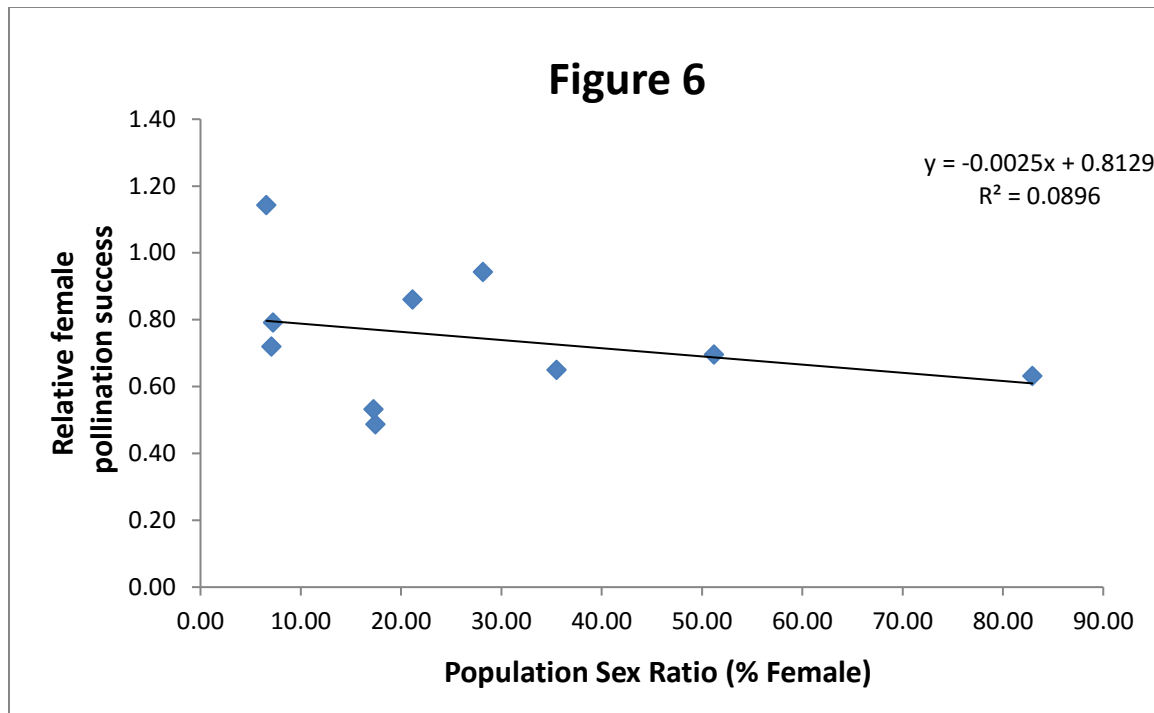


Figure 6. Relative female pollination success (female pollen receipt/hermaphrodite pollen receipt) as a function of population sex ratio shows a slight negative trend. This correlation is not significant. ($F_{(1,8)}=5.04$, $r^2=0.787$, $p=0.401$).

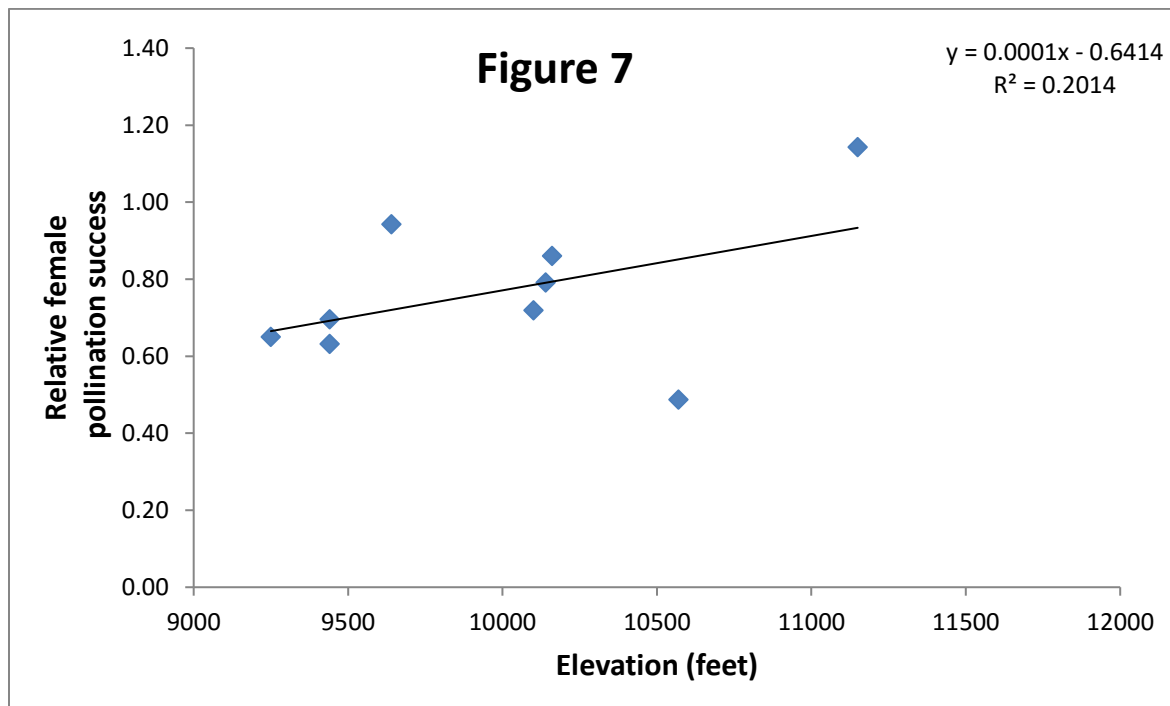


Figure 7. There is a weak, but non-significant positive correlation between elevation and relative female pollination success ($F_{(1,8)}=1.35$, $r^2=0.201$, $p=0.279$).

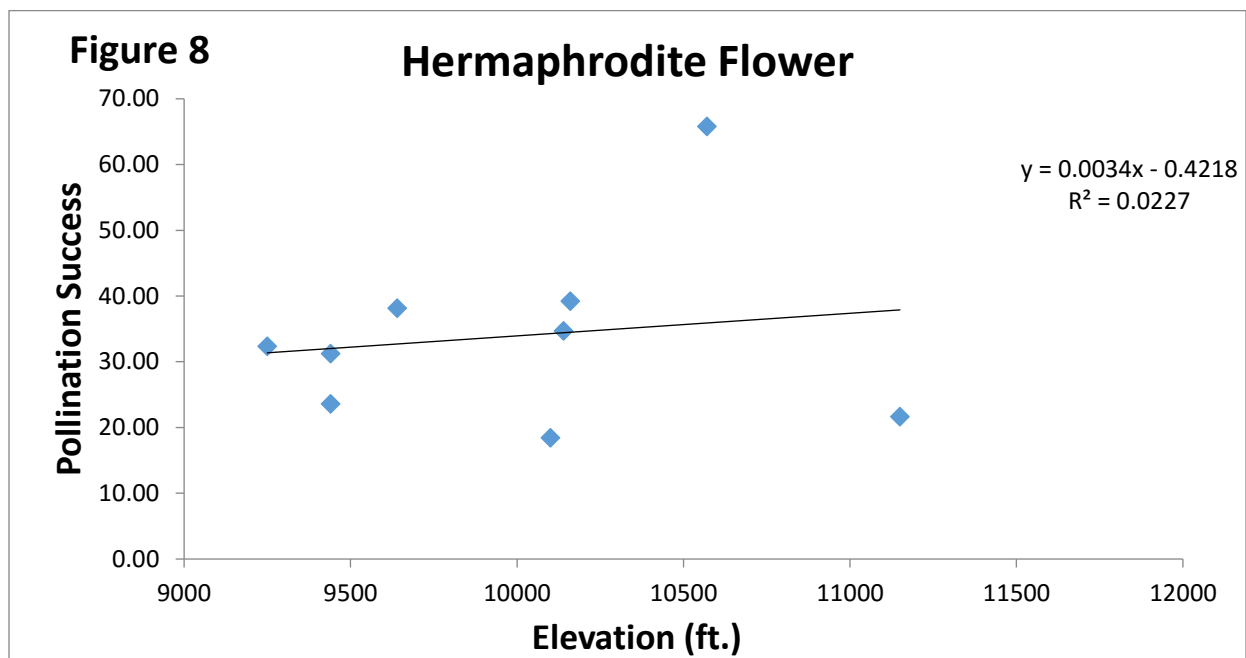


Figure 8. No significant correlation of Hermaphrodite pollination success with elevation ($F_{(1,8)}=0.178$, $r^2=0.023$, $p=0.684$).

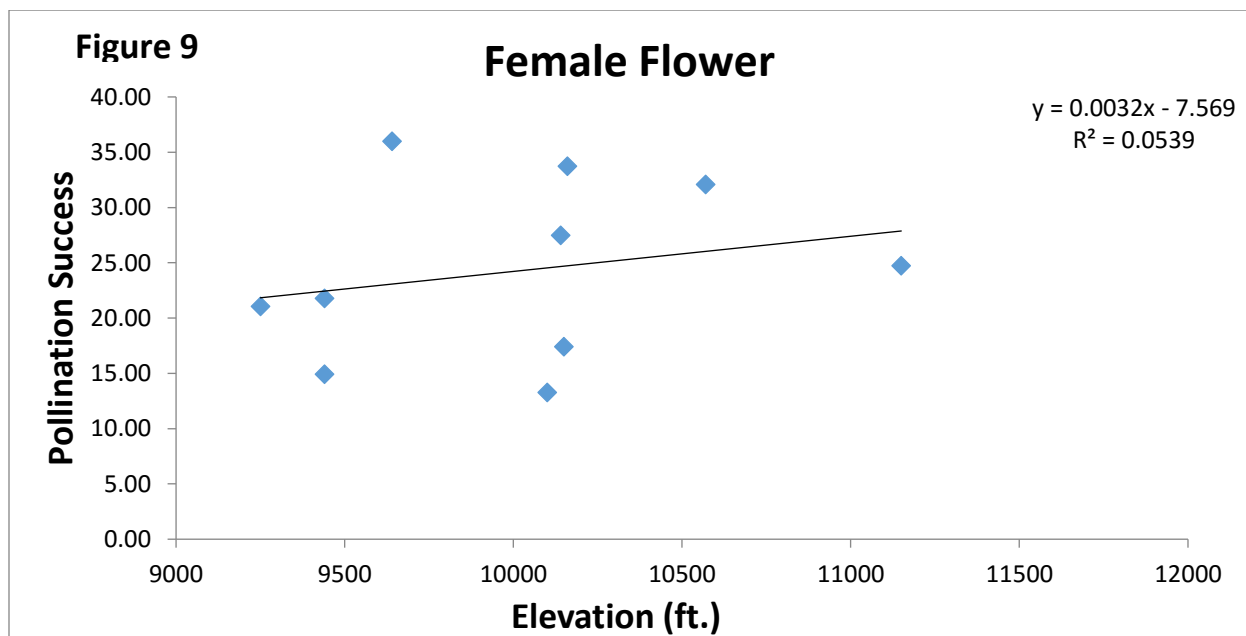


Figure 9. There is not significant relationship between relative female pollination success and elevation ($F_{(1,8)}=0.456$, $r^2=0.054$, $p=0.519$).