Investigating Sources of Phenotypic Variation in the Perennial Legume, *Lupinus polyphyllus*

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When developing new crops, breeders survey naturally occurring morphological variation and select individuals with desirable traits to found future generations. Domestication occurs as these traits are intentionally selected for over many generations. One organization that is actively trying to domesticate new plant species today is **The Land Institute** (Salina, KS). Scientists at the Land Institute seek to develop a sustainable form of agriculture that mimics natural systems: **perennial polycultures** (Wes Jackson 1980). However, herbaceous perennial plants that might be used in a perennial polyculture have not been developed.

Lupinus polyphyllus (Fabaceae), a perennial herbaceous legume species, is one candidate crop species, and some efforts to domesticate it are already underway (Kurlovich 2002). Extensive morphological variation has been documented in *L. polyphyllus*, but the evolutionary processes underlying the geographic patterns of variation are not well understood. Here, we investigate vegetative and reproductive traits of this species using herbarium records to shed some light on their evolutionary background.

Left. MOBOT Herbarium collection of *L. polyphyllus* (WI) & a patch of *L. polyphyllus* growing in Colorado.

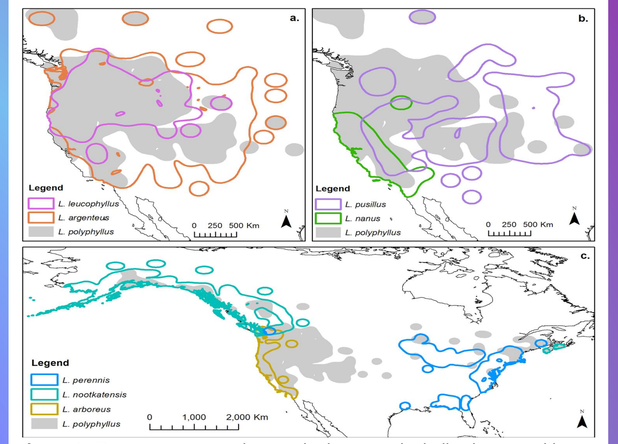

Figure 1. Range maps. Each map depicts *L. polyphyllus* in grey with **a.** two closely related perennial species, **b.** two annual species, **c.** three more distantly related perennial species in this study.

Does *L. polyphyllus* exhibit more morphological variation than the rest of the genus?

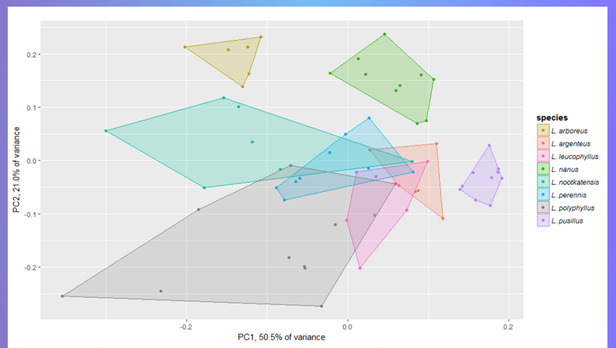

Figure 2. Principal Components Analysis (PCA) of morphological variation in eight *Lupinus* species. The two largest factors determining PC1 were leaflet width and leaflet number, and for PC2 it was seeds per pod and internode length.

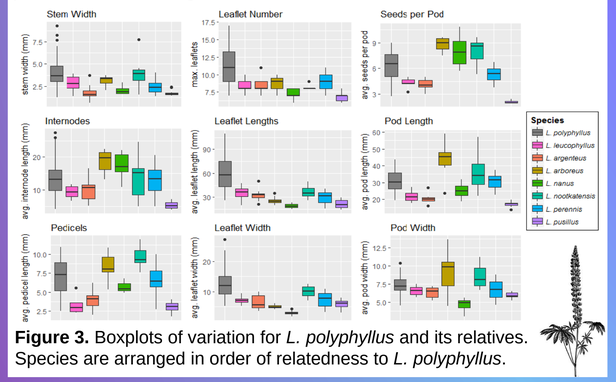

Figure 3. Boxplots of variation for *L. polyphyllus* and its relatives. Species are arranged in order of relatedness to *L. polyphyllus*.

Methods: For each specimen, other co-occurring (within 100km) lupinus species were recorded. Boxplots were made of 4 representative traits to visualize data (Figg. 4), and an AOV was run. Only trait/species combinations with significant differences between sympatric and allopatric *L. polyphyllus* were used. If the sympatric mean for a trait was between the two species allopatric means that difference was recorded as a signature of hybridization / convergence (Table 1).

Range overlaps

Background: The perennial section of the genus *Lupinus* is unresolved taxonomically, and multiple authors attribute this confusion to frequent hybridization between species (Dunn 1966, Isely 2002, Kurlovich 2002).

Hypothesis: Morphological variation in *Lupinus polyphyllus* is due to hybridization with other species of North American perennial *Lupinus*. I predict that in areas of sympatry, vegetative and reproductive traits will be intermediate to both parental phenotypes whereas in allopatric areas, traits will be distinct.

Results:	Life history (Drummond et al. 2012)	Aprx. date divergence from *L. polyphyllus* (Drummond et al. 2012)	Evidence found for:	
			Hybridization / convergence	Character displacement
L. arboreus	perennial	~ 2.5 mya	X	X
L. argenteus	perennial	~ 1.4 mya	X	
L. leucophyllus	perennial	~ 1.0 mya		
L. nanus	annual	~ 3.9 mya		X
L. nootkatensis	perennial	~ 4.2 mya	X	
L. perennis	perennial	~ 4.2 mya		X
L. pusillus	annual	~ 9.0 mya	X	X

Conclusions: There was no trend in where hybridization / convergence signatures were found. Going forward, **genetic work** should be done on those species pairs that showed possible signs of hybridization and introgression to differentiate between convergent evolution and actual gene flow. Signatures of character displacement were slightly more common in distantly related species pairs.

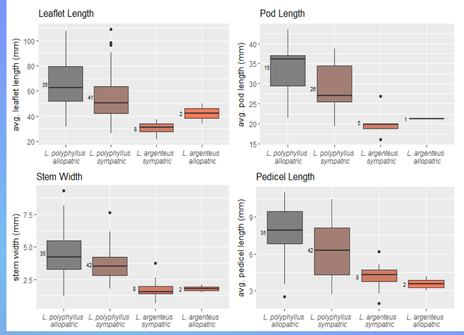

***Figure 4. Boxplot comparison of morphological traits for L. argenteus* x *L. polyphyllus*.** Intermediary traits of *L. polyphyllus* in sympatric areas suggest potential hybridization.

Climate

Hypothesis: Vegetative and reproductive structures are correlated with climatic conditions. I predict that sizes will be smaller in stressful environments such as high elevations, temperature extremes and little rainfall.

Results: Significant correlations were found for elevation, annual temperature, latitude and mean diurnal range.

Conclusions: In terms of crop development, this species shows promise for growth in colder areas that are not traditionally farmed. This is because annual temp. is positively correlated with leaflets, but does not seem to affect pods, so there would not be yield reductions at lower temperatures. In addition, pod length actually increased at higher latitudes. Further work investigating these four significant correlations, especially **common garden experiments** to determine if changes are due to phenotypic plasticity or fixed genetic changes.

Methods: For each coordinate, corresponding climate information was downloaded from the BioClim dataset. Climate variables were plotted against representative variables and linear regression analyses conducted (Fig. 5).

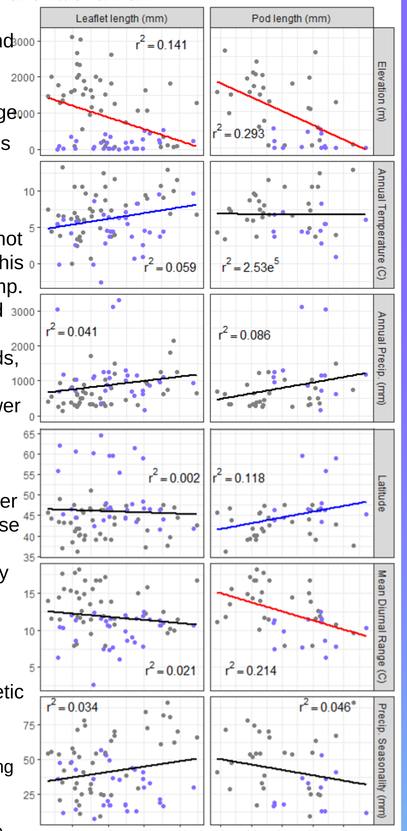

Figure 5. Correlations of one vegetative (leaf length) and one reproductive trait (pod length) with climate variables. Red line = sig. negative, blue line = sig. positive. Grey dots = *L. polyphyllus* in native range, purple dots = *L. polyphyllus* in non-native range.

In natural populations of *L. polyphyllus*, is there any evidence that increased reproductive output is associated with concomitant tradeoffs in vegetative structures?

Tradeoffs

Background: One theory as to why perennial herbaceous crops have not yet been developed is that there are significant tradeoffs between reproductive output and vegetative growth, so breeders cannot select for both.

Hypothesis: *Lupinus polyphyllus* will exhibit tradeoffs (negative correlations) between vegetative and reproductive traits and these relationships will be similar in other perennial *Lupinus* species.

Results: No evidence for tradeoffs found in *L. polyphyllus* (Fig. 6)

Conclusions: *Lupinus polyphyllus* makes an excellent crop candidate, as breeders should not see reduction in yields due to the perennial crop developing larger roots and stems. However, this property seems to be unique to *L. polyphyllus* out of the *Lupinus* analyzed in this study. Other perennial *Lupinus* had negative correlations between leaflet number and reproductive output. Annual species analyzed had even greater tradeoffs. What about *L. polyphyllus* allows it to avoid tradeoffs?

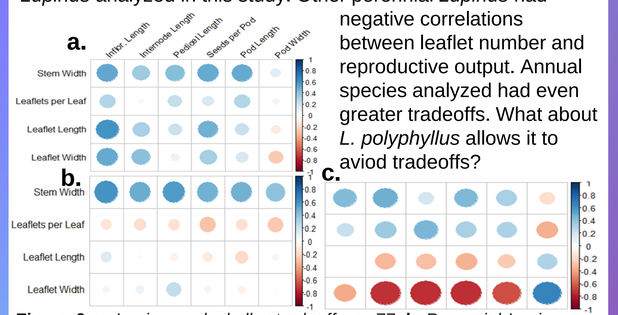

Figure 6. a. *Lupinus polyphyllus* tradeoffs, n=77. **b.** Perennial *Lupinus* tradeoffs. *L. arboreus* (n=11), *L. argenteus* (n=10), *L. leucophyllus* (n=9), *L. nootkatensis* (n=10) and *L. perennis* (n=20). **c.** Annual *Lupinus* tradeoffs. *L. nanus* (n=10) and *L. pusillus* (n=10).

Acknowledgements: MOBOT REU program, Miller lab personnel (Laura Klein, Sterling Herron, Summer Sherrod) for ideas help and explanations.

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