

Consequences of Pollination Neighborhood Composition and Pollinator Communities on the
Mating Success of the Dioecious Plant *Valeriana edulis*

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Full Time Independent Research

Summer 2014

Abstract

Climate change may affect individuals within a species differently dependent on sex, leading to skewed sex ratios. This paper considers the effect of mating environment on seed production in the dioecious plant species, *Valeriana edulis*. The study was conducted in female dominated populations where there could be sex ratio-mediated pollen limitation. We assessed five candidate indices describing the mating environment around focal females as predictors of focal female seed set including: density, pollen availability, pollen context with and without competition and sex-ratio. We also describe the daytime pollinators of *V. edulis* using pollen load counts. Plant density and pollen context were the best predictors of seed set individually and when combined into a single model. Sex-ratio did not have a significant effect on seed set though it is often used as the only factor to describe mating environment. We argue that mating environment should be quantified rather than assumed and suggest that future studies trace the effect of robustly characterized mating environments to population dynamics.

Introduction

Biological responses to climate change are occurring across a wide variety of taxonomic groups. Species range shifts, phenological change, plasticity, and resource limitation due to warming have been shown in many organisms since the early twentieth century (Parmesan 2006). Historical data and recent observational and empirical studies have shown that both plants and animals respond to yearly and long-term climate pattern change (Harte and Shaw 1995; Parmesan 2006). Investigation into precipitation loss (Barber, Juday and Finney 2000), and habitat unsuitability with temperature change (Parmesan and Yohe 2003, Burrows, et al. 2011) has shown that species respond differently to climate change both in phenology and population dynamics (CaraDonna, Iler and Inouye 2014). However, most studies of biological responses to climate change focus at the species level, neglecting parallel intra-specific variation.

There is significant variation between species in ecologically important traits and, as a result, responses to changing climate may differ among individuals (Cobben, et al. 2011). Diversity however may make some individuals within a species more or less sensitive to environmental changes. As the climate changes the environment may become unsuitable for a subset of the species forcing them to move or may pose an extinction risk (Bolnick, et al. 2003). Genotypes that can survive in the altered environment may then become spatially separated from other genotypes, in some cases the genotypic difference is sex which can create mate limitations (Bolnick, et al. 2003). Specifically in dioecious (having two distinct sexes) plants, the differences between males and females may result in spatial separation between the two sexes with changing climate (Hawkins, et al. 2009). If two sexes differ in sensitivity to stressors, one sex may not be able to sustain itself in an environment that the other gender can survive in. The gender that cannot survive may die off and make successful reproduction rare or impossible.

The vast majority of animals are dioecious and a large number of ecologically important plant species are dioecious as well (Ghiselin 1969; Renner and Ricklefs 1995). Dioecious organisms may be more susceptible to climate change because the resource requirements may be different between the sexes. If one sex cannot survive in a region, then reproduction cannot occur (Hultine, et al. 2007; Jung, et al. 2010). Studies have shown that stress in dioecious organisms may be handled differently between the two sexes both in plants (Hultine, et al. 2007; Field, Pickup and Barrett 2012) and in animals (Pistevos, et al. 2010). Males may have fewer stressors than their female counterparts in some cases (Carlsson-Graner, et al. 1998) and in others they may be equally or more stressed than females based on life-history style in both kingdoms (Border, et al. 2012; Field, Pickup and Barrett 2012). The different stress tolerance of one gender or the other may cause one sex to die off, which could create an unbalanced sex ratio.

Skewed sex ratios and resultant mate limitation may be of particular importance to sessile organisms where mating is limited to individuals in the relative vicinity. In plants, pollen limitation becomes a significant determinant of genetic diversity (Campbell 1991; Ghiselin 1969). This limitation may arise from too long of a distance from pollen source to stigma or by a lack of pollinators (Knight, et al. 2005; Oster and Eriksson 2007). Populations with highly biased sex ratios may have too few individuals of the rare gender to realize full reproductive potential. Furthermore, the reduced genetic diversity in populations without enough of one gender can slow the adaptation process as well as allow for the spread of deleterious alleles (López-Villavecencio, et al. 2013). Insufficient seed production could compromise the persistence of the species or lead to a decline in population size (Knight, et al. 2005). These limitations create an extinction risk in highly sex biased populations because without one gender in a dioecious community the other cannot reproduce.

Valeriana edulis is a native, wildflower species in Colorado with an elevational dependent sex-ratio that is changing with climate. This plant has increasingly female-biased sex ratios as elevation increases, starting at a nearly 1:1 ratio at low elevation (2000m) (Petty, unpublished data). The rarity of males at high elevation (only 20-25% of individuals at 3700m) is most likely pollen limiting and reduces fertilization likelihood (Knight, et al. 2005; Oster and Eriksson 2007; García-Camacho and Méndez 2009). It is unknown however what aspect of the elevation gradient (e.g. precipitation, soil composition) skews population sex ratios. If stressors raise beyond what is tolerable to the male plants due to climate change at the unbiased sex ratio populations then they may fail to persist at these sites leaving all populations female biased and potentially without necessary males. This study focuses on direct and indirect mechanisms affecting seed set success. This study answers the questions: **Is pollen limiting to female seed set success when neighborhood sex ratios are skewed? Does competition among females for mates further reduce seed set? How do pollinator communities change over the flowering period, and does this affect neighborhood size and subsequently seed set success?**

Methods

Study Site and Species: *Valeriana edulis* is a long-lived dioecious insect pollinated perennial found sporadically throughout the western United States at many elevations from subalpine to alpine, primarily in meadows (Soule 1981). *V. edulis* forms a leafy rosette at the base of the plant with oblong or spoon shaped leaves with white edges (Soule 1981). The inflorescences are clustered and produced on tall stalks that protrude from the leafy base and may have a few sparse forked leaves. Inflorescences for both sexes are small (<5mm diameter) with males being slightly larger than females. Flowering is temporally spaced within and between plants and occurs in June and July (Soule 1981). Plants do not flower every year and on

non-flowering years produce only the leafy base. The plant is believed to be pollinated by flies and small solitary bees (Soule 1981). We worked near Brush Creek (2780m) and Emerald Lake (3185m) outside of Crested Butte, Colorado (Figure 1). Brush Creek is in the mid to low elevation range of *V. edulis* habitat and may have slight female-biased sex ratio (60% of the population). Emerald Lake is in the mid to upper elevation range with a slightly greater female bias than Brush Creek (65% of the population; Petry, unpublished data).

Pollen Neighborhood: Pollen transfer distance will determine the scale of the mating environment or pollination neighborhood of female plants, and the composition of this neighborhood may drive variation in seed production. Pollen travel distance in female biased populations will be extremely important in determining seed set success, some may not be pollinated if there are no males within pollen travel distance. We applied florescent dye that mimics pollen to 12 male *V. edulis* plants to track pollen movement on July 17, 2014 (at Emerald Lake; Campbell and Waser 1989; Campbell, 1991; Inouye, et al. 1994). The small sample size is due to a mid-season site transfer from Brush Creek to Emerald Lake after plants began setting seed early. Approximately 24 hours after the dye was applied we returned to the field site and collected approximately half of the flowers on each female plant on four 10 meter transects spurring from the dyed male. The florescent dye was visually detected on the stigmas of the female flowers, and we counted dye particles under a dissecting microscope with a standard light source or UV light depending on dye color. Pollen transfer decline with distance was fit using a Lomax distribution, and the pollination neighborhood is defined as the distance within which 90% of dye was transferred. The Lomax distribution was used because it does not degrade as quickly as alternatives that may underestimate rare long-distance transfer events (Van Rossum, et al. 2010; Nathan, et al. 2003).

Intra-species Distances and Seed Set: Characteristics of the neighborhood of a plant should affect its reproductive success. Aspects of this neighborhood include competition between females for pollen as well as distance from males and total density of plants. We discreetly marked the calyx of ca. 40 open female flowers per stalk on a total of 15 focal females with a black Sharpie marker (Kearns and Inouye 1993). Sections of plants were delineated by small numbered tags to facilitate re-finding of marked flowers, and the number of open marked flowers was recorded. We then estimated the number of open flowers on all *V. edulis* plants within a 10 meter radius of the marked female. Estimations were made by counting the number of open flowers on a small section of the plant and multiplying by the approximated number of times the section was replicated on each stalk for each plant. Estimator bias among the four observers was evaluated by estimating 10 male and 10 female plants and then recording their true counts. Each estimator evaluated the same plants and then estimated values were compared to each other as well as to the true count. Model selection by Akaike Information Criterion (AIC) indicated estimator identity, the number of flowers estimated, and their interaction as the best model ($R^2 = 0.85$) and excluded main and interactive effects of inflorescence stalk number, inflorescence node number, and plant sex.

Seed set was determined by counting the number of seeds produced versus the number of marked open flowers at the time the neighborhood was assessed. The proportion of successful seeds produced was analyzed against multiple potential candidate indices (Table 1). Candidate indices were applied individually and then were applied in concert to look for possible joint effects. Candidates included were (Table 1) density (number of plants in the neighborhood), sex ratio (number of males per total population), neighborhood male flower count, pollen context (number of male flowers adjusted by Lomax proportion values per distance) (modified from

García-Camacho and Méndez 2009), pollen context with competition (pollen context over number of open female flowers adjusted by Lomax distribution values).

Pollinator Community Dynamics: Pollinator communities should change throughout the flowering season and across elevation. The maximum and minimum insect population sizes versus when the *V. edulis* is blooming may affect the amount of pollen that is being carried between plants. Collections were taken on June 30, July 6, 15 and 28, 2014. The first collection (June 30) was taken at Brush Creek and all subsequent collections were taken at Emerald Lake after the project was relocated. During each collection approximately 20 potential pollinators on ca. 15 *V. edulis* plants were collected and placed in individual vials. For the first two floral visitor collections there was no bias in collection based on gender. We then focused on floral visitors on females because pollen counts of insects on male plants may have been skewed. After collection approximately 0.25 mL of ethanol was added to each vial to wash the pollen off the insects (following Brosi and Briggs 2013). The alcohol was then poured onto clean microscope slides in two batches and allowed to evaporate. After the slides were dry we placed a few drops of heated fuchsin gel in the center of the slide (approx. 3cm square section) and added a cover-slip. Slides were allowed to sit for at least 20-30 minutes so pollen grains could absorb the dye before counting. *V. edulis* pollen grains were identified using a reference pollen library and counted. Insects were identified to family and compared by the number of pollen grains found on the slide. We used an unequal sample sizes t-test to compare mean pollen counts from the first collection and the last collection.

Results

Pollen Neighborhood: Pollen analog dye particles were most effectively transferred to the closest plants by pollinators. Pollen transfer dropped off extremely quickly with only a few particles traveling farther than a few meters. For example, 50% of pollen at Emerald Lake was transferred within approximately 2 meters using the Lomax distribution line (Figure 2).

However some particles may make it much farther with 90% of particles moving as far as 11.43 m. Pollen neighborhood size was used to determine competition neighborhood distances at the 90% demarcation.

Intra-Species Distances and Seed Set: Seed set should be influenced by aspects of the neighborhood of a particular female (Table 1). Density was determined to be the most influential single factor but density when combined with pollen context explained the data the most thoroughly. Pollination context is representative of the amount of pollen that is available to the focal female (García-Camacho and Méndez 2009) and is positively correlated with seed set ($p=0.048$; Figure 3). Density however, reduced the number of seeds produced ($p=0.020$; Figure 4). Both are referenced to deviation from mean seed set.

Pollinator Community Dynamics: Pollen load size as well as pollinator communities changed over the course of the summer (Figure 5). Between the first and last week of July the average number of pollen grains found on insects on female plants increased significantly ($\bar{x}_1=1.78\pm0.83$, $n=7$; $\bar{x}_2=39.17\pm21.85$, $n=19$ $p<0.001$, $df=19$). The first two collections have relatively few pollinators because we were not focusing on female plants and male data is excluded in Figure 2. Pollinators came from four orders; Diptera, Coleoptera, Hemiptera and Hymenoptera. Diptera carried the most pollen in every collection except the second which

showed neither Hymenoptera nor Diptera on female plants. Diptera were typically in the families Muscidae and Empididae. Empidids were dominant floral visitors across the collections whereas muscids increased in abundance throughout July. We did not look at potential night pollination, though moths have been reported to visit *V. edulis* (Soule 1981).

Discussion

Pollen Neighborhood: Insect pollinators predominantly move *V. edulis* pollen very short distances. Most pollen stays within 2 m of the male that it was produced on. This may be of more immediate importance to highly sexually biased populations where a male may not be within a few meters of a female. At higher elevations populations where sex ratios are more strongly skewed towards females, the pollen availability would be expected to be much lower. Fewer males in the pollen movement range of females could limit successful pollination. The short distance of pollen movement could be due to the fairly small size of the pollinators, their carrying structures or the way that they travel between plants (Borkent and Harder 2006). Fly pollinators – the predominant pollinators here – tend to be generalists, meaning that they travel between close plants with little to no preference or loyalty to plant species (Ashman 2000). Flies may only utilize a few flowers from each plant but leave when they encounter an empty flower (Borkent and Harder 2006). This type of dipteran movement may explain why pollen does not move very far, dipterans may not be choosing flowers based on their morphology or rewards but are traveling from one plant to the next closest (Borkent and Harder 2006). If there are no plants of the same species in the vicinity then they may be dropping the gathered pollen on other male *V. edulis* or heterospecific plants.

We cannot say definitively that the limited pollen movement will restrict fertilization in alpine populations because movement may be different per elevation, we saw a small change in movement distance between Brush Creek and Emerald Lake and do not know if this is attributed to better pollen movement, summer progression or insect community differences. The insect communities were slightly different between the two sites, both showed many dipterans but the single collection at Brush Creek had many more Empididae than Muscidae. Empididae tend to have a much smaller surface area than Muscidae which could account for the change in pollen movement between the two sites. Summer progression and warmer temperatures may affect when the pollinators are more active and which pollinators are active as well as how many male flowers are open and the subsequent amount of available pollen. Either of these factors could be contributing to change in pollen movement per site because the distances taken at Brush Creek were in June and the distances at Emerald Lake were taken in July.

Intra-Species Distances and Seed Set: We hypothesized that seed set and neighborhoods are highly correlated and that the females with more males within their pollen neighborhood will have the best seed production proportions. The alpine populations that are extremely female-biased will have extremely low pollen context values compared to the relatively unbiased sites that were observed in this study. Pollen context was a significant factor in determining female reproductive success, meaning that more pollen results in higher seed set as would be expected (Figure 3). However previous studies conducted on neighborhood factors and seed set tend to focus on sex-ratio rather than actual pollen availability (Oster and Eriksson 2007; Carlsson-Graner, et al. 1998; Field, Pickup and Barrett 2012). The present study suggests that future research may require a more in depth look at the mating environment of an organism to accurately describe potential seed set.

Competition for pollen between females in sex-biased populations was also hypothesized to affect their seed set. However there was no significant trend associating competition for pollen and seed set. Total plant density was a more important factor determining the reproductive success of the focal females (Figure 4), which is consistent with the only previous study relating mating environment to reproductive success (Miller and Inouye 2011). Aspects of density that may affect seed set include competition for pollinators or nutrient resources (Snell and Burch 1975). Female plants may not only be competing with other females but may also be competing with surrounding males in dense populations for necessary resources. If there is a pollinator bias by floral morphology or reward (Ashman 2000) then having more large male flowers in the vicinity of the focal female may be a disadvantage rather than an advantage. Further study with a larger sample size (especially at the larger neighborhoods) would need to be conducted to determine how females are competing with their neighbors for resources. Population density is currently unrelated to elevation, however demographic data suggests a trend toward more male abundance with time (Petty, unpublished data). This could increase the density of total plants and negatively impact seed set. Pollen context and density have opposing effects on seed set and could balance each other out or result in either more limited seed set or greater seed set. Based on our results, density should have the greater effect on seed set so an increase in male frequency may be detrimental to some populations. Further studies should be conducted at the alpine populations to determine how pollen limitation is affecting the seed set at these heavily sex-biased sites and if further population growth is possible or will be extremely limited.

Pollinator Community Dynamics: A previous study on *V. edulis* reported that Diptera and Hymenoptera are its primary pollinators; however this was based on observed floral visits

without assessing pollen loads (Soule 1981). The present study shows that although Diptera are important pollinators of *V. edulis*, Hymenoptera are not. Of the few Hymenoptera that were found on female plants with pollen on them, all had comparatively low amounts of pollen on their bodies. This suggests that although Hymenoptera may be frequent floral visitors, they tend to not be loyal or effective pollinators for this species. This may be due to the small size of the flowers on female plants that may be more suited to other insects (Borkent and Harder 2006). There were many more Diptera families present than any other order and they carried the most pollen. This suggests that *V. edulis* is reliant on the pollen transferred by Diptera (especially Muscidae and Empididae) for successful pollination. The plants produce an unpleasant floral scent which may be attracting the visitors that we saw (Chicas-Mosier, personal observation). Both muscid flies and carabid beetles are affiliated with the decomposition process (Dudareva and Pichersky 2006) and may be attracted to the foul scent of *V. edulis*. Finally, flies commonly replace bees as the most important pollinators at higher elevations, and the apparent lack of hymenopteran pollinators may be a consequence of the high elevation location of Emerald Lake (Dudareva and Pichersky 2006).

Hemiptera and Coleoptera are not traditionally associated with pollination and were not observed in previous pollination observation of this plant (Soule 1981). The male plants of *V. edulis* produce significant amounts of pollen and the females tend to produce highly concentrated nectar (Pettry, et al. 2013). It is possible that the plant is using these mechanisms to coax non-traditional pollinators onto both sexes of the plant for high calorie rewards. The bodies of these orders are not typically efficient carriers of pollen, but for *V. edulis* they appear to be important (Kevan and Baker 1983). The family Cerambycidae may be more suited for carrying pollen based on their head shape and proboscis length but they were not the most caught or the highest

pollen carriers of the coleopterans (Kevan and Baker 1983). During the second insect collection the only orders that were present with pollen on the female plants were Hemiptera and Coleoptera, suggesting that they may play an important role in the pollination of *V. edulis* despite not having specialized pollen carrying structures.

Climate change is affecting *V. edulis* in a sex-biased manner. Females are more abundant in the majority of studied populations near Crested Butte, Colorado but their frequency in populations is decreasing (Petry, unpublished data). This could mean that the sex-bias will reverse with time and create male dominated populations. Whether the populations are male or female dominated, the bias may result in genetic or distance related limitations to fertilization. Skewed sex-ratios may be the result of a bias in niche fit (Bolnick, et al. 2003), or pollinator induced selection (Ashman 2000). It is uncertain how the generalist pollinator communities of *V. edulis* are affecting the populations, and whether or not pollinators are creating competition both within and between sexes (Ashman 2000; Borkent and Harder 2006). The niche that *V. edulis* is currently occupying is changing with climate change (Harte and Shaw 1995) and there are estimates (e.g. sex-ratio change, range shift, mortality) of how the populations will change in the long-term due to the many dynamic factors that are shaping the populations and we know that there have been changes since previous work on *V. edulis* populations was conducted (Soule 1981; Petry, unpublished data).

Here we demonstrated how pollen neighborhood and neighborhood composition create mate limitation in sex-biased populations. We also show how focusing on sex-ratios may not accurately describe the mating environment of a species. There may be other factors that better explain variance in seed set such as density and pollen availability as we have shown in this system. Climate change may be producing skewed sex-ratios in other systems as a result of non-

parallel adaptation to changing environment by sex, adding to the need to address this shortcoming in our understanding of the population dynamics of dioecious organisms. Further study should be conducted to determine how mating environment shapes reproductive success and if it is affecting population growth or posing an extinction risk.

Literature Cited

- Ashman, T. "Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism." *Ecology*, 2000: 2577-2591.
- Barber, V.A., G.P. Juday, and B.P. Finney. "Reduced Growth of Alaskan White Spruce in the Twentieth Century from Temperature-Induced Drought Stress." *Nature*, 2000: 668-673.
- Bolnick, D.I., et al. "The Ecology of Individuals: Incidence and Implications of Individual Specialization." *The American Naturalist*, 2003: 1-28.
- Border, F., N. Ponlet, J. Gouy de Bellecq, A. Ribas, and S.M. Krasnov. "Is there sex-biased resistance and tolerance in Mediterranean wood mouse (*Apodemus sylvaticus*) populations facing multiple helminth infections?" *Oecologica*, 2012: 123-135.
- Borkent, C.J., and L.D. Harder. "Flies (Diptera) as pollinators of two dioecious plants: behavior and implications for plant mating." *Canadian Entomologist*, 2006: 235-246.
- Brosi, B.J., and H.M. Briggs. "Single Pollinator Species Losses Reduce Floral Fidelity and Plant Reproductive Function." *PNAS*, 2013: 13044-13048.
- Burrows, M.T., et al. "The Pace of Shifting Climate in Marine and Terrestrial Ecosystems." *Science*, 2011: 652- 655.
- Campbell, D.R. "Comparing Pollen Dispersal and Gene Flow in a Natural Population." *Evolution*, 1991: 1965-1968.
- Campbell, D.R., and N.M. Waser. "Variation in Pollen Flow Within and Among Populations of *Ipomopsis aggregata*." *Evolution*, 1989: 1444-1455.

- CaraDonna, P. J., A.M. Iler, and D.W. Inouye. "Shifts in flowering phenology reshape a subalpine plant community." *Proceedings of the National Academy of Sciences*, 2014: 4916-4921.
- Carlsson-Graner, U., T. Elmqvist, J. Agren, H. Gardfjell, and P. Ingvarsson. "Floral Sex Ratios, Disease and Seed Set in Dioecious *Silene dioica*." *Journal of Ecology*, 1998: 79-91.
- Cobben, M.M.P., et al. "Projected Climate Change Causes Loss and Redistribution and Genetic Diversity in a Model Metapopulation of a Medium-Good Disperser." *Ecography*, 2011: 920-932.
- Dudareva, N., and E. Pichersky. *Biology of Floral Scent*. Boca Raton: CRC Press, 2006.
- Field, D.L., M. Pickup, and S.C. Barrett. "Comparative Analyses of Sex-Ratio Variation in Dioecious Flowering Plants." *Evolution*, 2012: 661-672.
- García-Camacho, R., and M. Escudero, A. Méndez. "Pollination Context Effectuated in the High-Mountain Dimorphic *Armeria caespitosa* (Plumbaginaceae) Neighborhood is Something More Than Density." *American Journal of Botany*, 2009: 1620-1626.
- Ghiselin, M.T. "The Evolution of Hermaphroditism Among Animals." *The Quarterly Review of Biology*, 1969: 189-208.
- Harte, John, and Rebecca Shaw. "Shifting Dominance with a Montane Vegetation Community: Results of a Climate-Warming Experiment." *Science*, 1995: 876-880.
- Hawkins, T.S., et al. "Growth and Intraspecific Competitive Abilities of the Dioecious *Lindera melissifolia* (Lauraceae) in Varied Flooding Regimes." *Journal of the Torrey Botanical Society*, 2009: 91-101.

- Hultine, K.R., S.E. Bush, A.G. West, and J.R. Ehleringer. "Population Structure, Physiology and Ecohydrological Impacts of Dioecious Riparian Tree Species of Western North America." *Oecologia*, 2007: 85-93.
- Inouye, D.W., D.E. Gill, M.R. Dudash, and C.B. Fenster. "A Model and Lexicon for Pollen Fate." *American Journal of Botany*, 1994: 1517-1530.
- Jung, V., C. Violle, C. Mondy, L. Hoffman, and S. Muller. "Intraspecific Variability and Trait-Based Community Assembly." *Journal of Ecology*, 2010: 1134-1140.
- Kearns, C.A., and D.W. Inouye. *Techniques for Pollination Biologists*. Niwot: University Press of Colorado, 1993.
- Kevan, P.G., and H.G. Baker. "Insects as Flower Visitors and Pollinators." *Annual Review of Entomology*, 1983: 407-453.
- Knight, T.M., et al. "Pollen Limitation of Plant Reproduction: Pattern and Process." *Annual Review of Ecological Evolution*, 2005: 467-497.
- Langenheim, J.H. "Vegetation and Environmental Patterns in the Crested Butte Area, Gunnison County, Colorado." *Ecological Monographs*, 1962: 249-285.
- Legendre, S. "Age Structure, Mating System, and Population Viability." *Evolutionary Conservation Biology*, 2004: 41-58.
- López-Villavecencio, M., A.J.M. Debets, M. Slakhorts, T. Giraud, and S.E. Schoustra. "Deleterious Effects of Recombination and Possible Nonrecombinatorial Advantages of Sex in a Fungal Model." *Journal of Evolutionary Biology*, 2013: 1968-1978.

- Miller, T.E.X., and B. D. Inouye. "Confronting the two-sex demographic models with data." *Ecology*, 2011: 2141-2151.
- Nathan, R., G. Perry, J.T. Cronin, A.E. Strand, and M.L. Cain. "Methods for estimating long-distance dispersal." *Oikos*, 2003: 261-273.
- Oster, M., and O. Eriksson. "Sex ratio mediated pollen limitation in the dioecious herb *Antennaria dioica*." *Ecoscience*, 2007: 387-398.
- Parmesan, C. "Ecological and Evolutionary Responses to Recent Climate Change." *Annual Review of Ecology, Evolution and Systematics*, 2006: 637-669.
- Parmesan, C., and G. Yohe. "A Globally Coherent Fingerprint of Climate Change Impacts Across Natural Systems." *Nature*, 2003: 37-42.
- Petry, W. "unpublished data."
- Petry, W.K., et al. "Mechanisms underlying plant sexual dimorphism in multi-trophic arthropod communities." *Ecology*, 2013: 2055-2065.
- Pistevos, J.C., P. Calosi, S. Widdcombe, and J.D.D. Bishop. "Will Variation Among Genetic Individuals Influence Species Responses to Global Climate Change?" *Oikos*, 2010: 675-689.
- Renner, S.S., and R.E. Ricklefs. "Dioecy and its correlates in the flowering plants." *American Journal of Botany*, 1995: 596-606.
- Snell, T.W., and D.G. Burch. "The effects of resource partitioning *Chamaesyce Hirta* (Euphorbiaceae)." *Ecology*, 1975: 742-746.

Soule, J.D. *Ecological Consequences of Dioecism in Plants: A Case Study of Sex Differences, Sex Ratios and Population Dynamics of Valeriana Edulis Nutt.* Crested Butte, 1981.

Van Rossum, F., I. Steiers, A.V. Geert, L. Triest, and O.J. Hardy. "Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb." *Oecologia*, 2010: 663-674.



Figure 1: Map of *Valeriana edulis* field sites; Brush Creek and Emerald Lake

Table 1: Defined equations for candidate indices and their observed ranges, where: n_m and n_f are the total number of male and female individuals, respectively; f is the number of flowers on individual i ; $p(d)$ is the probability density of Lomax at distance d meters

Candidate index	Formula	Range of Our Neighborhoods
Density of Individual Plants	$n_m + n_f$	22-294 plants
Sex Ratio	$\frac{n_m}{n_m + n_f}$	0.26 - 0.45
Number of Male Flowers	$\sum_{i=1}^{n_m} f$	2979.38 - 32397.26
Pollen Context	$\sum_{i=1}^{n_m} fp(d)$	250 - 3055.65
Pollen Context with Competition	$\frac{\sum_{i=1}^{n_m} fp(d)}{\sum_{i=1}^{n_f} fp(d)}$	0.18 - 1.82

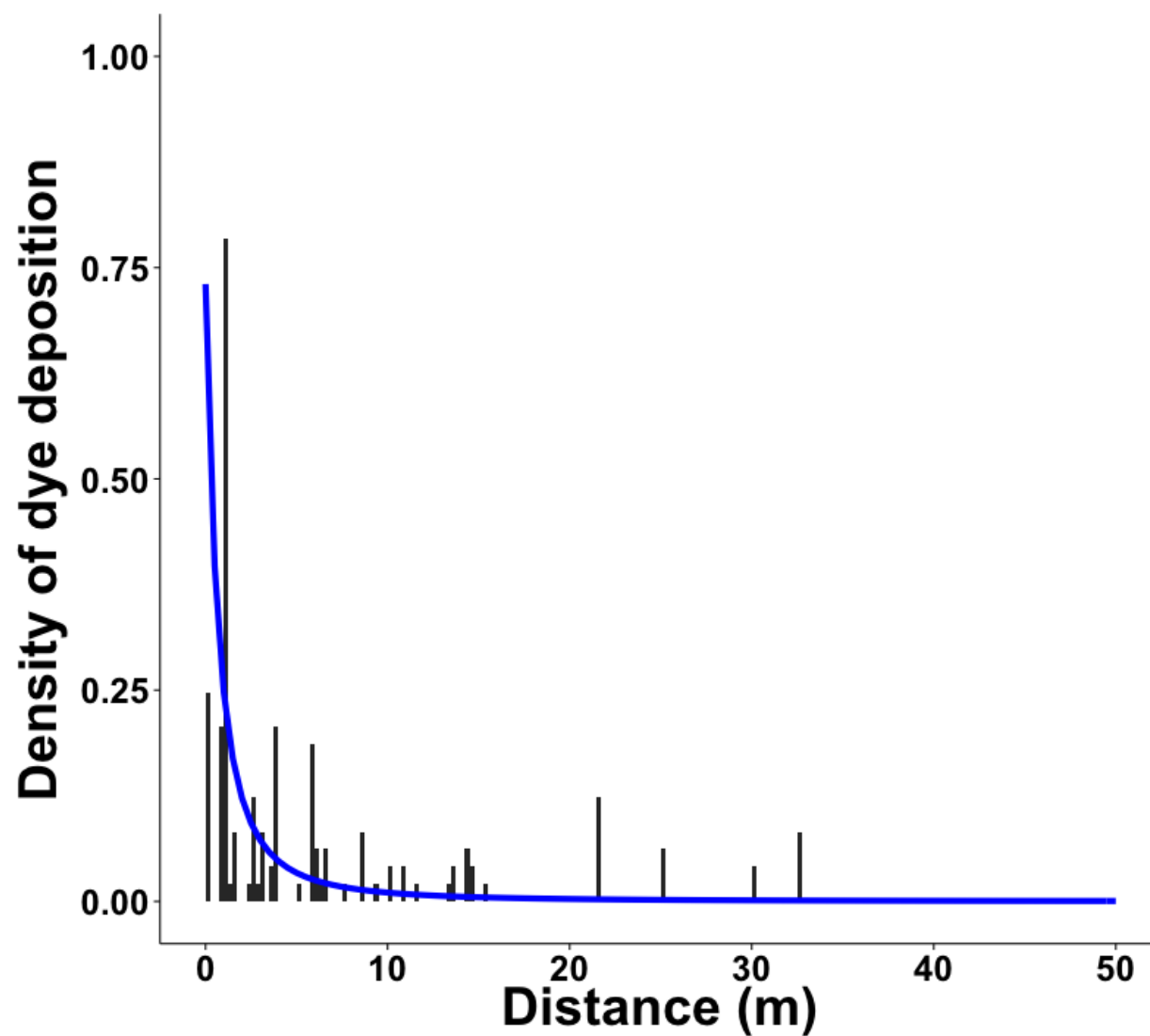


Figure 2: Lomax distribution of pollen analog movement at Emerald Lake, Colorado. Approximately 90% of pollen travel is within 10 meters of the focal male.

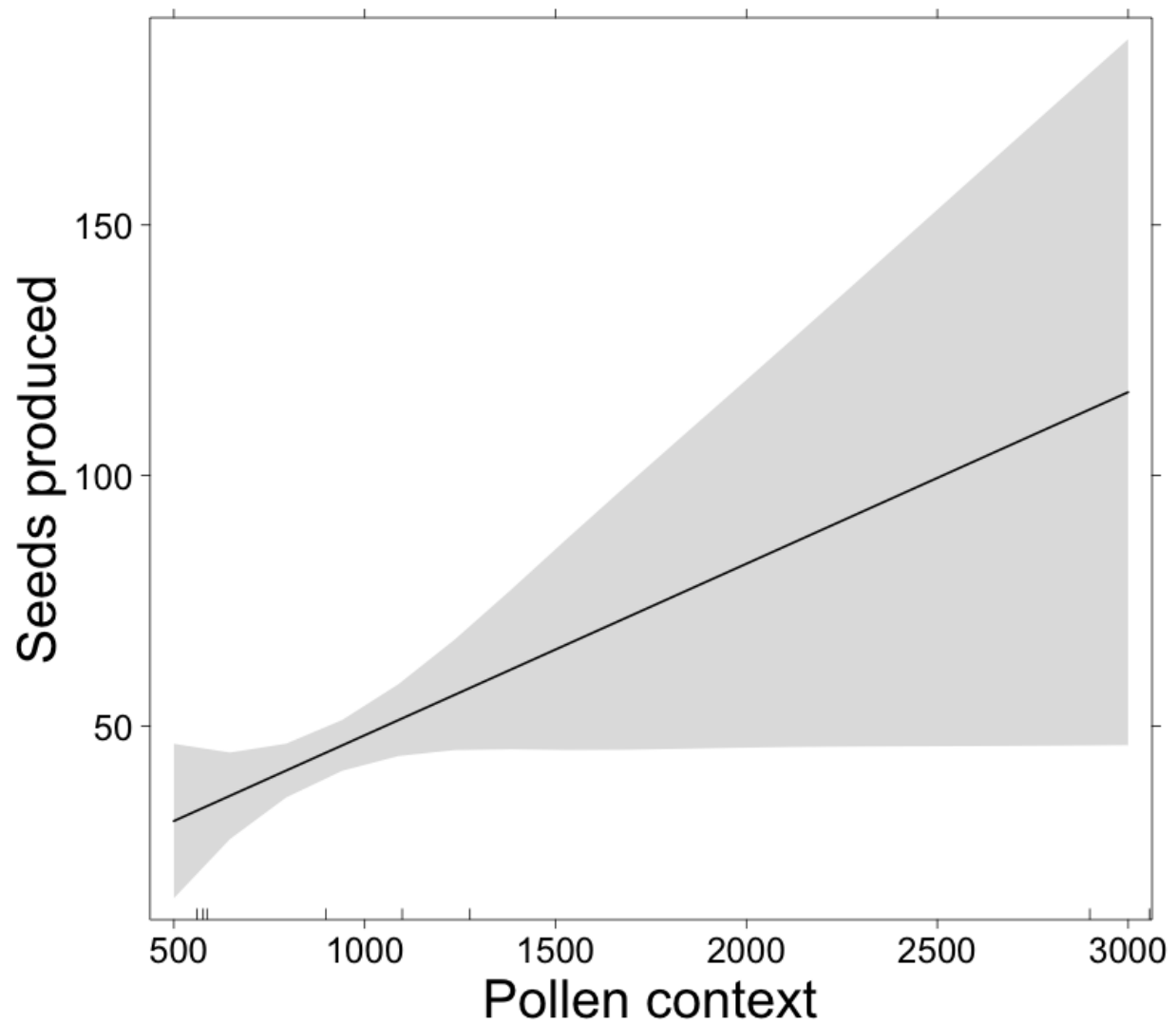


Figure 3: Pollen availability affects the proportion of seeds produced by a female; pollination context (Table 1) is modified from Garcia-Camacho & Mendez (2009) and is representative of the availability of pollen in the focal female's immediate vicinity at Emerald Lake, Colorado.

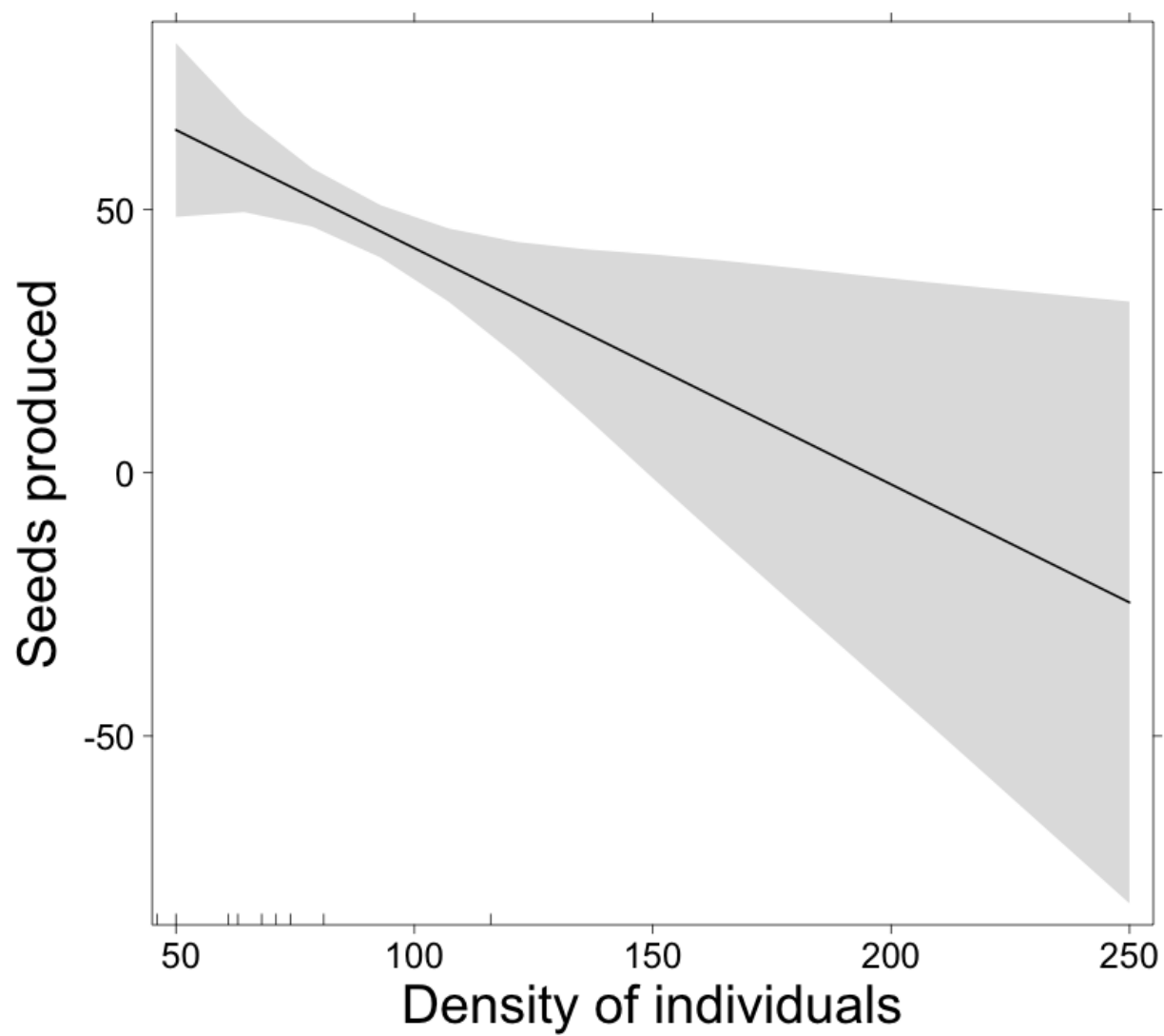


Figure 4: Effect of density of individual plants at Emerald Lake, Colorado on seed set.

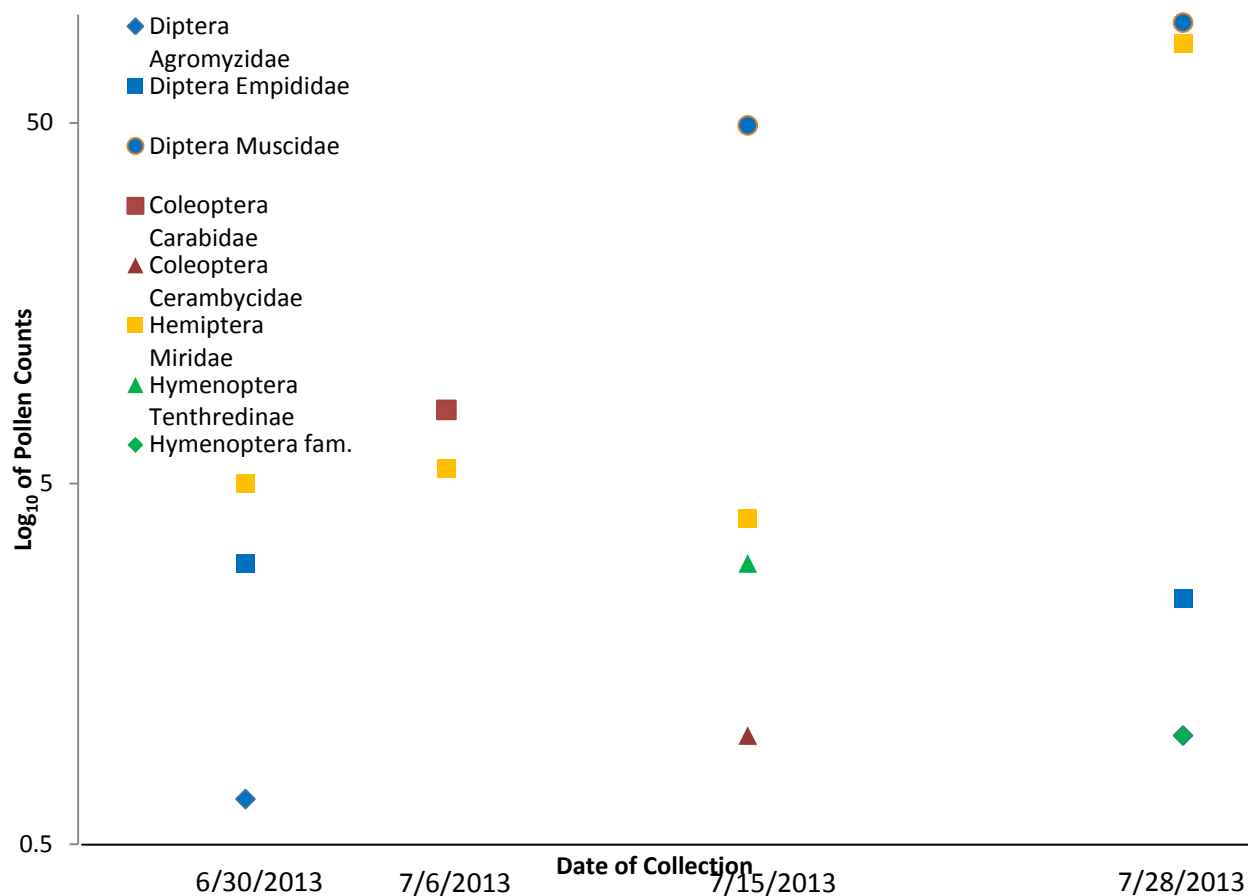


Figure 5: Logarithmic mean pollen counts by family removed from insects caught on female *V. edulis* plants during late June and July 2014. The first day of collection was at Brush Creek while subsequent collections were at Emerald Lake.