

## First Steps of Diversification? Relationships Among the Many Varieties of Pacific Lupine

Washington and Oregon are home to many species of native lupine (*Lupinus*), a striking group of flowers that have beguiled gardeners and botanists with their beauty, and bedeviled taxonomists with their complex and ambiguous relationships. Western North America has been identified as a center of diversity for perennial lupines. This means that this group has rapidly evolved many species compared to perennial lupines from other regions; and the processes of diversification may be continuing today. Western North America itself is a fascinating region to study evolution and diversification due to its varied geography, abundance of different ecoregions, and dramatic history of geographic and climatic change.

As a botanist and a student interested in the processes affecting and driving the diversification of species, I was happy to turn to lupines as an enticing system. The particular species I chose to study goes by many names, including Pacific, Prairie, Alpine, Dwarf, Desert, Dry Ground, or Tidy lupine.



*Lupinus lepidus* var. *aridus*, a variety of dry regions east of the Cascades, this photo was taken near Goldendale, WA. © 2008 Kevin Weitemier

However, I know it best as *Lupinus lepidus*. (Although botanical nomenclature only allows one name to be ‘accepted’, members of this species have in the past been known under *many* different scientific names.) Part of the reason this lupine has earned so many names is that this species has incredible morphological and ecological diversity: alpine members, for example, have a tight clump of foliage with a small inflorescence of blue to purple flowers suspended above the leaves; members from the dry side of the Cascades come in at least three varieties ranging from much smaller than the alpine variety with flowers held deep within the clump of leaves, to forms that are knee-high or larger with long racemes of flowers. Some members live in the moister areas of the Willamette Valley or the Puget Trough and are taller, not as clumped, and may have more leaves along the flowering stem. The differences between these forms are so great that, in the past, most of the forms were named as separate species. But then observant botanists found many intermediate populations that they couldn’t place neatly into one group or another, so today all of these forms are named as different varieties within the species *Lupinus lepidus*.

*Lupinus lepidus* ranges from British Columbia through central California, is found on both sides of the Cascades, and inhabits the Great Basin as far east as Wyoming and Utah. Three varieties occur within Washington: the moist valley variety *lepidus*, the alpine variety *lobbii*, and one of the arid varieties *aridus*. These varieties also occur in Oregon along with varieties *sellulus* (a southern, generally montane variety), *utahensis* (a minute variety of the Great Basin), and two narrow endemics: *ashlandensis* and *cusickii*. Three more varieties are found in California and Nevada.

I was intrigued both by the morphological diversity of this species and by its distribution along the top of, and on either side of the Cascades. While researching the biogeography of the region, I found that there had been numerous studies investigating the Pacific Northwest region as a whole, but few that focused on the Cascades themselves, and those that had usually involved organisms found only on one side of the range. An important study I did find investigated the genetics of seven plants distributed along the Cascade axis. The authors found that in each species there was a north-south disjunction, such that northern populations had less genetic diversity than southern populations, and the genes that were present in the north were generally different than those in the south (Soltis *et al.* 1997). They attributed this pattern to the effect of glaciation during the last ice age: While the glaciers were present, the northern populations would have been wiped out, but as the glaciers receded northern areas would be recolonized. The authors hypothesized that it was the recolonization that produced the observed disjunction, and that the recolonization could have occurred in one of two ways: the remaining southern populations may have been the only plants left, and as the glaciers receded the species slowly expanded northward; alternatively, there may have been one or a few populations that were able to remain in the north during the glaciation which then provided the material to recolonize the northern regions (these are known as refuge populations, and several areas have been proposed as potentially suitable sites).

*Lupinus lepidus* began to look like a good candidate to ask two questions related to diversification: What are the relationships between the different varieties of this species,

and does it display the same north-south pattern as seen in other Cascade axis plants?

To answer these questions I obtained samples from *L. lepidus* populations along the Cascades, from Mt. Baker to Mt. Lassen in northern California, along with populations east and west of the Cascades. I mainly focused on *L. lepidus* var. *lobbii*, the alpine variety, due to its presence along the length of the Cascades, but included samples from most of the other varieties as well. I then examined the genetic material from these collections using two methods.

The first method, DNA sequencing, can provide good information on how genes are related to each other, and for groups that have been separated for an extended period can provide a history of those separation events. However, DNA sequencing focuses on areas of the genome that might mutate too slowly to manifest differences between newly separated populations. In this case newly separated groups may share identical sequences and it would be impossible to tell if the groups are still exchanging genes or newly isolated.

The second method, known as a microsatellite analysis, also examines genetic material, but in a very different way. A microsatellite analysis examines many separate locations in the genome, and at each location measures a length. This isn't done on just a single member of a population, but on a sample of many individuals from each population. Microsatellites are thought to be invisible, or neutral, to natural selection, so different lengths can exist in a population, even if the environment is the same. By comparing the sets of lengths found in each population, a measure of similarity can be calculated. While it isn't necessary for mutations to occur to distinguish



*Lupinus lepidus* var. *lobbi*, the alpine-subalpine variety. © 2009 Kevin Weitemier

separated populations, length mutations do occur, and at a much higher rate than the mutations generally found in DNA sequencing. This provides added sensitivity to detect separation between groups. Since it was unknown if *L. lepidus* varieties had been separated for a long time, or were still exchanging genes, I employed both DNA sequencing and microsatellite analysis.

I found that despite the strong morphological differences within this species, the varieties are very similar genetically: For

both the DNA sequencing and the microsatellite analysis most of the genetic diversity was found between individuals in a population or between populations, with only a very small percentage found between varieties. Given the morphological differences between varieties (and some indications that those differences have a genetic basis) it is somewhat surprising to find little varietal genetic differentiation. These results indicate that gene flow between varieties is either still occurring, or has stopped only very recently. Natural selection in the different environments occupied by each variety may be selecting for specific genes that control the morphology of the plant, but leave most of the genome free to mix between varieties. In this situation the different entities can be referred to as 'ecotypes,' which have been documented in other plants of the region (e.g. yarrow, *Achillea millefolium* [Ramsey *et al.* 2008]).

There may be little genetic differentiation between *L. lepidus* varieties, but what about the north-south differences found for other plants of the region? The DNA sequencing results did not show any strong differences between northern and southern groups of *L. lepidus*. (They were, in fact, very similar, with some identical sequences being shared between groups.) The microsatellite analysis, on the other hand, did show a very strong distinction between far-northern populations (Mt. Rainier and north) and southern populations, including unique mutations (lengths) in the northern group.

What can account for the different results between the two tests? Recall that the microsatellite analysis examines regions of the genome that mutate at a much higher rate than regions used in the DNA sequencing, and that

there are two hypotheses for how plants recolonized northern areas: expansion from the large southern population, or expansion out of a northern refuge population. It turns out that one of these, the northern refuge hypothesis, includes a period that is well-suited to accumulating mutations. While the small refuge population is separated from the southern population it can accumulate novel mutations, and these mutations will then be passed to the areas that get recolonized. Finding novel mutations with the quickly-mutating microsatellite analysis, and none with the slowly-mutating sequencing analysis, agrees well with a scenario where a northern population of *L. lepidus* was separated from southern populations for a brief period of time (on an evolutionary time scale).

The microsatellite analysis was able to provide additional clues to the biogeographic history of *L. lepidus*: Patterns in the data indicate that the Oregon and Washington regions were colonized rapidly from California, where this species probably originated. This original colonization (taking place before the last ice age) probably occurred independently on either side of the Cascades, with little mixing between the two branches.

Applying DNA sequencing and microsatellite analysis to *Lupinus lepidus* has uncovered detectable genetic patterns within this protean group. These patterns, however, reflect geographic and historical trends much more than the morphological trends that might be expected. The morphological groups are probably best described as ecotypes, and should retain their rank as varieties within *L. lepidus*. Since differential natural selection among the varieties may be acting on specific areas of the genome (that may not be picked up



*Lupinus lepidus* var. *lepidus*, this variety is from mesic regions west of the Cascades, although it may be nearly extirpated from the Willamette Valley. © 2009 Kevin Weitemier

by these tests) restoration efforts involving reintroductions should probably use material from the same variety that's being restored. This is relevant to var. *cusickii* (unfortunately not sampled for this study), var. *ashlandensis*, and perhaps in the future var. *lepidus*, which has been almost entirely extirpated from Oregon (although is more stable in Washington's Puget Trough).

This study has begun to uncover the answers to the exciting question of diversification in *Lupinus lepidus*, but even before those answers are fully formed, new questions arise to entice the investigator: What other organisms display a similar pattern of

spread along the Cascades? What traits are being selected between the varieties? How much gene flow is there between them? The answers to these and other questions will surely raise more questions of their own. The knowledge that such puzzles exist only adds to the beguiling nature of these flowers, and happily, one does not need to know the solutions in order to be drawn in by the beauty and charm of our western lupines.

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