**A geographic mosaic of hybridization: phenotypic evidence from two North American species of *Aquilegia***

**Jeffrey S. Groh1 & Quentin C.B. Cronk1**

1*Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada*

Author for correspondence: Jeffrey S. Groh, j.groh@alumni.ubc.ca

**Abstract**

As species ranges can be spatially complex and dynamic, so then can hybrid zones between them. To illustrate one such example, we present phenotypic evidence for a complex spatial distribution of morphological introgression between two North American species, *Aquilegia formosa* Fisch. ex DC. and *A. flavescens* S. Watson. Through analysis of over 250 herbarium specimens from across the entire range of these, we found evidence of extensive phenotypic intergradation in several contact zones in western North America. Using a geospatial morphology diagram (GMD), we show that spatial proximity to the alternative species predicts the extent of phenotypic discrimination, suggesting introgression beyond initial hybridization events is common. We further show with phenotypic data from field-sampled populations the varying degrees of morphological introgression in separate contact zones, and discuss how complex landforms and variable selection pressures might influence these patterns. Taken together, we characterize these patterns as a “geographic mosaic of hybridization”. Lastly, we present evidence that a historically disputed taxon, *A. flavescens* var. *miniana* J.F. Macbr. & Payson, is indeed of hybrid origin, as intended by the original authors. We therefore provide the hybrid binomial *Aquilegia* × *miniana*, hybr. et stat. nov.

**Keywords:** *Aquilegia*; geolocation; geospatial morphology diagram; herbarium; hybridization; mosaic hybrid zone; western North America

**Running Title:** AGeographic Mosaic of Hybridization

**Introduction**

Few studies have taken a wide landscape-level phenotypic approach to understand the structure of hybrid zones in geographically complex regions. The increasing application of large genomic datasets to the study of hybrid zones should ideally be accompanied by the use of large and geographically diverse phenotypic datasets. Herbarium collections are poised to make a substantial contribution in this regard (Lexer & al. 2009). To illustrate this approach, we investigated hybridization in North American *Aquilegia*, a genus with a rich history of research on interspecific hybridization (e.g. Grant, 1952; Chase & Raven, 1975; Hodges & Arnold, 1994; Filiault & al., 2018). A hybrid population between *Aquilegia formosa* (red-flowered, lowland to submontane) and *A. flavescens* (yellow-flowered, montane) in British Columbia has previously been studied in detail (Groh & al. 2018). Here, we present analyses of floral phenotypic data from herbarium specimens of *Aquilegia formosa* and *A. flavescens* across western North America. We calculated a phenotypic hybrid index for all specimens using discriminant analysis and combined these data with geolocations to reveal signatures of introgression in several independent contact zones between the speciesin question*.* We furthermore conducted field sampling of two populations of *A. flavescens* in regions of contact with *A. formosa* and two populations of *A. formosa* near to a previously studied hybrid populationto characterize the distribution and extent of phenotypic intergradation between these species.

Over a century ago, MacBride and Payson collected specimens from central Idaho of what to them represented an advanced fusion of the typical parental forms of *Aquilegia formosa* and *A. flavescens*. Their description of this entity (1917) as a new variety of the latter, *A. flavescens* var*. miniana,* conveys their impression of its greater morphological affinity to *A. flavescens*, but with clear indication of introgressive variation in the direction of *A. formosa*, particularly in the way of sepal coloration. This interpretation was reinforced in a monograph by Payson (1918) which details his observations of the two species growing in close proximity within the same region from which the putative hybrids were collected. In spite of the ensuing use of this name to refer to hybrids between the parental species, and the clear intention by the authors of the name to refer to such, the hybrid nature of MacBride’s and Payson’s type specimens has come into question. The treatment of *Aquilegia* in Flora of North America (Whittemore, 1997) takes the view that the type specimens represent a pink color variant of typical *Aquilegia flavescens*, and that there is no evidence of hybridization or introgression. A similar view on pink-flowered *A. flavescens* populations in southwestern Montana was reached earlier by Culver (1994) who wrote: “*Invoking* *hybrid origins… is ad hoc and should be considered only if some other evidence rather than reddish pigment is found.”* Following the Flora of North America treatment, herbarium specimens originally identified as *A. flavescens* var*. miniana* have often been reclassified in herbaria simply as *A. flavescens*, leaving confusion as to the actual extent of interspecific hybridization. In an effort to shed light on this situation we re-examined the type specimens of *A. flavescens* var*. miniana* with a robust morphometrics approach, taking advantage of the range of variation present in our dataset of herbarium specimens.

**Methods**

**Herbarium data collection. –** We used a dataset of 151 floral phenotypes of *Aquilegia formosa* and *A. flavescens* collected as part of a previous study (Groh & al., 2018), supplemented with an additional 107 specimen phenotypes, which were either accessed through online databases – Consortium of Pacific Northwest Herbaria specimen database (http://www.pnwherbaria.org/data.php) and the Global Biodiversity Information Facility database (https://www.gbif.org) – or provided directly by herbaria in the Pacific Northwest. We focused on covering the entire geographical extent of each species and increasing sampling in potential contact zones between the focal species. Herbarium specimens without previous geolocations were assigned estimated coordinates according to information from herbarium sheet labels. Previously geolocated specimens whose coordinates did not match herbarium sheet labels were reassigned coordinates. The phenotype dataset consists of seven length measurements and one categorical measurement as previously described (Groh & al., 2018). Details of herbarium specimens are deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.XXXXXXXX).

**Construction of the discriminant function.–** To view phenotypes along an axis which separates the typical forms of the species, we took the approach of constructing a linear discriminant function based on a training set of herbarium specimen phenotypes. To remove potential effects of introgression or misidentification in the discriminant function, we focused on allopatric specimens by filtering out 40% of the data points with the least minimum geographic distance to a record of the alternative species in the data set. We additionally filtered out any specimens which were labelled as putative hybrids based on a visual assessment of floral phenotype. Linear discriminant analysis (LDA) was implemented using the R package MASS (Venables & Ripley, 2002). Discriminant scores for all specimens were then predicted according to this model, with the prior probability of group membership set to be equal for both species. Note that this hybrid index is therefore not influenced by potential identification bias. It does however, ignore other pertinent information for species identification, such as floral color and habitat, and as such it is strictly a measure of the extent of intermediacy in floral morphology rather than a complete predictor of species identity.

**Size correction. –**Due to allometry of species-specific trait combinations, variation in overall flower size can potentially mislead interpretation of a hybrid index based on morphology. Indeed, we found that smaller flowers showed less divergent phenotypes, even for populations for which recent hybridization seemed unlikely. General floral size differs within individuals and within populations and does not appear to be a taxonomically informative character, unlike floral shape. All individuals with complete data from the herbarium data set and across two sampling seasons (522 individuals in total), were submitted to a principal component analysis (PCA), with each trait scaled to unit variance. While the first axis (PC1) separated the two species, all measured traits loaded with the same sign onto the second axis (PC2), indicating that this axis captured variation in overall flower size (Electr. Suppl.: Fig. S1). Notably, within each species and populations, we found significant correlations between our predicted discriminant scores and size values given by PC2 within both species. We therefore used linear regression to determine the scaling of discriminant scores with size for each species separately, then used the residuals from within-species regressions to displace individuals from the discriminant score predicted by their species regression model at the overall mean size. Size-adjusted discriminant scores were re-scaled between zero and one using two different methods. To mimic the classic hybrid index, we used a sigmoid transformation, which collapses the variation at the extremes of the phenotype distribution of each species. For regression analysis, we applied a translation and linear transformation by subtracting the minimum value and dividing by the range so as to preserve the entire range of variation within the phenotype distribution of each species.

**Biogeographic analysis. –** To investigate patterns of morphological introgression in contact zones between *Aquilegia formosa* and *A. flavescens*, we assessed whether the extent of phenotypic discrimination of herbarium specimen phenotypes was negatively associated with spatial proximity to records of the alternate species. For each specimen, we calculated geographic distance to all records of the alternate species in the dataset using the *distHaversine* function in the R package *geosphere* (Hijmans, 2016), which assumes a spherical earth. We used the R package *ggmap* (Kahle & Wickham, 2013) to plot directly on a map the geolocations of the specimens in our data set, using a color gradient to indicate the range of sigmoid-transformed hybrid indices. To further illustrate the occurrence of intermediate morphology in contact zones, we produced a geospatial morphology diagram (GMD) by plotting linearly-transformed discriminant scores against the log-transformed minimum distance to a record of the other species (using original species identifications from herbarium sheets). This was followed by linear regression within each species to examine the relationship between morphological and spatial distance.

**Sampling of natural populations. –**In addition to populations previously sampled (Groh & al., 2018), we sampled two further *Aquilegia flavescens*-like populations in regions of range contact with *A. formosa* to investigate whether these populations showed signals of phenotypic and genetic introgression. Similarly, we sampled a further three populations of plants resembling *A. formosa*: two introgressed populations and one apparently pure population.

The two *A. flavescens*-like populations were located at Mission Ridge in the Wenatchee Mountains of Washington State, USA, and on Cheops Mountain within Glacier National Park in the Columbia Mountains of British Columbia, Canada. The Mission Ridge *Aquilegia* plants were observed at the eastern and western edges of the Skookum ski run on June 21, 2018. The Cheops Mountain site was accessed by proceeding from the beneath the bridge where the Trans-Canada Highway crosses the Illecillewaet River at 51.2619°, -117.5482° up a south-facing avalanche slope between the peak of Cheops Mountain and the adjacent Napoleon Spur (observed 11 July 2018).

The three *A. formosa*-type populations include one “pure” population (Manning Park, British Columbia, Canada) and two introgressed populations. One of these latter was in an adjacent valley (hereafter “Adjacent Valley population”) to the hybrid population at Porcupine Ridge, Marble Range, BC, Canada detailed in another study (Groh & al., 2018). The other was located along the Pavilion-Clinton Highway, approximately 20 km away from the Marble Range hybrid population. All populations are listed in Table 1.

To survey the sites, we used a plotless sampling (point-centered quarter) method to estimate density of plants within the *A. flavescens* populations, and to estimate the census size of each. We then haphazardly sampled plants with post-anthesis flowers for phenotypic data. For each plant, we first photographed the outer face of the sepal whorl and a SpyderCheckr 24 color card (DatacolorTM, Lawrenceville, New Jersey) under the same lighting conditions and camera settings for color standardization. Plants were then collected at the crown and pressed.

To quantify human-visible variation in sepal coloration from red to yellow, we extracted relative values of red and green reflectance from color calibrated images in the RGB color space (Bergman and Beehner, 2008, Groh & al., 2018). The ratio of red to green reflectance was centered around zero by log-transformation. We measured the same eight characters from which the discriminant function was constructed and predicted the phenotypes of individuals within sampled populations using an equal prior probability for each species. A size-correction procedure was performed as described above, but with regressions within populations rather than within species. Size-adjusted discriminant scores of sampled populations were transformed by the sigmoid function to range between zero and one.

**Type specimen analysis of *Aquilegia flavescens* var*. miniana*. –** We accessed digital images of all 13 type specimens of *A. flavescens* var*. miniana* from JSTOR (<https://plants.jstor.org>, accessed Aug 31, 2018), which include the holotype, eight isotypes from the same location, and four paratypes from different locations (listed in Table 2).

**Results**

**Geographic zones of floral introgression. –**The spatial distribution of discriminant scores (Fig. 1) reveals evidence of hybridization between *Aquilegia formosa* and *A. flavescens* in several independent contact zones in western North America. The most striking of these occurs in central Idaho and southwestern Montana, where the phenotypes of the two species homogenize to a considerable extent. Indeed, MacBride and Payson (1917) directly described this phenomenon over a century ago, and, even then, noted that botanists had for some time recognized that these two species seem to merge in this territory and become virtually indistinguishable. In spite of this longstanding acknowledgement, not a single herbarium specimen that we examined from this region was directly identified as a hybrid. Rather, taxonomists appear generally reluctant to make such a claim, despite the most recent taxonomic treatment confirming that “*Aquilegia flavescens* sometimes forms hybrid swarms with *A. formosa* var. *formosa*, which grows at lower elevations through much of its range” (Whittemore 1997). Instead, the reluctance of taxonomists to determine herbarium material as hybrid may be influenced by the assessment of Whittemore (1997) that the specimens collected by MacBride and Payson from this region as hybrid, and named *A. flavescens* var. *miniana*, simply represent a pink-flowered variant of “pure” *A. flavescens,* rather than being of hybrid origin.

A second major zone of introgression occurs in the Wenatchee Mountains of central Washington, a region known to host many endemic and disjunct plant species. In this region *A. flavescens* occurs disjunct from the main body of its distribution, separated to the east by the desert environment of eastern Washington. Thus isolated, and in close proximity to the coastal distribution of *A. formosa,* the *A. flavescens* plants in this region evidently have been subject to considerable introgression from the lowland species. These plants are often characterized by pink-tinged sepals, which in conjunction with the prevalence of intermediate floral morphology, strongly implicates a history of hybridization.

A third zone of introgression occurs where the two species come into contact in the Columbia Mountains and Rocky Mountains of southeastern British Columbia. Here, the two species can be found in close proximity, and all manner of intermediate-looking plants have been observed in such areas (Jamie Fenneman, personal communication). While collections of *Aquilegia* from this area are notably less extensive, a number of intermediate morphologies are represented among them (Fig. 1).

**Spatial proximity predicts morphological similarity. –** The geospatial morphology diagram (GMD) shows clear regression patterns (Fig. 2).While the hybrid index shown in Fig. 1 collapses variation at the phenotypic extremes of each species, our regression analysis, which uses a morphology index that preserves this variation, shows that spatial proximity to a record the other species can be used to predict the extent of phenotypic discrimination (Fig. 2). Notably, the apparent linear trend within each species suggests that this effect is not purely driven by strongly intermediate early-generation hybrids which occur in close contact zones, but that the introgressive effects of hybridization permeate within each species beyond areas of immediate proximity, likely through continued backcrossing.

**Introgressed natural populations. –**The *Aquilegia* population at Cheops Mountain, British Columbialies within the broad zone of intergradation seen in southeastern BC (Fig. 1). The plants at this site were distributed along the western edge of a waterfall in between 1200 m and 1350 m in elevation. No *Aquilegia* plants were observed outside this altitudinal range, and above 1400 m the slope becomes too steep to ascend. Within this region, the plants occur within one main cluster at 1200 m and in smaller subsidiary clusters along the upstream bank of the waterfall. Our site survey estimated the population size at c. 900 individuals.

The discriminant scores of individuals within this population confirm greater affinity to *A. flavescens,* but with noticeable variation in the direction of *A. formosa.* In comparison with the “pure” *Aquilegia flavescens* population at Mt. Kobau, the discriminant scores are more highly variable and intermediate, implicating introgression from *A. formosa.*

Color values of the Cheops Mountain population and other *Aquilegia* populations of the two focal species are shown in Figure 3. Compared to “pure” *Aquilegia flavescens* represented by Mt. Kobau, we found elevated values of red reflectance in the sepals of the Cheops Mountain population, which was observed visually during the sampling procedure. Notably, some individuals within the population had completely pink or nearly red sepals, indicated by higher values on the y-axis in Figure 3. While we observed a similar phenotype in one individual in the Mt. Kobau population, the frequency of pink-colored morphs appears much higher in the Cheops Mountain population, and the intensity of red reflectance also exceeds that seen at Mt. Kobau. The color values of pink-flowered individuals at Cheops Mountain are consistent with the range of color variation present in hybrids (Porcupine Ridge), even falling within or close to what is typical of “pure” *A. formosa* (e.g. Robert’s Lake). While attributing this pink coloration to hybridity has been treated with caution (Whittemore, 1997), the morphological intermediacy of this population, and its geographic location – within the range of *A. formosa –* strongly suggests that it represents introgression from the latter species. Indeed, *A. formosa* has been found in a valley on the other side of Cheops Mountain (Jamie Fenneman, personal communication).

The *Aquilegia* population at Mission Ridge, Washington Statelies within the region of intergradation in the Wenatchee Mountains of Washington State. The plants in this population occurred on a ski run bordered on the west by a service road, beyond which no *Aquilegia* were found to occur, and to the east by dense coniferous forest, in which *Aquilegia* does not grow. After snowmelt, the ski run consists of scree with sparse herbaceous vegetation. The population size is estimated at c. 100 – 150 individuals. Similar to the Cheops Mountain population, this population shows evidence of introgression from *A. formosa*, on the basis of intermediate hybrid index scores (Fig. 3A) and a higher frequenc of individuals with elevated red reflectance in the sepals (Fig. 3B). Furthermore, herbarium specimens of both species have been collected in close proximity to the sampling site, and these collections show intermediate hybrid index values (Fig. 1).

During the course of a previous study of a hybrid population in the Marble Range of BC (indicated by diamond in Fig. 1), we encountered nearby populations of *A. formosa* which appeared to vary phenotypically in the direction of *A. flavescens.* One of these was in a nearby valley to the focal hybrid population (the Adjacent Valley population), and another was found along the Pavilion Clinton Highway, approximately 20 km away from the hybrid population. We obtained morphometric data from the former and colorimetric data from the latter. The morphometrics of the Adjacent Valley population show a clear signal of variation in the direction of *A. flavescens*, but with greater affinity to *A. formosa* than plants of the nearby hybrid population beneath Porcupine Ridge (Fig. 3). For reference, Manning Park, Clearwater and Roberts Lake are all considered “pure” populations of *A. formosa* (Fig. 3)*.* Color values of the population of *A. formosa* along the Pavilion Clinton Highway, which appeared visually to vary morphologically in the direction of *A. flavescens,* also show intermediacy and suggest introgression from the latter species (Fig 4).

**Type specimen analysis of *Aquilegia flavescens* var*. miniana*. –** Hybrid index values of the type specimens of *A. flavescens* var. *miniana* J.F.Macbr. & Payson are shown in Figure 4. The specimens (MO) annotated by Whittemore, on which the judgement was based that this name refers to typical *A. flavescens,* have the most extreme *A. flavescens-*like phenotypes out of all specimens collected from the type locality. However, the population as a whole clearly shows variation in the direction of *A. formosa*, suggesting that this name does accurately refer to the hybrid between *A. formosa* and *A. flavescens,* as originally intended by the collectors. Four collection numbers (Challis Creek, Bonanza, Sawtooth Peaks and Smoky Mts) are cited in the protologue (Table 2), with the Challis Creek gathering being the main one on which the name is founded. Numerous duplicates were taken in the Challis Creek gathering but only one specimen (GH [photo!]) is singled out, and must be considered the holotype. The others are from the same gathering and must be considered isotypes. The holotype is a clear intermediate (hybrid index 0.32), although with more affinity with *A. flavescens*. Pure *A. flavescens* is expected to have a hybrid index of c. 0.1 or less (Fig. 3).

**Discussion**

***A Geographic Mosaic of Hybridization*** – Characterizing the structure of hybrid zones is vital to an understanding of how they originate, are maintained, and what their eventual fate will be (Barton & Hewitt, 1985; Harrison, 1990). Earlier authors have duly pointed out that the structure of hybrid zones may conform to different models at different levels of spatial resolution (Harrison, 1990). For instance, when considering the entirety of western North America, the hybrid zone between *A. formosa* and *A. flavescens* appears as a clinal transition between the two forms (Groh & al. 2018). However, by restricting the spatial scale to the region in which these species are broadly sympatric, and taking into consideration the peripheries and pockets of their respective ranges, their regions of intersection can be seen to form dissected zones of introgression in varying degrees, resembling a geographic mosaic of phenotypes.

On yet a finer spatial scale, *A. formosa* and *A. flavescens* typically occupy different altitudes, allowing for extensive interdigitation of their respective habitats across the terrain of western North America. In this way, the varied topography of mountainous areas likely plays a determining role in structuring the hybrid zone. Not only does the mosaic of parental and hybrid populations coincide with a mosaic of habitats to which the species have adapted divergently, but also the complex pattern of rivers and mountain ridges may physically impede gene flow between populations. To illustrate the first point, Payson (1918) observed habitat association in the hybrid zone in central Idaho, noting *A. flavescens* in higher areas, *A. formosa* in the valley bottoms, and intermediate forms in between these two altitudes. Furthermore, the observation that putative hybrids west of the main body of the *A. flavescens* range only occur in high elevation areas provides additional evidence for habitat association. This raises the strong possibility of selection for or against immigrant alleles as a primary factor in the maintaining the structure of the hybrid zone (Nosil & al., 2005; Suarez-Gonzalez & al., 2018a; Suarez-Gonzalez & al., 2018b).

While we have characterized the hybrid zone as a geographic mosaic, it should be noted that the various models of hybrid zone structure are not mutually exclusive. At a highly local scale, where the species come into contact, clines may be established along altitudinal gradients. At other localities plants with intermediate floral morphology may almost entirely supplant parental types, as in central Idaho (Figure 1; MacBride & Payson, 1918).

Floral morphology traits, as investigated here, are directly relevant to pollinator use, and hence potentially to reproductive isolation. Floral morphology is a candidate trait for showing evidence of “reinforcement” (Hopkins, 2013) in sympatric taxa, as it can directly influence differential pollinator use, and therefore reduce the production of potentially unfit hybrid offspring. It might be predicted that *A. formosa* and *A. flavescens* should show increased divergence in traits related to pollinator attraction or pollen placement in sympatry as opposed to allopatry, as is seen in *Mimulus* (Grossenbacher and Whittall, 2011). Our finding of the extensive intergradation of forms between the two species is not consistent with reinforcement. Instead it suggests that reproductive isolation between *A. formosa* and *A. flavescens* is incomplete, or even lacking, across areas of sympatry.

***The correct name for the hybrid*** – The morphometric analysis provides strong evidence that the holotype of *A. flavescens* var. *miniana* is intermediate between *A. flavescens* and *A. formosa*. Furthermore, the specimen originates from a region in which considerable introgression is common (Fig. 1). The holotype is therefore most likely of hybrid origin, as suggested by the original authors. We therefore re-confirm this as a valid name for hybrid specimens. There remains the issue of establishing it as a hybrid binomial rather than a variety. Accordingly, we raise it to hybrid status:

*Aquilegia* × *miniana* (J.F.Macbr. & Payson) Groh & Cronk, hybr. et stat. nov.

Basionym: *Aquilegia flavescens* S.Watson var*. miniana* J.F.Macbr. & Payson (1917) *Contr. Gray Herb. Harvard Univ.* 49: 60–72.

Holotype: Macbride and Payson 3326 (GH). A hybrid of the parentage: *Aquilegia flavescens* S.Watson × *A. formosa* Fisch. ex DC.

**Acknowledgements**

We thank Linda Jennings and Erin Manton for facilitating study of UBC herbarium specimens. We also thank David Giblin, Kimberley Hansen, and Sophie DeLuca for sending digitized images of requested herbarium sheets from WTU, WS, and V herbaria, respectively. We thank Diana Percy and Marius Roesti for assistance with field surveys.

**Literature Cited**

**Barton, N.H., & Hewitt, G.M.** 1985. Analysis of hybrid zones. *Annual Rev. Ecol. Evol. Syst.* 16: 113--148. https://doi.org/10.1146/annurev.es.16.110185.000553

**Bergman, T.J., & Beehner. J.C.** 2008. A simple method for measuring color in wild animals: validation and use on chest patch color in geladas (*Theropithecus gelada*). *Biol. J. Linn. Soc.* 94: 231--240. https://doi.org/10.1111/j.1095-8312.2008.00981.x

**Chase, V.C., & Raven, P.H.** 1975. Evolutionary and ecological relationships between *Aquilegia formosa* and *A. pubescens* (Ranunculaceae), two perennial plants. *Evolution* 29: 474--486. https://doi.org/10.1111/j.1558-5646.1975.tb00837.x

**Consortium of Pacific Northwest Herbaria.** Specimen database. http://www.pnwherbaria.org/data.php (accessed 15 Aug 2018).

**Culver, D.R.** 1994. *Floristic analysis of the Centennial Region, Montana.* Dissertation, Montana State University, Bozeman, Montana, U.S.A.

**Filiault, D.L., Ballerini, E.S., Mandáková, T., Aköz, G., Derieg, N.J., Schmutz, J., Jenkins, J., Grimwood, J., Shu, S., Hayes, R.D., Hellsten, U., Barry, K., Yan, J., Mihaltcheva, S., Karafiátová, M., Nizhynska, V., Kramer, E.M., Lysak, M.A., Hodges, S.A., & Nordborg, M.** 2018. The *Aquilegia* genome provides insight into an adaptive radiation and reveals and extraordinary polymorphic chromosome with a unique history. *eLife* 7: e36426. https://doi.org/10.7554/eLife.36426

**Grant, V.** 1952. Isolation and hybridization between *Aquilegia formosa* and *A. pubescens.* *Aliso* 2: 341--360. https://doi.org/10.2307/2407586

**Groh, J.S., D.M. Percy, D.M., Bjork, C.R. & Cronk, Q.C.B.** 2018. On the origin of orphan hybrids between *Aquilegia formosa* and *Aquilegia flavescens*. *AoB Plants*: ply071. https://doi.org/10.1093/aobpla/ply071

**Grossenbacher, D.L., & Whittall, J.B.** 2011. Increased floral divergence in sympatric monkeyflowers. *Evolution* 65: 2712--2718. https://doi.org/10.1111/j.1558-5646.2011.01306.x

**Harrison, R.G.** 1990. Hybrid zones: windows on evolutionary process. *Oxford Surv. Evol. Biol.* 7: 69--128.

**Hijmans, R.J.** 2016. geosphere: Spherical trigonometry. R package version 1.5--5. https://CRAN.R-project.org/package=geosphere.

**Hodges, S.A., & Arnold, M.L.** 1994. Floral and ecological isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Proc. Natl. Acad. Sci. U.S.A.* 91: 2493--2496. https://doi.org/10.1073/pnas.91.7.2493

**Hopkins, R.** 2013. Reinforcement in plants. *New Phytol.* 197: 1095--1103. https://doi.org/10.1111/nph.12119

**Kahle, D. & Wickham, H.** 2013. ggmap: Spatial visualization with ggplot2. *The R Journal* 5: 144--161.

**Lexer, C., Joseph, J., van Loo, M., Prenner, G., Heinze, B., Chase, M.W. & Kirkup, D**. 2009. The use of digital image-based morphometrics to study the phenotypic mosaic in taxa with porous genomes. *Taxon* 58: 349--364.

**MacBride, J.F., & Payson, E.B.** 1917. New or otherwise interesting plants from Idaho. *Contr. Gray Herb.* 49: 60--72.

**Nosil, P., Vines, T.H., and Funk., D.J.** 2005. Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* 59: 705--719.

**Payson, E.B.** 1918. The North American species of *Aquilegia. Contr. U.S. Natl. Herb.* 20: 140--144.

**Suarez‐Gonzalez, A., Hefer, C.A., Lexer, C., Douglas, C.J., & Cronk, Q.C.B.** 2018a. Introgression from *Populus balsamifera* underlies adaptively significant variation and range boundaries in *P. trichocarpa*. *New Phytol.* 217: 416--427. https://doi.org/10.1111/nph.14779

**Suarez-Gonzalez, A., Lexer, C., & Cronk, Q.C.B.** 2018b. Adaptive introgression: a plant perspective. *Biology Letters* 14: 20170688. https://doi.org/10.1098/rsbl.2017.0688

**Venables, W.N. & Ripley, B.D.** 2002. *Modern Applied Statistics with S-Plus*. New York: Springer.

**Whittemore, A.T.** 1997. *Aquilegia.* In: Flora of North America Editorial Committee (ed.) *Flora of North America north of Mexico*, vol. 3. New York, Oxford: Oxford University Press. http://www.efloras.org/florataxon.aspx?flora\_id=1&taxon\_id=102388 (accessed 1 Jan 2018).