

Comparing floral morphology and inflorescence structure of *Lupinus argenteus* and *L. bakeri* in sympatry and allopatry.

Noah Olson

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Dr. Jacob Heiling

RMBL

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ABSTRACT:

Differences or similarities in floral morphology can affect the interactions between plants and shared pollinator communities. As such, plant species with similar floral morphology may be competing for pollinators or facilitating pollinator attraction. Thus, pollinators mediate selection on floral morphology. In the Mountain West, two closely related species, *Lupinus argenteus* and *Lupinus bakeri*, co-occur, share flower phenology, and have similar floral morphology and inflorescence structures. However, the degree of shared floral morphology and inflorescence structure in areas where both species cooccur compared to areas of where only one, or the other, occurs is unknown. With pollinator mediated selection in mind, this leads to the question, does sympatry predict variation in floral morphology and inflorescence structure of *Lupinus bakeri* and *Lupinus argenteus* populations? We took population census data from 5 sites paired across elevation, 3 sympatric populations and 2 allopatric populations, and measure inflorescence structure and floral morphological traits to attempt to answer this question. Based upon our results we found that *L. argenteus* shows evidence of divergence when in sympatry for the two variables: floral volume and banner to wing gap angle. Which suggests that pollinator sharing between *L. argenteus* and *L. bakeri* is competitive. Furthermore, the directionality of this divergence is a trend towards lower floral volume and smaller gap angles, which also suggest that *L. argenteus* is pollinator filtering. The outcomes of our study, highlights the interactions between floral morphology and pollinator communities, which can lead to potential studies that explore the degree and direction of selection that may, or may not, be present. Furthermore, future studies could use the findings of this project to aid in assessing pollinator communities for each species of subject.

INTRODUCTION:

Plant communities demonstrate a wide range of differing flower morphologies and inflorescence structures, influencing pollinator foraging behavior (Pyke, 1984). All of which may influence reproductive success, and thus, subsequent selection on these traits. For example, in a system where all other factors are equal, but the floral density of the inflorescence is high in one flowering species and low in another. Then, pollinators may be able to maximize their foraging efficiency by favoring the species with higher floral density. If that is the case, we may expect selection against lower floral density. Thus, pollinator mediated selection may result in the evolution of higher floral density in one or both of these co-flowering populations.

Differences or similarities in floral morphology can affect the interactions between plants and shared pollinator communities as well. For example, if two plant species in the same area share flowering phenology, floral morphology, and pollinator communities then two species could be competing for pollinator attraction in their co-flowering partner. In this case, pollinator mediated selection may cause the two species to diverge in floral traits. Or, if pollinator sharing facilitates attraction and pollination success in one or both species, the opposite could occur, and floral traits could converge. For example, Schemske (1981) described pollinator sharing between two closely related understory herbs that benefitted both species. The two herbs share similar floral morphologies, habitats, and floral phenology. Furthermore, both grow at low densities. In this case, Schemske suggests that floral convergence increases the rate of pollinator visitation.

In the Mountain West, the ranges of two closely related species, *Lupinus argenteus* and *Lupinus bakeri* partially overlap (Weber & Wittmann, 2012). In the West Elk Mountains of Colorado, sympatric populations occur at certain elevations, but at other elevations they are isolated in allopatric populations, especially at elevations above 3,000 m.a.s.l., where *L. bakeri* populations become rare (Heiling, *pers comm.*). Moreover, both *L. argenteus* and *L. bakeri* are bee pollinated, pollen-rewarding, share floral phenology, and broadly have similar floral morphology and inflorescence structure. However, floral volume and banner-to-wing gap angle, both of which influence pollinator handling, are known to differ between these species; however, the degree to which these characteristics converge or diverge in areas where both species co-occur compared to areas of where only one, or the other, occurs is unknown. If they are competing for, or facilitating, pollinator attraction differentiation of such floral traits may be expected to limit or promote pollinator sharing between species. This leads to the question: *Does sympatry predict variation in floral morphology and inflorescence structure of Lupinus bakeri*

and *Lupinus argenteus* populations? A few possible selective hypotheses present themselves. First, in the null hypothesis, if there is no pollinator sharing between these species, we would expect no intraspecific differences in floral morphology or inflorescence structure between sympatric and allopatric populations of *Lupinus argenteus* and *L. bakeri*. Alternatively, if *Lupinus argenteus* and *L. bakeri* compete for pollinators, we would expect divergence in floral traits in sympatry (H1). Finally, if pollinator sharing is facilitative *Lupinus argenteus* and *L. bakeri* may converge in sympatry (H2).

The aim of this study is to compare floral traits and inflorescence structure within and between species in sympatry vs. allopatry in an effort to identify signatures of pollinator mediated selection and to determine the direction of said selection should such signatures exist.

METHODS:

Population Censusing and Measurement Gathering

During the summer of 2024, from the middle of June and to the end of July, populations of *Lupinus argenteus* and *Lupinus bakeri* were censused and inflorescence clippings were taken for floral morphology measurements, and inflorescence structure measurements. 5 sites total were used, (Table 1) for locations based on previous studies. The 5 sites consisted of 2 allopatric populations (UPL, and BVB) and 3 sympatric population sites (BRC, LERB, and MAR), that span a portion of the elevational distribution of both species. Within each site, 10, roughly 30 × 3-meter belt transects, spaced approximately 10 meters apart, terrain depending, were used to census *Lupinus argenteus* and *Lupinus bakeri* populations. This resulted in a total of 50 belt transect polygons that were censused. All sites, as well as the 50 belt transect polygons, were

marked via GPS. From each site, 20-10 total inflorescences, 10 per species, were clipped and then marked with tape to prevent resampling. This resulted in a total of 80 inflorescence clippings. The inflorescence and floral measurements included: (1) the total number of open flowers; (2) the inflorescence length, from the base of the pedicel of the bottom open flower to the base of the pedicel of the top open flower; (3) banner-to-wing height; (4) claw-to-wing length; (5) flower width; and (6) banner-to-wing gap angle (Fig 1). The first 5 viable flowers per stalk were used for measurements, beginning from the base-most flower and moving up the inflorescence. Flowers with deformities, or potential fruits were skipped.

full site name	abbreviation	N	W	Elevation (masl)
Brush Creek Trail Head	BRC	38.86367	- 106.91402	2723.6928
Lower East River Bridge	LERB	38.95225	- 106.98946	2862.986
Marmont Mesa	MAR	39.00485	- 107.03181	3128.4672
Bellview Bench - Southern Draw	BVB	39.00648	- 107.02795	3200.4
Upper Loop	UPL	38.88315	- 106.96015	~2801

Table 1: Site Locations

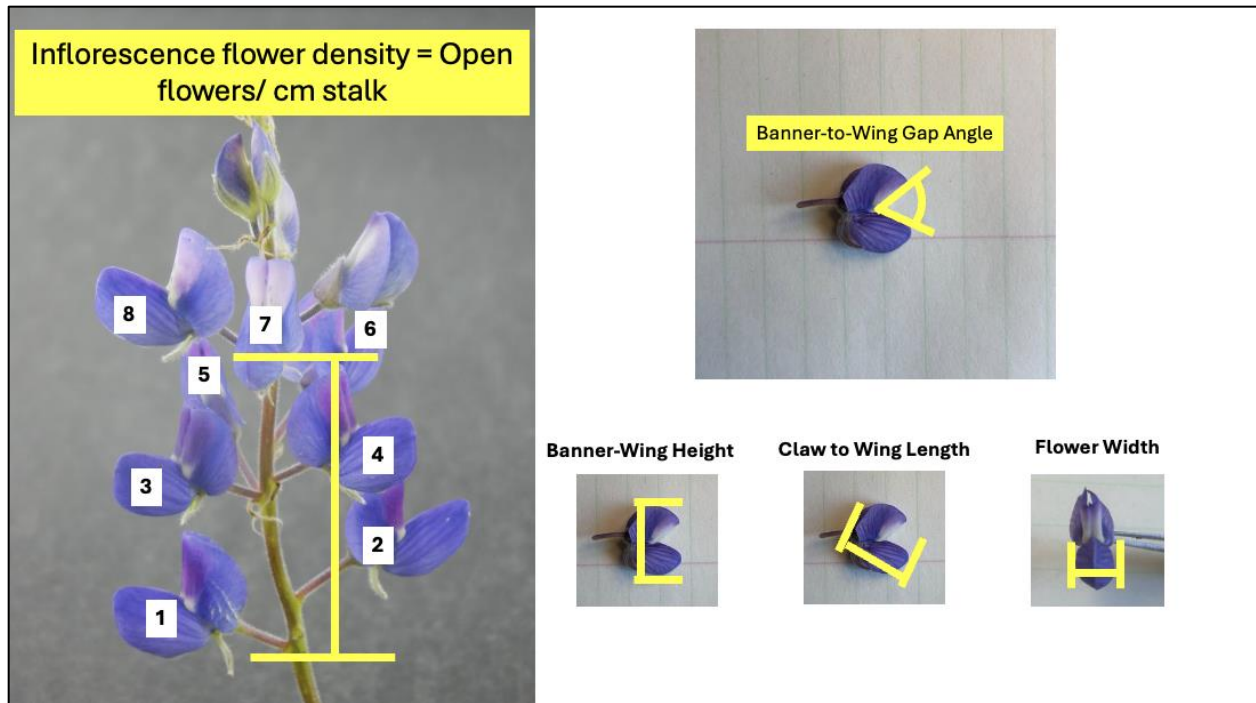


Figure 1: Floral Morphological and Inflorescence Structure Measurements

Inflorescence Structure and Floral Morphology Measurement Analyses

Inflorescence Flower Density Analysis

Inflorescence measurements, the total number of open flowers and the inflorescence length, were used to obtain an inflorescence density (flower number / inflorescence length) for each population type, either *L. argenteus* sympatric, or allopatric, or *L. bakeri* sympatric, or allopatric. We performed a one-way ANOVA to compare floral density between groups with population type (e.g., sympatric vs. allopatric) as a predictor and flower density as the response. We then performed a Tukey's HSD to compare inflorescence densities between all groups.

Floral Volume Analysis

We used the product of banner-to-wing height $\times$ claw-to-wing length $\times$ flower width to estimate floral volume, and derived the mean individual floral volume of 5 flowers per individual. Mean floral volume was then natural log transformed to achieve a normal

distribution. We performed a one-way ANOVA on a linear model with population type as a predictor and with natural log transformed floral volume as the response. Again, a Tukey's HSD was performed to compare the floral volume means between all population types.

Banner-To-Wing Gap Angle Analysis

The banner-to-wing gap angles were averaged per stalk per site and natural log transformed, as with floral volume. Then compared via one-way ANOVA on a linear model with population type as the predictor and natural log transformed banner-to-wing gap angle as the response. We then used a Tukey's HSD to compare banner-to-wing gap angle between all population types.

RESULTS:

Inflorescence Structure and Floral Morphology Results

Inflorescence Flower Density Analysis

Inflorescence flower density did not differ significantly between *L. argenteus* sympatric (mean density_{sympatric} = 3.665 ± 1.242) and allopatric populations (mean density_{allopatric} = 3.649 ± 0.743) was (Tukey's HSD $P = 1.000$; 95% CI = -0.087, 0.90), nor between *L. bakeri* sympatric (mean density_{sympatric} = 2.42 ± 0.726) and allopatric (mean density_{allopatric} = 2.129 ± 0.471) populations (Tukey's HSD $P = 0.825$; 95% CI = -0.596, 1.181). However, we did find that inflorescence flower densities differed between species, regardless of population type, *L. argenteus* populations had significantly higher inflorescence flower densities than *L. bakeri*

populations (Tukey's HSD $P = < 0.003$; 95% CI = -2.640, -0.400) (Fig. 2).

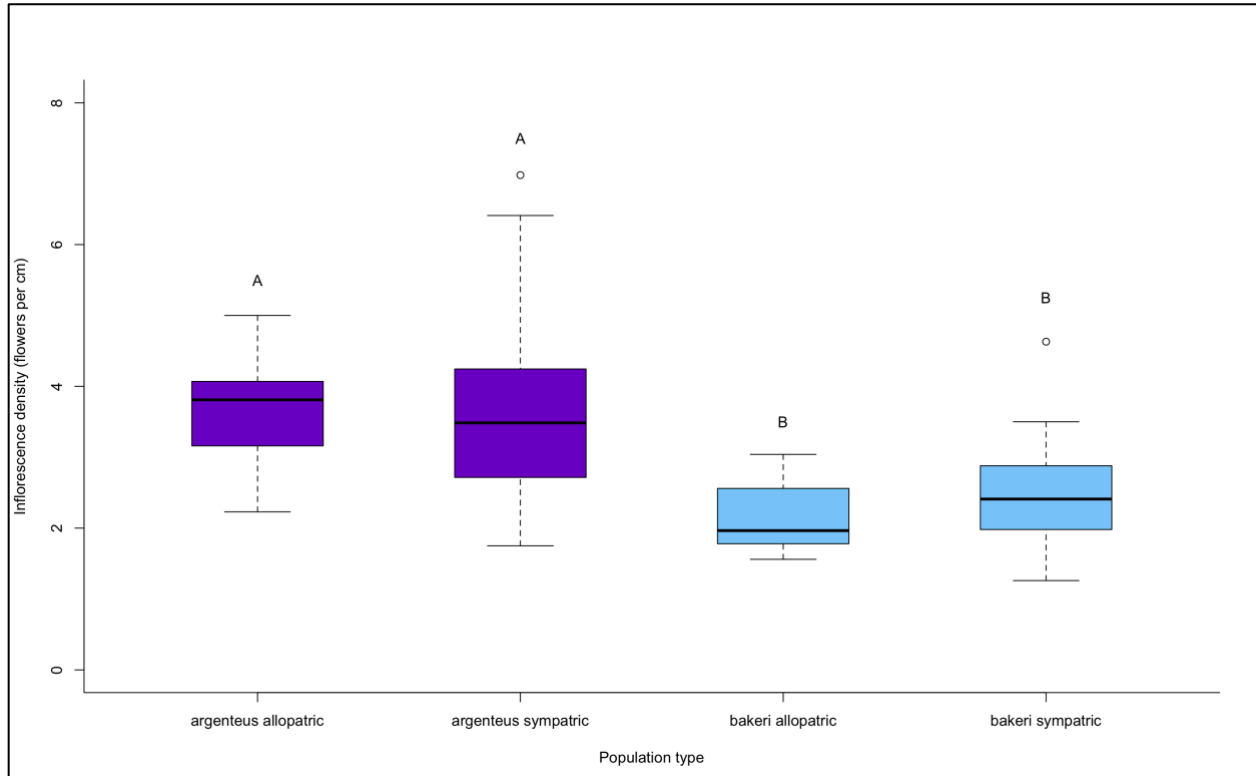


Figure 2: Inflorescence Flower Density Histogram

Floral Volume Analysis

Floral volume was lower for *L. argenteus* compared to *L. bakeri* in sympatric populations (mean volume_{allopatric} = 0.667 ± 0.155), likewise, *L. argenteus* floral volume was significantly lower in sympatric compared to allopatric populations (mean volume_{sympatric} = 0.500 ± 0.117) (Tukey's HSD $P = < 0.001$; 95% CI = 0.951, 1.377). No significant difference was found between *L. bakeri* in sympatric populations (mean volume_{sympatric} = 1.396 ± 0.252) and *L. bakeri* in allopatric populations (mean volume_{allopatric} = 1.649 ± 0.558) (Tukey's HSD $P = 0.411$; 95% CI

= -0.034, 0.087) (Fig. 3).

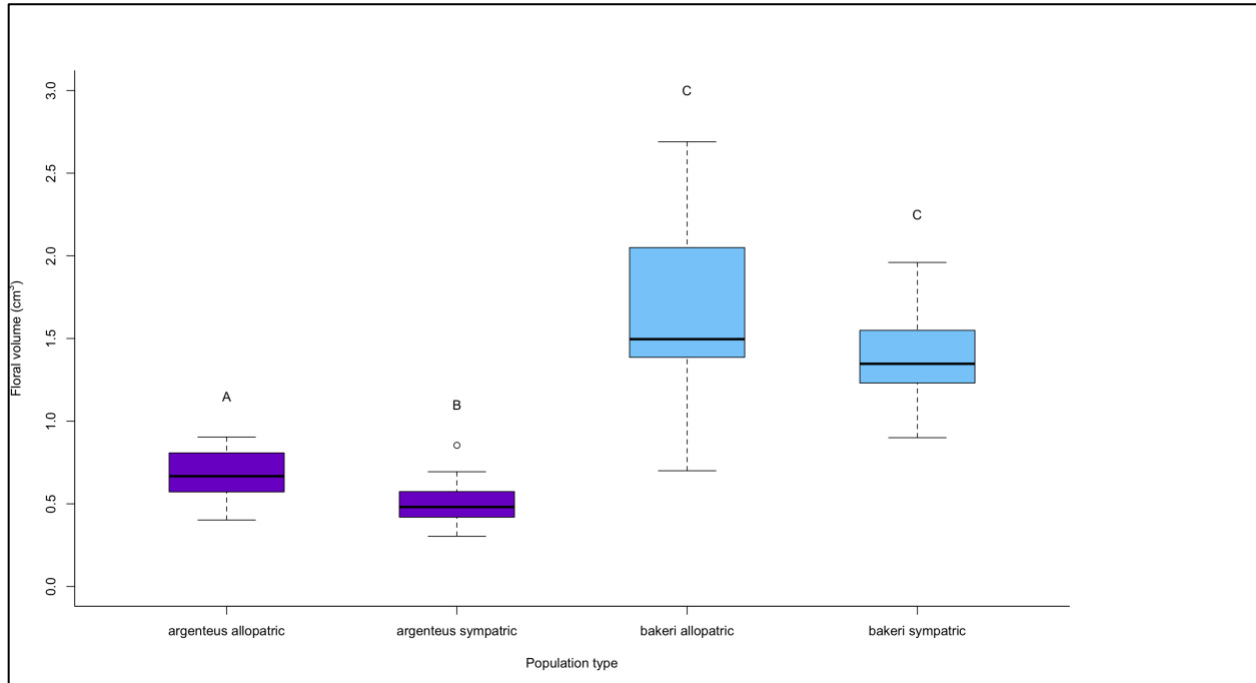


Figure 3: Floral Volume Histogram

Banner-To-Wing Gap Angle Analysis

Furthermore, we found both *L. argenteus* population types to have a more acute angle than that of *L. bakeri* for both population types (Tukey's HSD $P = <0.001$; 95% CI = 0.372, 0.615). Gap angle was more acute in *L. argenteus* in sympatric populations (mean angle_{sympatric} = 42.930 ± 9.30) when compared to *L. argenteus* in allopatric population (mean angle_{allopatric} = 54.650 ± 7.49) (Tukey's HSD $P = 0.002$; 95% CI = -0.442, -0.074). Gap angle did not differ between population types in *L. bakeri* (mean angle_{sympatric} = 69.650 ± 11.45) (mean angle_{allopatric}

$= 74.425 \pm 6.62$) (Tukey's HSD $P = 0.686$; 95% CI = -0.264, 0.107) (Fig. 4).

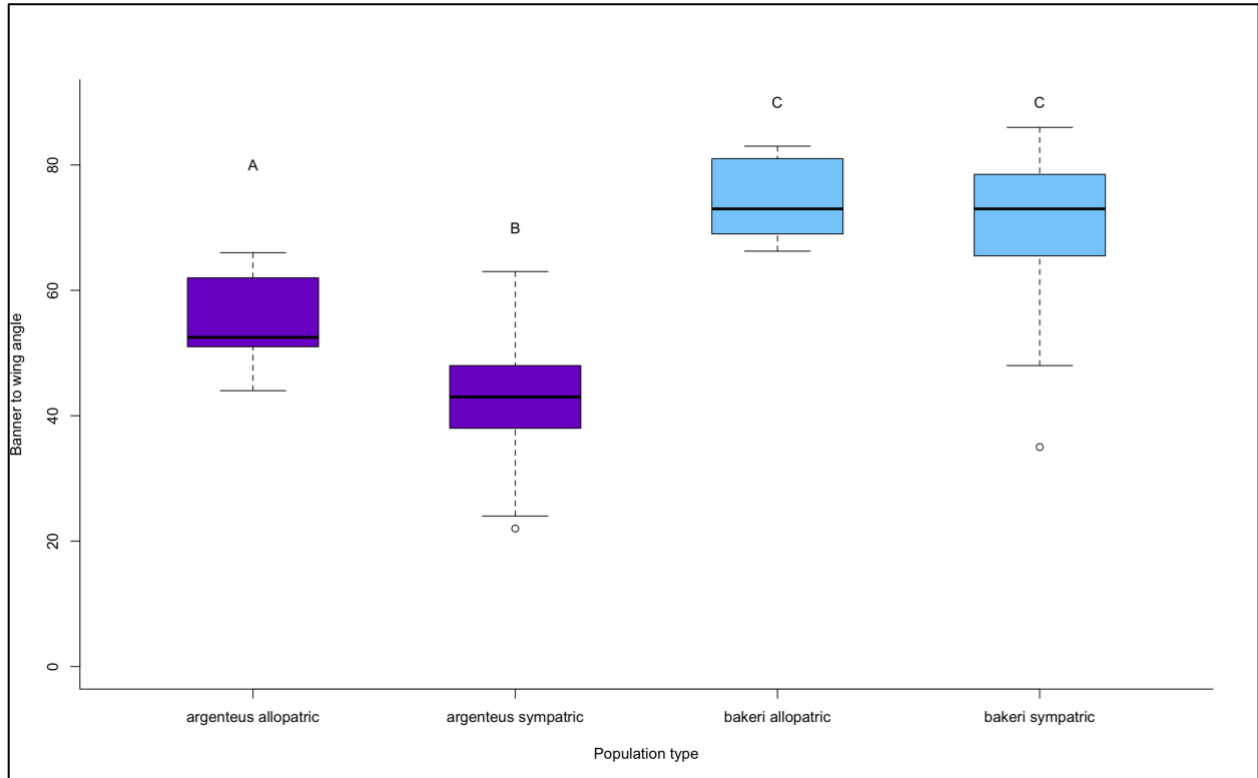


Figure 4: Banner to Wing Gap Angle Histogram

DISCUSSION:

In this study we assessed three competing hypotheses. First, our null hypothesis, if there are no shared pollinators, and no selection, then no difference is expected between sympatric and allopatric populations of *Lupinus argenteus* and *L. bakeri*. Second, (H1), if pollinators are shared and sharing is competitive, *L. argenteus* and *L. bakeri* diverge when in sympatric populations. Finally, (H2), if pollinators are shared and sharing is facilitative, then *L. argenteus* and *L. bakeri* converge in sympatric populations. We found possible evidence for divergent selection on floral morphology in *L. argenteus* as a function of co-occurrence with *L. bakeri*. There was no evidence of selection on inflorescence flower density; however, for both floral volume and banner-to-wing gap angle, there was evidence of sympatric *L. argenteus*

diverging from the *L. argenteus* allopatric populations and *L. bakeri* sympatric and allopatric populations (Fig. 3 & 4). Due to this separation of *L. argenteus*, there is evidence for *L. argenteus* in sympatric populations to be experiencing selective pressures against similarity to *L. bakeri*. Which, based upon the evidence for divergence, is consistent with H1 predicting divergence in sympatry for the two of the three variables: floral volume and banner-to-wing gap angle. Interestingly, *L. bakeri* traits did not differ between sympatric and allopatric populations. This suggests that *L. bakeri* floral morphology is not under pollinator-mediated selection in relation to the co-occurrence of *L. argenteus*. A possible reason for this lack of difference is that reproductive costs are negligible for *L. bakeri*. Yet, the difference between sympatric *L. argenteus* and the remaining populations suggests reproductive costs of co-occurrence *L. argenteus* and thus selection should favor *L. argenteus* with floral morphology that is even more divergent from that of *L. bakeri* than the morphology observed in allopatric populations. .

Potential interspecific costs could include pollen deposition costs, fertilization costs, and hybridization costs. A few examples of one of these potential costs could be if *L. bakeri* pollen has no impact on the fertilization of the ovules in *L. argenteus*, *L. bakeri* pollen deposition could block future *L. argenteus* pollen deposition and thus block viable fertilization within *L. argenteus*. Furthermore, if interspecific pollen deposition fertilization is successful then another possible fitness cost arises—hybridization (Burke & Arnold, 2001). If the two species are successful in hybridization, based upon our results, hybridization would not positively impact the fitness of *L. argenteus*. There is evidence for differentiation within *L. argenteus* populations however it is unclear the mechanisms, possibilities, and selective pressure of hybridization for *L. bakeri*. A pilot study has shown zero seed set between the two species (Heiling, unpublished data). It is possible but unlikely that hybrids do occur. Hybrids could have both direct (unviable

seeds) and indirect (generational infertility) effects on fitness. If hybridization is costly within this lupine system, then reinforcement of reproductive isolation would be expected (REF).

In the case of this study, based on previous observations, we assumed that *L. argenteus* and *L. bakeri* share a sub-set of the pollinator community. This sharing could be either either facilitative or competitive. Floral morphology is essential for both overall attraction and mechanical fit of pollinators (REF). Based upon our results of the divergence of sympatric *L. argenteus*, it appears likely that, (1) sharing is competitive and (2) *L. argenteus* is filtering the pollinator community through divergence from *L. bakeri* in sympatry. *Lupinus argenteus* in both populations, sympatric and allopatric, has an overall smaller floral volume (Fig. 3) and a more acute banner-to-wing gap angle (Fig. 4), when compared to *L. bakeri* of both populations.

Another important feature for pollinator attraction is inflorescence density, which correlates with pollinator foraging bout efficiency. In our study we found no intraspecific difference of inflorescence flower density, however; there was a significant difference between species. With *L. bakeri* having an overall lower density compared to *L. argenteus*. If the nutritional value of pollen from both species is equal, we could expect selection for a higher density when in sympatry. However, this is not observed. This could be a result of pollen amount differences a pollinator can forage in a single bout, given the larger floral volume of *L. bakeri*. Indeed, *L. bakeri* produces considerably more pollen per-flower than *L. argenteus* (Heiling, unpublished data). Thus, a higher floral volume being correlated to a lower density. So, why might there not be any differences between sympatric inflorescence flower densities? The difference between *L. argenteus* and *L. bakeri* flower densities could be due to the significant difference present between the two species. Inflorescence structure energy costs could have a neutral or negative fitness impact, whereas flower production energy may have neutral or

beneficial fitness impacts. So, in the case of *L. argenteus* the energy used for the production for more flowers could be cheaper and have less costs to fitness than the energy cost relating to inflorescence structure. For instance, *L. bakeri* has a significantly higher floral volume, whereas *L. argenteus* has higher inflorescence density, potentially representing equivalent reward volumes on a per-stalk basis. Both species could have already maximized their inflorescence densities by reaching an equilibrium between costs and benefits between floral rewards and inflorescence density.

Potential limitations of this study include the sample size, timeframe, and the absence of an assessment of pollinator sharing. The sample size we used for this study included 5 populations. From the populations a total of 80 individual inflorescences were collected. Although providing insight on what could be occurring between these populations, to have a more accurate picture of what could be happening within this lupine system a larger study sample would need to be collected. This means more individuals collected from a larger geographical range. A caveat to increasing geographical sampling is the possibility that there could be abiotic factors influencing floral traits that coincide with sympatry and allopatry but are not related to pollinator mediated selection.

Although this study highlights potential pollinator mediated selection, multiple areas within this system still need exploration. A more in-depth study to analyze the degree of selection could be done in the future. This would include increasing the study timeframe, geographical sampling size, and conducting pollinator observations, as well as analyzing the strength of selection on floral characteristics. An example of how strength of selection could be measured could include the manipulation of certain floral morphological traits, such as banner-to-gap angle, and seeing the effects of said manipulations on reproduction success, thus giving an

evaluation of fitness. Another study that could be done would be to assess the possibility and success of hybridization between *L. argenteus* and *L. bakeri*. As mentioned previously, there was unsuccessful hybridization in a pilot study (Heiling, unpublished data). However, a larger scale experimental study could yield differing results which would provide insight into the possibilities of hybridization and well as the success of hybrids if they do occur. From our results, i.e. divergence of *L. argenteus* in sympatric populations, we may infer that if hybridization is possible, it is likely costly (at least from the perspective of *L. argenteus*). Another future study includes a Network analysis, determining the degree of overlapping pollinator communities and assessing the fitness affects pollinators have across elevation. This would also include measuring and analyzing the morphology of pollinators, and their effectiveness on both lupine species.

In all, our results are consistent with the idea that pollinator sharing is competitive in this system, alluding to the possibilities of divergence of sympatric *Lupinus argenteus*. Although our study had its limitations due to sampling size and timeframe there is still evidence showing selection within this system, specifically regarding pollinator-mediated selection, such as the possible presence and directionality of selection and population differences in the strength of said selection. This study also highlights the possible importance of floral morphology on pollinator attraction and pollinator mechanical fit, as well as potential pollinator behavior impacts on floral morphology. Future studies could benefit by expanding the timeframe, sampling size, and analyzing the pollinator communities, which could have implications for species interactions and evolutionary studies.

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