

Selection on floral traits of *Linus lewisii* along an elevational gradient

Anna DiPaola

Ken Whitney

Full-time Independent Research

Summer 2014

Selection on floral traits of *Linum lewisii* along an elevational gradient

Abstract: There is a recorded shift from bee-dominated pollinator assemblages at lower elevations to fly-dominated pollinator assemblages at higher elevations. In this study, we asked whether there are patterns of selection on *Linum lewisii*'s floral traits, and whether these patterns differ over an elevational gradient. We measured traits of *Linum lewisii* at two lower elevation sites (~2800m) and two high elevation sites (~3400m), all near Gothic, Colorado, USA. We will collect seeds from each plant in order to correlate each plant's traits with its fitness, using phenotypic selection analysis. We will also collect *Linum* seeds along an elevational gradient, grow these plants in a greenhouse, and measure their traits to determine if the observed differences in traits are genetic or environmental. Preliminary analyses of selection on traits have been carried out using counts of fruits per plant at the lower two sites. Site 1 has shown significant positive selection for height, number of stalks, and floral diameter using both selection differential and selection gradient methods of analysis. At site 2, height, number of stalks, floral diameter, and petal width are undergoing positive selection that is significant using selection differential analysis, but only number of stalks shows significant positive selection using selection gradient analysis. In order to compare selection among the low elevation and high elevation sites, as is the focus of this study, fruit and seed data must be collected for sites 03 and 04.

Introduction

Insect-pollinated plants have adapted to the selective pressure of pollinator assemblages by evolving floral traits that maximize pollen transfer to plant conspecifics, ultimately increasing an individual plant's reproductive output. Since the composition of pollinators may vary across altitude or habitat, local populations of plant species may evolve traits that are attractive to their assemblage of pollinators. Traits that are known to modify in response to pollinator-driven selection include

phenology, flower proportions, overall flower size, and floral scent (Ackerman, Melendez-Ackerman, and Salguero-Faria 1997).

Many mountain ranges show a similar pattern of changing pollinator composition as elevation increases. In some high-elevation systems in which bees are less abundant, flies fulfill a pollinating role and become increasingly abundant as the elevation increases (Ssymank et al. 2008; Larson, Kevan & Inouye 2001). Although the effectiveness of fly pollination is highly variable between species, in systems where bee pollinators are scarce, flies are a vital supplement to bees' pollination, making up for their ineffectiveness with abundance (Kearns 2001). This pattern is observed in various systems. As elevation increases in the Chilean Andes, the proportion of plants pollinated by flies and butterflies increases, although bees, flies, and butterflies all experience an overall reduction in diversity (Arroyo, Primack and Armesto 1982). A survey of montane habitats in New Zealand found that dipterans were the most abundant insects on flowers, making up 50-80% of all visits to flowers (Primack 1983). Within the order Diptera, families are represented in different proportions depending on the elevation and local system. Certain families (Anthomyiidae, Tachinidae, and Muscidae) are able to carry more pollen, so they may make up higher proportions of fly diversity arctic and alpine regions around the world (Larson, Kevan, and Inouye 2001). Research in the White Mountains of Australia found the pollination services of muscid and anthomyiid flies to be the most important, compared to other pollinators (Inouye and Pyke 1988). This pattern of fly importance at high elevations and changing species composition also applies to the Rocky Mountains in Colorado. In a study of *Linum lewisii* near Gothic, CO, rates of fly visitation and the proportion of pollen deposited by flies increased at high elevations (Kearns and Inouye 1994). An earlier study of the same area around Gothic, CO found that with an increase in elevation, overall insect abundance decreased, but the proportion of flies increased (Kearns 1992). Kearns suggested that anthomyiids, muscids, and tachinids functionally replace the small bees that were prevalent at lower elevations. She also

attributed the increase in fly importance to the decreasing in abundance of other insects (Kearns 1992).

Dominance of flies relative to other pollinators at high elevations is hypothesized to be mainly due to flies' physiological traits (Kearns 2001). First, relative to bees, flies can maintain higher levels of activity in cold temperatures (Szymanski et al. 2008). Flies also have lower energy needs than bees because flies do not collect nectar and pollen to feed a brood. Instead of collecting resources and frequently returning to a nest, flies are able to conserve energy and by resting on sunny flowers to thermoregulate with little metabolic investment (Arroyo, Primack and Armesto 1982).

The effects of pollinator-driven selection on plant characteristics are well known in various systems, but studies specifically examining potential changes in selection with the bee-to-fly-transition are rare. A study done across high elevation sites in the Rocky Mountains examined the two morphs of *Polemonium viscosum*, a sweeter-scented morph that appeals to bumblebees, and a skunky-scented morph known to attract flies, mostly Muscoid flies (Galen, Zimmer, and Newport 1987; Galen and Newport 1988). They found that the sweet-scented morph correlated with other traits that attracted bumblebees, such as longer and wider corollas. The skunky morph's corolla length and width had no effect on the rate of pollination, meaning flies aren't selecting on these corolla traits. Another study done in the Rocky Mountains determined that bumblebee-driven selection could be responsible for *Polemonium viscosum* being larger at higher elevations. (Galen 1989). In cages containing larger *Polemonium* collected from higher elevations and smaller *Polemonium* collected from lower elevations, Galen observed bumblebees preferentially visiting flowers with longer and wider corollas, and longer stem length. Galen later found that *Polemonium viscosum* populations are also known to be able to adapt to a rapid change in selective pressure from the pollinator assemblage within one generation, as shown with an experiment that excluded all pollinators but bumblebees and observed a

9% increase in corolla flare (Galen 1996). Waser and Price (1980) found at RMBL that the random mutation causing pale flowers in *Delphinium* was associated with lowered seed set, suggesting that pollinators select against pale flowers. In the seacliffs of England, bumblebees preferentially pollinated the rarer white morph of *Cirsium palustre* in times of pollen-limitation (Mogford 1974). Further investigation of variation in selection due to differing pollinator assemblages will help us better understand pollinators' role in plant adaptations.

In this study I aim to see if the difference in pollinator composition that corresponds with elevation is driving differences in selection on floral traits. The questions I pose are 1) Do patterns of selection on floral traits exist, and do those patterns vary with elevation? 2) Is there evidence of past selection, manifested as correlations between floral trait values and elevation?

Methods

Linum lewisii, or Blue Flax, was chosen because it experiences different pollinator assemblages across an elevational gradient in the Gothic area. Kearns and Inouye (1994) found that as elevation increases, there are higher rates of fly visitation to *Linum lewisii*. They also found that the proportion of pollen delivered to *Linum* by flies increases with elevation: averaging across three years of data, they found that flies deliver 3.6, 1.1, 28.5 times as much conspecific pollen to *L. lewisii* as do bees at low, middle and high elevations (data from Table 3 of Kearns and Inouye 1994). This makes it ideal for comparing pollinator-driven selection at different elevations.

Site Number	Lat	Long	Aspect (deg)	Aspect (cardinal)	Slope	Elev (m)	Elev (feet)
2	38.86135	-106.91882	166.0	South	15.1	2739.6	8988
1	38.89585	-106.88950	189.5	South	9.2	2795.4	9171
1	38.89631	-106.89041	188.1	South	17.9	2808.6	9215
1	38.89608	-106.88995	188.8	South	13.6	2802.0	9193
3	38.97731	-106.97629	208.3	SW	18.5	3463.9	11365
3	38.97756	-106.97613	206.6	SW	15.8	3473.5	11396
3	38.97744	-106.97621	207.5	SW	17.2	3468.7	11380
4	38.97687	-106.97802	172.5	South	30.0	3424.9	11237

Table 1, Site information.

All four study sites were near Gothic, CO. Sites 1 and 2 were considered low elevation sites, and were at Brush Creek. The two high elevation sites, sites 3 and 4 were located at Virginia Basin. Sampling at two sites of the same elevation allows us to compare selection at the same elevation, with hypothetically the same selective pressures from similar pollinator assemblages.

The numbers reported above for sites 1 and 3 are averages of each plot from each site. Site 1 included 70 flowers in a flat meadow and 24 flowers on a similarly vegetated hill. The 100 plants at Site 2 grew in a drier sagebrush community approximately 5955m away from site 1. Site 3, containing 100 plants was located in a subalpine meadow, with its upper plot having more sparse vegetation and being more distant from trees. At Site 4, 100 *Linum* plants were on a steeper, rocky slope in a subalpine meadow with no trees nearby. Site 3 was 216m from Site 4. These sites and plots were chosen deliberately to maximize variation within each site and between each elevation.

Four sites along an elevational gradient were sampled with 100 plants per site. All plants were left open to allow for pollination by the local assemblage. Every three days, number of flowers open were counted and seeds were checked for ripeness. Seeds were collected as they appeared to be ripe, which included seeds affected by insect predation.

To verify that the selected sites actually do follow the pattern of more flies at high elevations, I surveyed the species composition of pollinators at each site by netting 20 insects on *Linum* per site.

The traits that were measured to examine selection were number of stalks, plant height, flower color, flower number, petal length, petal width, flower depth, flower diameter, stigma length and anther length. A spectrometer was used to quantify floral color, and the flower counts appear as the “Peak flowering date” and “Average flowering date” metrics.

We used phenotypic selection analysis (PSA; Lande and Arnold 1983) in the program SAS to analyze the data. Fitness was the response variable, and the predictor variables were the traits listed above. The predictor variables were first standardized to a mean of zero and a standard deviation of one. Fitness was relativized to the mean fitness at each site. PSA was run separately at each of the four sites.

In a common-garden greenhouse experiment, 30 plants from at least two additional populations of *Linum* will be grown and the same traits measured as above. These collections will be taken from Cement Creek and Ohio River Valley. Fixing the environmental conditions of these plants will allow us to measure the traits, knowing that variation should be due to genetic adaptation that may have arisen from pollinator-driven selection.

We will use SAS to examine correlations between trait values and elevation. If such correlations exist, we will then evaluate whether the correlations match the predictions generated by our field selection analysis: e.g. if our 2014 selection analysis indicated stronger selection for larger flowers at high elevation, do we see a matching pattern where high elevation populations have larger flowers?

Note that this greenhouse experiment will be part of a longer-term continuation of the project and will not be conducted this summer.

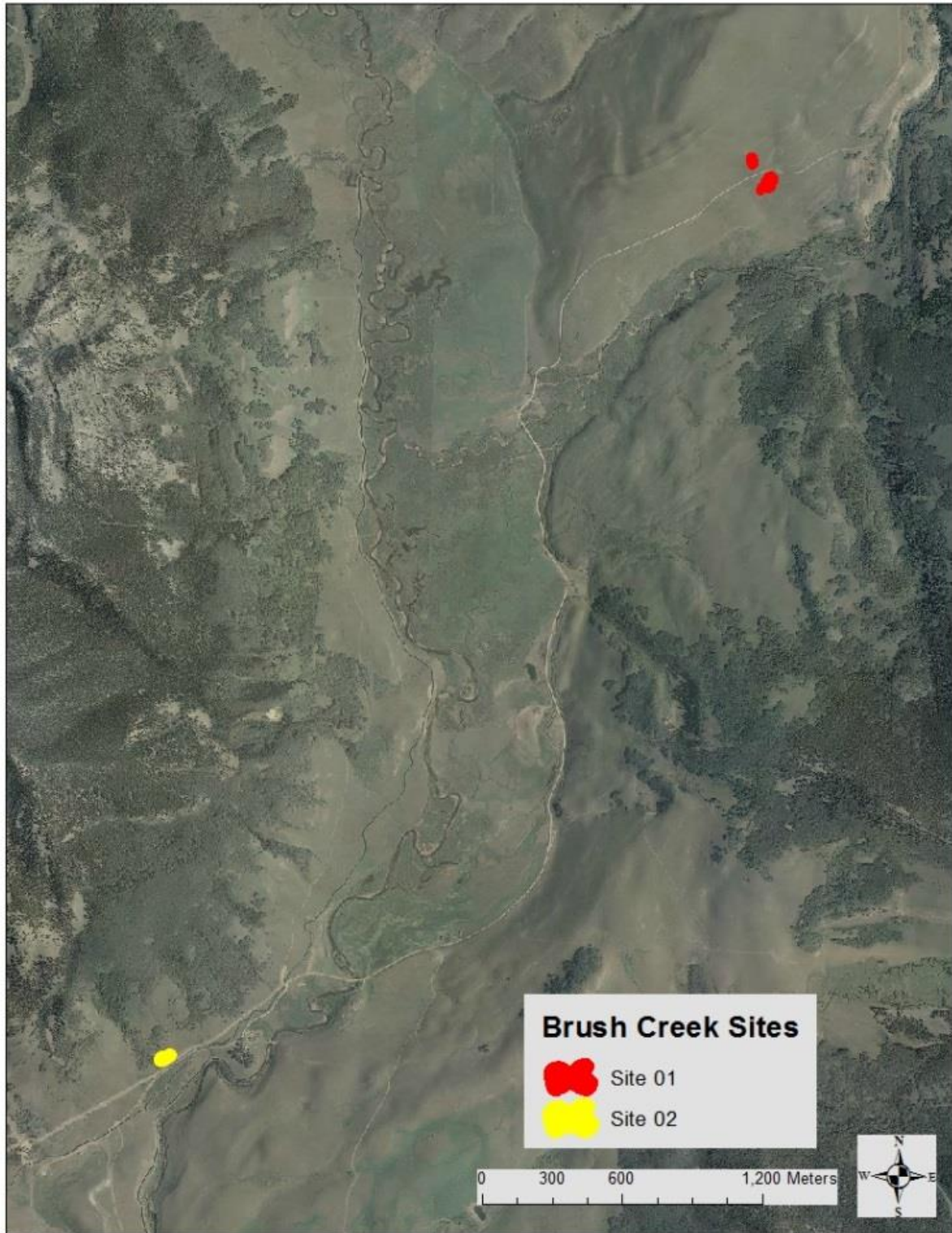


Fig. 1 Low elevation Brush Creek site

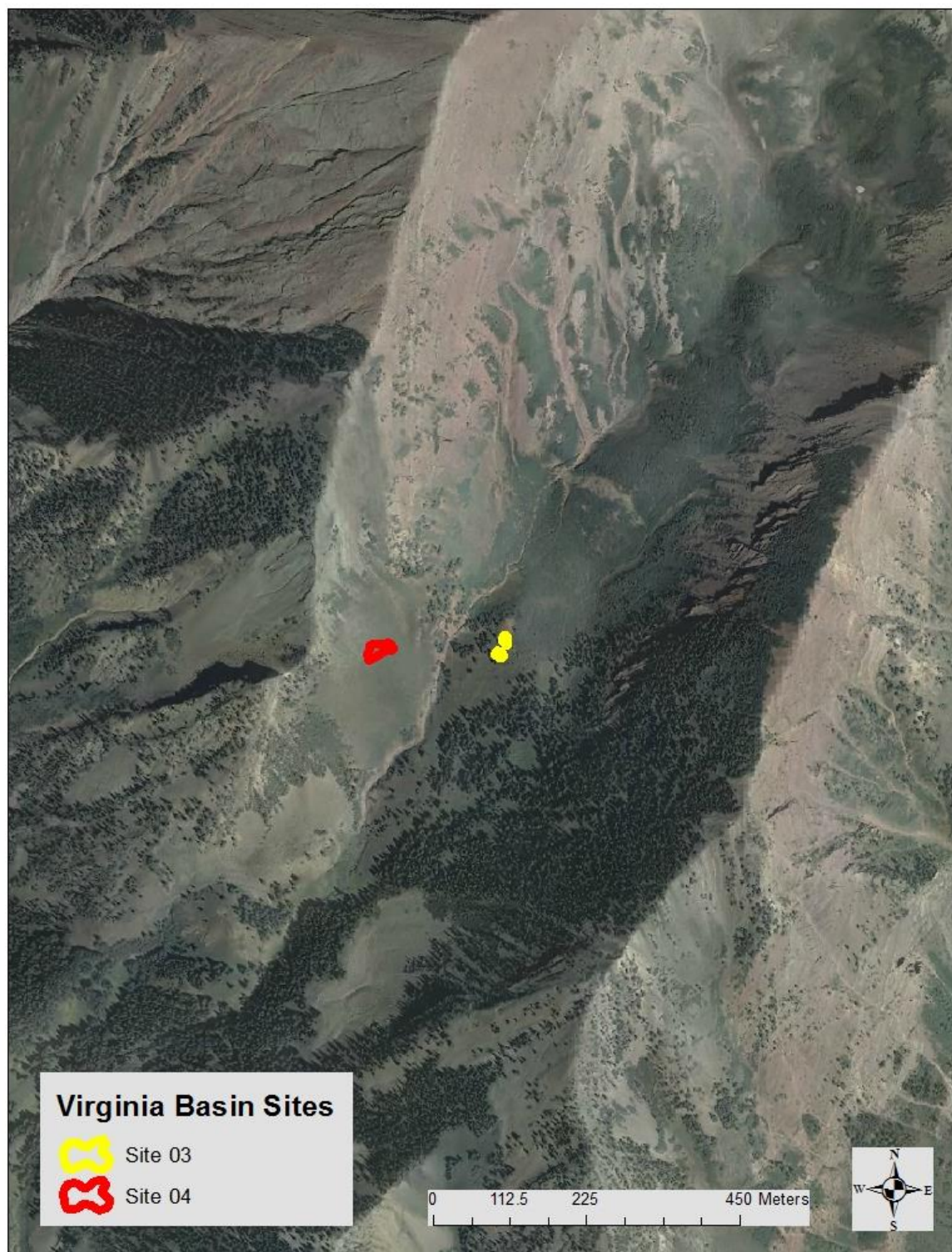


Fig. 2 High elevation Virginia Basin sites

Results

Site 1 – 2802m in elevation

Trait	S	p-value	β	p-value
Height	0.6524	<.0001	0.4583	<.0001
# Stalks	0.6708	<.0001	0.5615	<.0001
Floral Diameter	0.3731	0.0034	0.4659	0.0039
Floral Depth	0.0237	0.8564	0.0095	0.9336
Petal Length	0.2246	0.0831	-	0.2948
Petal Width	0.2007	0.1222	-	0.5176
Stigma-Anther Separation	0.0053	0.9680	-	0.0804
Average Flowering Date	0.2129	0.1008	0.0968	0.4113
Peak Flowering Date	0.1457	0.2637	0.1212	0.2770

Table 2

Site 2 – 2739m in elevation

Trait	S	p-value	β	p-value
Height	0.2986	0.0033	0.0678	0.4232
# Stalks	0.6181	<.0001	0.5857	<.0001
Floral Diameter	0.2894	0.0045	0.2215	0.0814
Floral Depth	0.0705	0.4989	0.1352	0.3125
Petal Length	0.1919	0.0633	-	0.2470
Petal Width	0.3296	0.0011	0.1220	0.2622
Stigma-Anther Separation	0.1956	0.0583	0.0143	0.8604
Average Flowering Date	-	0.8475	0.0835	0.4449
Peak Flowering Date	-	0.9479	0.0384	0.7082

Table 3

Plant height, number of stalks, and floral diameter were found to be experiencing significant positive selection when analyzing using the selection differential (Table 2). Selection gradient analysis showed that again, height, number of stalks, and floral diameter were also under significant positive selection (Table 2). Selection differential analysis showed that plants at Site 2 were undergoing significant positive selection on height, number of stalks, floral diameter, and petal width (Table 3).

However, using the selection gradient, only number of stalks appeared to be experiencing significant positive selection.

Discussion

So far, preliminary fruit counts have shown that at Site 1, taller plants, plants with more stalks, and flowers with larger diameters are being selected for using both selection differential and selection gradient analysis. At Site 2 the same trait values are possibly being selected for, but only number of stalks is experiencing direct selection according to the selection gradient analysis. While the preliminary data begins to reveal that patterns of selection do exist in the study populations at low elevations, complete seed and fruit counts are needed to compare trends in selection among the high and low elevation sites.

Although plant height is greatly influenced by resource availability, pollinators are known to select for plant height (Peakall and Handel 1993). A taller plant or flowers with large diameters may be more conspicuous and therefore receive more pollination. Similarly, a plant with more stalks may produce more flowers in total over the flowering period. More total flowers would give a *Linum* plant the opportunity to produce more fruits, so a plant with many stalks may receive more pollination and produce more fruits than a plant which only has a few stalks and only produces a few flowers.

At site 2, plant height, flower diameter, number of stalks and petal width were all found to be undergoing positive selection using selection differential analysis. However, using the selection gradient, only number of stalks was experiencing direct selection. In this case, petal width may be significant using the selection differential because it is correlated with floral diameter. Plant height, flower diameter, and number of stalks may turn out to be significant using selection gradient once

seed data is used in the analysis. Some additional traits may become significant with the incorporation of seed count in data analysis.

The second part of our study will help verify that the measured differences in traits are due to genetics. Low vs. high differences in plant traits from the observed plots could be misleading because of the differences in resources that each plant receives. Certain plants may be flourishing in a microhabitat, making it seem as if that plant has a genotype that is highly selected-for, while a plant with a very similar genotype may happen to be competing with nearby plants or be living on a drier area with less nutrients. Growing seeds from different elevations in a greenhouse with controlled environmental conditions will allow us to see how much of the measured differences in traits are due to genotype.

The impact of pollinator composition on plant traits is important to measure so that we can answer basic questions about evolution. If we see certain patterns of selection at different elevations, we may better come to understand the beginning of speciation. Climate conditions that are drastic enough to increase the disparity between fly-dominated assemblages and bee-dominated assemblages could potentially lead to speciation of *Linum*. However, if climate change causes bee pollinators to expand their range to higher elevations, the *Linum* that was previously adapted to fly-dominated pollinator assemblages may face new selective pressures. Further research must be done that involves pollinator exclusion experiments so that we can observe changes in traits directly due to changes in pollinator assemblage.

Literature Cited

Ackerman, J. D., Melendez-Ackerman, E. J., Salguero-Faria, J. (1997). Variation in pollinator abundance and selection on fragrance phenotypes in an epiphytic orchid. *American Journal of Botany* 84(10) 1383-1390.

Arroyo, M.T.K., Primack, R., Armesto, J. (1982). Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. *American Journal Botany* 69(1) 82-97.

- Galen, C. (1996). Rates of floral evolution: adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution*, 50(1), 120-125.
- Galen, C. (1989). Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* 43(4), 882-890.
- Galen, C. & Newport, M. A. (1988). Pollination quality, seed set, and flower traits in *Polemonium viscosum*: complementary effects of variation in flower scent and size. *American Journal of Botany*, 75(6) 900-905.
- Galen, C., Zimmer, K. A., Newport, M.E. (1987). Pollination in floral scent morphs of *Polemonium viscosum*: a mechanism for disruptive selection on flower size. *Evolution* 41(3) 599-606.
- Inouye, D.W. & Pyke, G.H. (1988). Pollination biology in the Snowy Mountains of Australia: comparisons with montane Colorado, USA. *Australian Journal of Ecology* 13, 191-210.
- Kearns, C. A. (2001). North American dipteran pollinators: assessing their value and conservation status. *Conservation Ecology*, 5(1).
- Kearns, C. A. (1992). Anthophilous fly distribution across an elevation gradient. *American Midland Naturalist*, 127(1), 172-182.
- Kearns, C. A., & Inouye, D. W. (1994). Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany*, 81(9), 1091-1095.
- Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution*, 37(6), 1210-1226.
- Larson, B. M. H., Kevan, P. G., Inouye, D. W. (2001). Flies and flowers: taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist* 133, 439-465.
- Mogford, D. J. (1974). Flower colour polymorphism in *Cirsium palustre*. *Heredity* 33(2), 257-263.
- Peakall, Rod, & Handel, S. N. (1993). Pollinators discriminate among floral heights of a sexually deceptive orchid: Implications for selection. *Evolution* 47(6), 1681-1687.
- Primack, R. B. (1983). Insect pollination in the New Zealand mountain flora. *New Zealand Journal of Botany*, 21, 317-333.
- Ssymank, A., Kearns, C. A., Pape, T. & Thompson, F. C. (2008). Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. *Biodiversity* 9, 86-89.

Waser, N. M. & Price, M. V. (1981). Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution* 35(2), 376-390.