

*Multi-Intron Analysis of *Aquilegia flavescens* Across the Central and Northern Rocky Mountains*



*Peter Billman and Dr. Matt Lavin,
Montana State University-Bozeman*

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The field of phylogenetics studies the relationships among groups of organisms to give insight into the evolutionary processes that have taken place. Phylogenetics, and more specifically gene flow, has not previously been a focus of research across populations of *Aquilegia flavescens* before. The following research examines the relationships between populations of the yellow columbine (*Aquilegia flavescens*) plant across the Central and Northern Rocky Mountains. We examined and compared 46 distanced populations and their genetic variance in trnL and ITS intron regions to better understand connectivity among populations.

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SPECIES OF INTEREST-

The yellow columbine (*Aquilegia flavescens*) is a perennial native plant to the mountainous regions of western North America, with a range extending from British Columbia to Utah. Commonly found in open conifer forests, talus slopes, and wet meadows in montane and subalpine zones, this plant grows 20-70 cm tall.

The nodding flowers are pollinated by both bumblebees and hummingbirds (Bastida *et al.* 2010). Dispersal occurs when the seed drops from the dried follicle directly below the plant, and it then is subjected to abiotic factors such as wind, snow melt, and animals

BACKGROUND-

Evolutionary events in the lives of plants include isolation, genetic mutations, migration, and genetic drift. These events are dependent not only on the biotic factors of the plants, but also the abiotic factors such as migratory boundaries (i.e. mountain ranges). *Aquilegia flavescens*, the yellow columbine, is an example of a species not well adapted for long distance dispersal.

The family *Ranunculaceae* consists of 60 genera and comprises an estimated 1,700 species flowering plants globally. The genus *Aquilegia*, referred to as columbines, contains approximately 75 species distributed across the Northern Hemisphere.

The genus emerged 6.18-6.57 million years ago and reached North America 1.4-5.0 million years ago (Bastida *et al.* 2010). *Aquilegia* most likely originated in Central Asia, as genetic evidence

Pollinator Syndromes and *Aquilegia* Species ...

Each species of *Aquilegia* has its specialized pollinators which prohibits most interspecific hybridization (Tang *et al.* 2007).

Speciation of columbines in North America and Eurasia is hypothesized to have occurred due to pollinator shifts and species extinctions. Each pollinator (such as bumblebees, hummingbirds, and hawkmoths) is subject to their own ability to travel long distances between populations, climatic limits such as temperatures, and other geographical barriers (Brunet *et al.* 2012). Shifts in these pollinators' ranges may isolate and fragment *Aquilegia* species for extensive periods of time causing speciation to slowly occur. Pollinator extinctions also may also affect genotypes and more importantly phenotypes of species on the landscape.

demonstrates this region forms a basal group (Hodges and Arnold 1994). Once established in Asia, *Aquilegia* subsequently migrated to Europe and North America where it experienced many radiation events (Hodges and Arnold 1994).

Being a genus of recent and rapid divergence, *Aquilegia* species have not been given ample time to gain many mutations in their DNA; therefore, many of these species can interbreed freely (Hodges and Arnold 1994). Speciation was likely related to reproductive isolation due to pollinator syndromes, alongside diversification of nectar spurs (Bastida *et al.* 2010).

METHODS-

During the summer months in 2014 and 2015, I hiked to subalpine-alpine regions that were historically documented for containing *Aquilegia flavescens* populations on the Consortium of Pacific Northwest Herbaria's website (<http://www.pnwherbaria.org>). Several sites were selected that appeared to have suitable climate, terrain, and elevation (but weren't yet noted in the websites database for having *Aq. fl.*) and these areas were also sampled to complement the previously documented sites.

I collected healthy tissue from one basal leaf per plant. These leaves were then immediately placed in envelopes and pressed to preserve the DNA. 46 populations were sampled across the U.S. and Canadian Rockies and one sample of *Aquilegia coerulea* was used as an outgroup. The *A. coerulea* sample was collected from the Wind River Mountains of Wyoming. Glacier National Park in Montana was generous enough to allow me to collect three leaves from plants that were grown at their greenhouse location. These plants were grown from seeds collected near the Big Bend area of the park. The University of British Columbia (UBC) in Vancouver allowed me to obtain leaf tissue from seven herbarium-pressed specimens that spanned across the Canadian Rockies. Without their assistance, we would not have been able to analyze this expansive region of the continent that we weren't able to access in only two summer seasons.

Back in the lab, we isolated the DNA utilizing a DNEasy Plant Mini Kit (Qiagen, Valencia, CA, USA). We used the instructions included in the kit to isolate and amplify the DNA. trnL primers and ITS 6 and 9 primers were then added and the polymerase chain reaction (PCR) was ran. The samples were then sent off to Washington state to be sequenced.

We analyzed the sequenced DNA using Sequencer and Se-Al software after we received the DNA back.

RESULTS-

The ITS region of all samples sequenced was conserved with no mutations. After isolating samples from 2014's field season, we noticed that one sample in south central Montana (Bridger Mountains near Bozeman) and one sample in the Swan Range (Clayton Lake, Jewel Basin) had similar mutations to one another and these mutations were distinct from all other samples. We decided to rerun these samples in 2015 and found that these mutations do not actually exist and there was a human or sequencer error originally.

The trnL intron region, although usually conserved through generations, showed several variations of mutations. The most notable mutation occurred in the form of a 26 base pair (bp) deletion depicted as red (derived/deleted) vs. black dots (ancestral/not deleted) below in Figure 1. The base pairs lost were TGATTGGGTTGGGCCTTAATTATGGA as seen in Figure 2. *Aquilegia coerulea*, the outgroup species, was documented as black in Figure 1 which is ancestral DNA, which supports that the 26 bp event was a deletion event and not an addition since two addition events would have had to occur, one in each species. This deletion event appears in a northeastern and southwestern fashion. The cause of this genotypic variation is unknown. It is not split by the continental divide, nor the UBC samples vs. fresh samples, nor by pollinator differences. A key point to make is that the black dot in Waterton National Park, Alberta was a UBC sample collected in 1969 and if sampled today, potentially may be red/derived DNA type now. This large deletion

likely occurred in one event and spread from that individual or population long ago. A note to remember is that most of the red dots in Canada and the Yellowstone region were covered by ice sheets 15,000-25,000 years ago and those areas had no plant life for those 10,000 years.

Two other variations in the DNA occurred in the trnL region and occurred Utah (Figure 3. and noted as green and maroon). The maroon variation was a single nucleotide substitution of a cytosine to an adenine. The green dot variation in Figure 1 and 3 is a five base pair deletion (AAGGA). This site is the furthest south documented population of *Aquilegia flavescens* and is in an aspen-conifer grove in the rolling hills of the Wasatch Plateau. The likely cause of these mutations is unknown as well.

APPLICATION AND CONCLUSION:

Further research should be conducted on the region between derived and ancestral genotypes (black vs. red dots) to better understand where the boundary lies and what may have caused these two types of DNA to persist across the landscape. Sampling should also occur north of Banff National Park, Alberta to better map the specie's range and genotypes more north. Other intron and exon regions of *Aquilegia flavescens* should also be sequenced and analyzed to understand whether or not this may be the start of cryptic speciation event. If this is indeed a cryptic speciation event, mapping the genotypes on the landscape may allow botanists and paleoecologists to better understand how plants historically shifted under changing climate regimes

FIGURES:

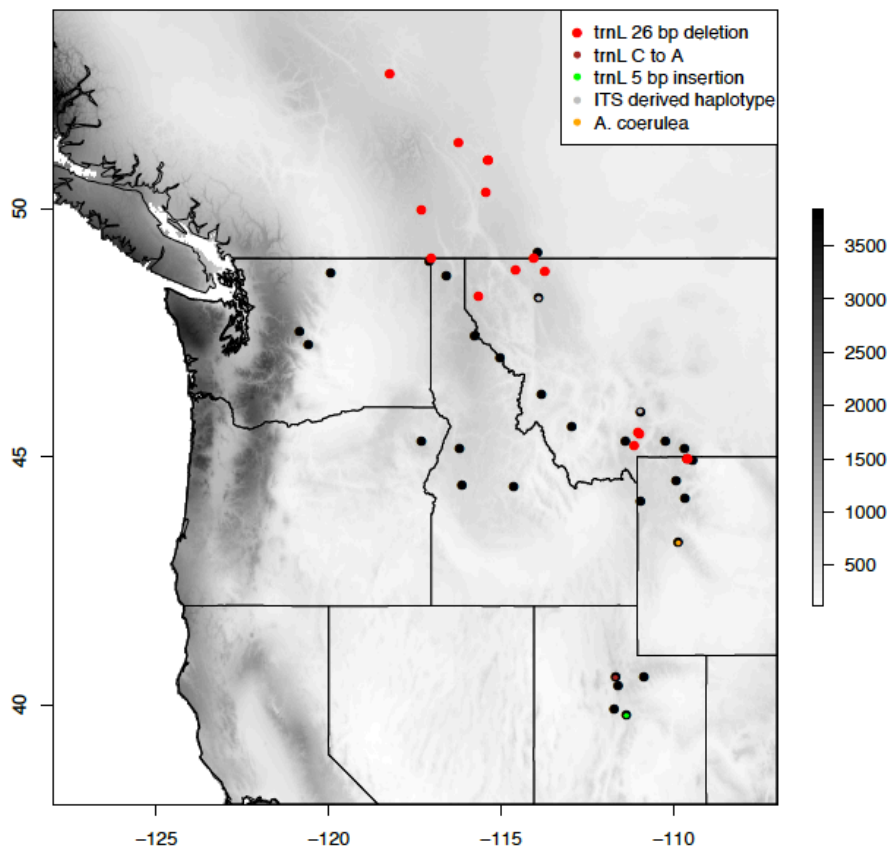


Figure 1.) Geospatial results of three genotypes. The two genotypes in respect to the 26 base pair deletion are depicted here in a NE vs. SW fashion.

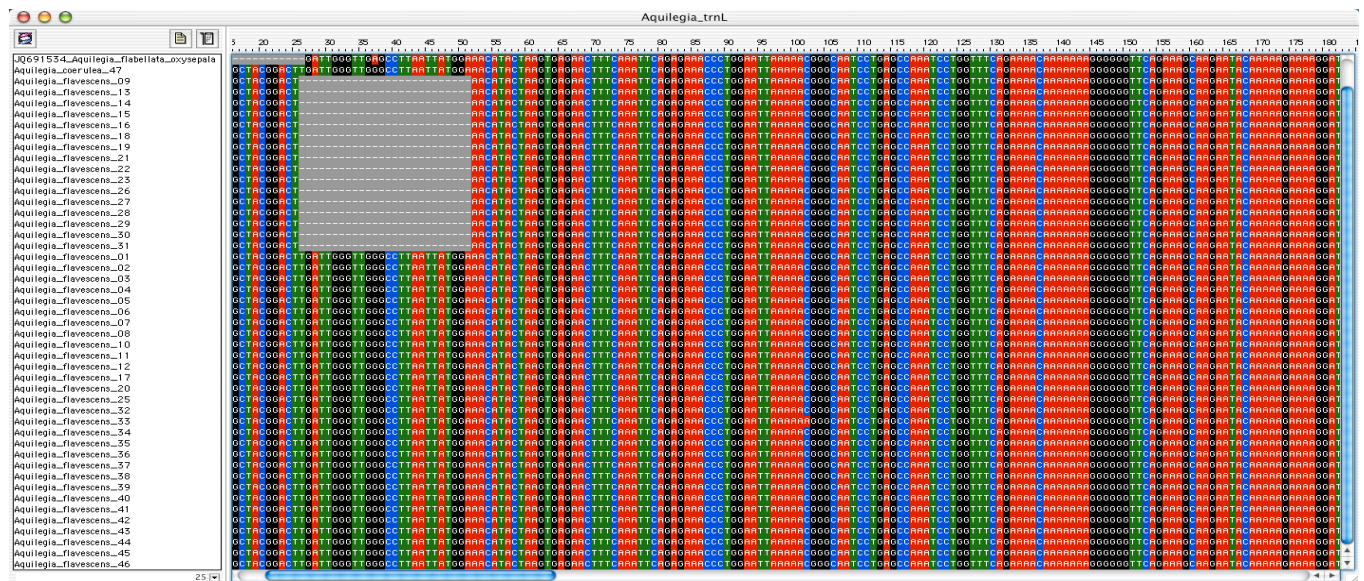


Figure 2.) trnL sequences with the 26 base pair deletion noted by gray space. The cytosine to adenine substitution can also be seen on this figure.

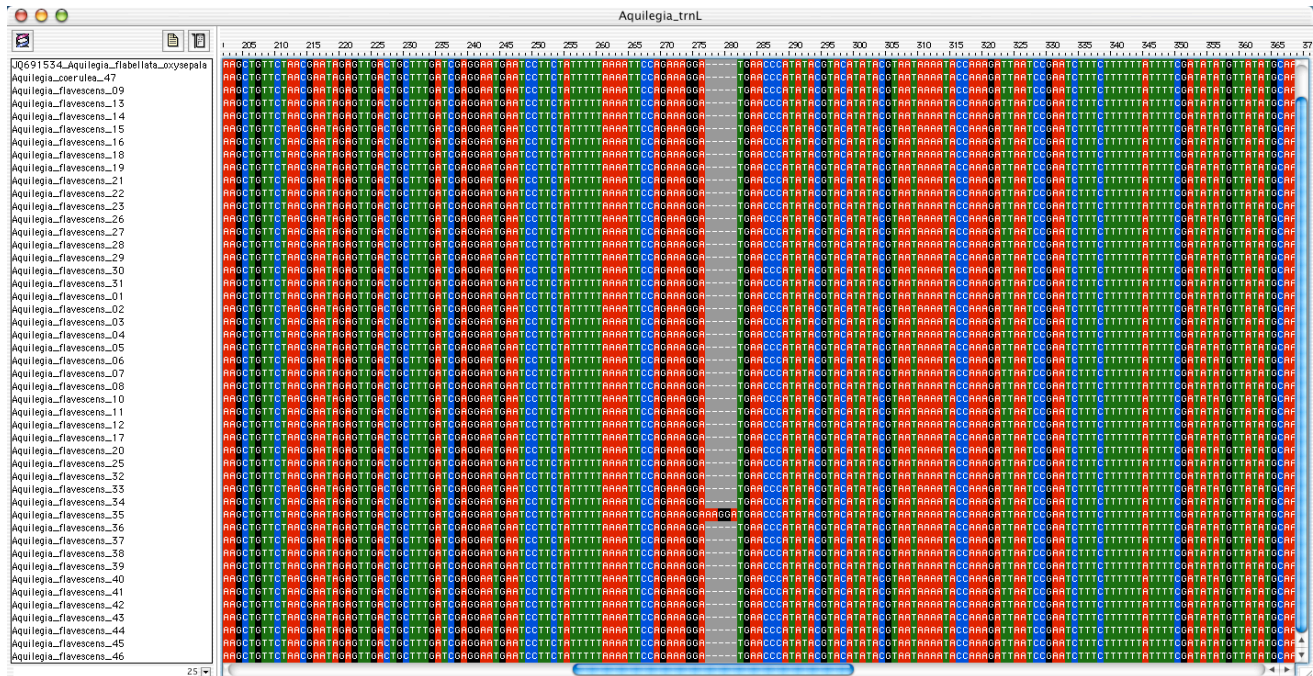


Figure 3.) Five base pair addition in the population at the southern most sample site on the Wasatch Plateau, UT.

WORKS CITED:

- Bastida, JM, *et al.* (2010) Extended phylogeny of *Aquilegia*: the biogeographical and ecological patterns of two simultaneous but contrasting radiations. *Plant Systematics and Evolution*: 284:171–185
- Brunet, J, Larson-Rabin, Z., Stewart, CM. (2012) The distribution of genetic diversity within and among populations of the rocky mountain columbine: the impact of gene flow, pollinators, and mating system. *International Journal of Plant Science* 173(5):484-494
- Hodges SA, Arnold ML (1994) Columbines: a geographically widespread species flock. *Proc Natl Acad Sci USA* 91:5129–5132
- Tang, L.-L., Yu, Q., Sun, J.-F., Huang, S.-Q. (2007) Floral traits and isolation of three sympatric *Aquilegia* species in the Qinling Mountains, China. *Plant Systematics and Evolution* 267: 121-128