**Phenotypic evidence for an extensive mosaic hybrid zone between two species of columbine, *Aquilegia flavescens* and *A. formosa***

Jeffrey S. Groh1,2 and Quentin C.B. Cronk1,3

2 [jgroh@ucdavis.edu](mailto:jgroh@ucdavis.edu), 3quentin.cronk@ubc.ca

1Department of Botany and Beaty Biodiversity Research Centre, University of British Columbia. 3156-6270 University Blvd., Vancouver, BC, Canada V6T 1Z4

2Currently at Center for Population Biology and Department of Evolution and Ecology, University of California, Davis. 2320 Storer Hall, 1 Shields Avenue, Davis, CA 95616

2Author for correspondence.

**Abstract**

Describing the structure and dynamics of hybrid zones has important consequences for our understanding of lineage diversification. Herbarium collections constitute an extensive, though often underutilized, morphological resource which can potentially be leveraged to identify and describe hybrid zones. We demonstrate the use of herbarium collections to describe the structure of a hybrid zone between two North American columbines, *Aquilegia* *flavescens* and *A. formosa*. Hybrid indices were calculated from floral morphology measurements and mapped using geolocation data, revealing an extensive and consistent pattern of floral intermediacy in several contact zones. Population phenotype samples from contact zones corroborate these findings and strongly suggest introgression. At one locality with a confirmed hybrid population, floral phenotypes show clinal variation in parental-type morphology along an elevational gradient, in the direction predicted by the divergent elevational distributions of these taxa. We argue that these patterns together reflect a habitat-associated mosaic hybrid zone, consistent with divergent ecological selection maintaining the structure of the hybrid zone.

**Keywords:** columbines, herbarium, morphometrics, introgression, mosaic hybrid zone, refusion, cline, clinal variation, contact zone

**Introduction**

Hybrid zones refer to regions where genetically diverged taxa overlap and produce offspring. Because of the fundamental role of reproductive isolation in determining the structure and dynamics of hybrid zones, describing hybrid zones can lend important insights to our understanding of lineage diversification. A detailed knowledge of hybrid zone structure can serve as the basis for models of evolutionary forces fundamental to speciation (Barton and Hewitt 1985; Szymura and Barton 1986), and aid in identifying genomic regions underlying reproductive isolation (Rieseberg et al. 1999; Sung et al. 2018; Tavares et al. 2018). Even without an understanding of introgression at the genomic level, an ecologically-based understanding of hybrid zone structure can yield insights about the nature of selection as a mechanism of reproductive isolation (Mallet and Barton 1989; Nosil et al. 2005).

Hybrid zones structured as a monotonic cline between two parental taxa fit the ‘tension-zone’ model, which describes a balance between gene flow into the contact zone and selection against hybrids (Barton and Hewitt 1985). In this model, selection against hybrids can be environment-independent, for example through hybrid male sterility (Turner and Harr 2014). Alternatively, in the ‘mosaic’ model, the distribution of parental types and hybrids is patchy across a landscape (Harrison 1986). While this pattern could result from priority effects of colonization, if the spatial distribution of genotypes is associated with an underlying habitat mosaic it is more likely that variable ecological selection structures the hybrid zone (Rand and Harrison 1989; Vines et al. 2003; Nosil et al. 2005). An important consequence of this spatial pattern is that multiple regions of secondary contact can behave to some degree independently (Harrison 1986), potentially giving rise to parallel or contingent outcomes throughout the region of secondary contact. While not mutually exclusive in a given system (Riquet et al. 2019), these models are a useful first step in descriptions of hybrid zones.

Herbarium records and museum collections provide a rich yet often underutilized resource for the analysis of morphological variation (McAllister et al. 2018). When available, the association of geolocation with phenotype across a broad geographic range can be a powerful tool for identifying and describing hybrid zones. Floral phenotypes offer several advantages in this enterprise, in that collectors have a bias towards collecting plants in flower (Daru et al. 2018), and these characters are often among the most taxonomically informative. Closely-related taxa are often better differentiated by floral characters than vegetative characters (Grant 1971), in line with the commonly-held opinion that divergence in floral morphology underlies much of angiosperm diversification (Crepet and Niklas 2009). Indeed, hybridization studies through the lens of floral morphology have provided important insights into the evolutionary process, often focusing on the role of divergent pollination systems in maintaining reproductive isolation (Grant 1949, 1952; Fulton and Hodges 1999; Schemske and Bradshaw 1999; Milano et al. 2016). In contrast to revealing hybrid intermediacy, floral phenotypes may instead exhibit exaggerated divergence in sympatry, a pattern consistent with character displacement or reinforcement (van der Niet et al. 2006; Grossenbacher and Whittall 2011; Hopkins 2013; Grossenbacher and Stanton 2014; Runquist and Moeller 2014), although it is possible that both patterns might be observed over different time scales. While careful experiments are necessary to address these hypotheses, documenting phenotypic patterns in areas of sympatry is a prerequisite step toward understanding the course and fate of trait divergence during secondary contact between hybridizing taxa.

The columbines (*Aquilegia*) are well-known for impressive interspecific floral diversity despite broad similarity at the genomic level and extensive natural hybridization (Hodges and Arnold 1994; Filiault et al. 2018). Previously, we described in detail a hybrid population between *Aquilegia flavescens* S. Watson and *A. formosa* Fisch. ex DC., two species which occur in sympatry in montane regions of western North America (Groh et al. 2019). In the present work, we use floral phenotypes of herbarium specimens to describe more broadly the structure of the hybrid zone between these two species. We combine these herbarium data with phenotypic analyses of additional population samples from identified contact regions, including the original type specimens of the controversial taxon *A. flavescens* var. *miniana* J. F. Macbr. & Payson, to corroborate our biogeographic analyses and test for evidence of introgression in these regions. Last, we show results from an analysis of phenotypes along a local elevational gradient in a previously identified hybrid population in British Columbia, Canada (Groh et al. 2019), to examine evidence for divergent ecological selection between parental species as an explanation for the observed mosaic hybrid zone structure.

**Materials and Methods**

*Herbarium data collection*

As part of our previous study (Groh et al. 2019), we compiled floral trait data from 191 herbarium specimens of *A. flavescens* and *A. formosa*.In this study weuseda subset of these data for which complete floral trait measurements were available (151 specimens,) and added an additional 107 herbarium specimens with complete floral trait measurements to the data set. The latter specimens 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 as images provided directly by staff from herbaria in the Pacific Northwest (WTU, WS, V). We focused on obtaining specimens from the entire range of each species and increasing sampling in potential contact zones between them. Herbarium specimens without previous geolocations, or whose geolocations were clearly inaccurate, were assigned estimated coordinates according to the information given on the herbarium sheet labels. The phenotype dataset consists of seven continuous characters (spur length, anther exsertion, petal lamina length, lamina width, corolla width, sepal length, and sepal width (Fig. S1)).

*Construction of the hybrid index*

We used linear discriminant analysis (LDA) on a training set of herbarium specimen floral phenotypes to calculate hybrid index values for all herbarium specimens. This method is a dimensionality-reduction technique that finds a linear combination of original trait variables maximizing the distance between group means while minimizing within-group variance, making it useful for multivariate studies of introgression between predefined taxa (Namkoong 1966).

We sought to restrict the training data set to specimens clearly representing the parental phenotypes in order to remove potential effects of misidentification or introgression in the discriminant function. We thus filtered out a subset of specimens on the basis of proximity to records of the alternative species, which we understood a priori to be associated with phenotypic intermediacy. We filtered out 40% of all individuals (half of this number was filtered from each species) with the least minimum distance to a record of the alternative species, guided by a heuristic visual assessment of which percentage value yielded nearly completely non-overlapping distributions of species discriminant scores in the training data set, while retaining as much data as possible. We also filtered out any specimens labelled as putative hybrids based on a preliminary visual assessment. The final training set consisted of 149 individuals (*A. flavescens*, 77; *A. formosa*, 72). Linear discriminant analysis was implemented on raw trait values with the lda function in the R package MASS (Venables and Ripley 2002; R Core Team 2018). Discriminant scores for all herbarium specimens were then predicted under equal prior probability for each species. Note that this hybrid index for those specimens not in the training set is therefore agnostic towards initialidentification status. It ignores other information pertinent to species identification, such as floral color and habitat, and as such is strictly a measure of morphological intermediacy for pressed specimens, rather than a comprehensive predictor of species identity.

Due to allometry of species-specific trait combinations, variation in overall flower size can potentially mislead interpretation of a hybrid index based on morphology, as morphological differences become more pronounced throughout development. General floral size differs within individuals and within populations and does not appear to be a taxonomically informative character in these species, unlike floral shape. To verify that our hybrid index metric was robust to size variation, we therefore sought to correct for the covariation of the discriminant scores with size. To capture variation in size, we submitted traits of 522 individuals (all individuals with complete phenotype data from herbarium collections and from two sampling seasons) to a principal components analysis (PCA) with traits mean-centered and scaled to unit variance. While the first axis (PC1) clearly separated the two species, trait loadings onto PC2 all showed the same sign, indicating that PC2 captured variation attributable to the joint increase of all trait measurements throughout development, which we hereafter interpret as size. As we found significant correlations between our predicted discriminant scores and PC2 (size) scores for each species (size scores were not significantly different between species in the training set), we implemented a regression-based size correction procedure in of two ways. (1) For the first, we took residuals from a regression of discriminant scores onto PC2 scores (calculated from the pooled PCA), and added these residuals to the discriminant score predicted by the regression line at the overall mean size (mean value of PC2 scores from all specimens). This was done separately for each species. These size-adjusted discriminant scores were re-scaled between zero and one. To position the parental phenotypes at the extremes, we used a sigmoid transformation, which collapses the variation at the extremes of the phenotype distribution of each species. The parameters of this function are such that the average hybrid index in the training data set was approximately 0.1 for *A. flavescens* and 0.9 for *A.* formosa. (2) For our analyses using linear regression, we applied a linear transformation directly to discriminant scores by subtracting the minimum value and dividing by the range in order to better meet the regression assumption of normality. Size (PC2) was then explicitly taken into account as a covariate in the regression. All analyses were run with and without size-correction step. All results were highly similar. Results reported below use the correction.

*Biogeographic analysis*

To investigate patterns of morphological introgression in contact zones between *A. flavescens* and *A. formosa,* we first used the R package ggmap(Kahle and 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 (method 1) hybrid indices. Next, for each specimen, we calculated the minimum geographic distance to a record of the other species in the dataset using the distHaversine function in the R package geosphere (Hijmans 2017). To formally test for an association of reduced floral divergence and sympatry, we used the lm function in R (R Core Team 2018) to fit a linear model predicting the linearly-rescaled hybrid index (method 2), using our distance metric, size, and species as predictors, and including interaction terms distance\*species and size\*species.

*Sampling of natural populations*

As herbarium specimen collections generally capture only a single individual from one locality, we sought to corroborate the patterns revealed in our biogeographic analysis of herbarium specimens by collecting population samples from regions of sympatry and allopatry. In addition to reference populations of the pure species and one hybrid population previously sampled (Groh et al. 2019), we sampled two further *A. flavescens*-like populations in regions of range contact with *A. formosa* where introgression was previously suspected (Curtis Björk and Jamie Fenneman, personal communication 2017) . The two *A. flavescens*-like populations were located at Mission Ridge ski resort in the Wenatchee Mountains of Washington State (WA), USA, and on Cheops Mountain within Glacier National Park in the Columbia Mountains of BC. We also included data from a suspected introgressed *A. formosa* population along the Pavilion-Clinton Highway, approximately 20 km away from the previously characterized hybrid population in the Marble Range, BC (Groh et al. 2019). All sampled populations are listed with details in Table 1.

We haphazardly sampled plants with post-anthesis flowers for phenotypic data. For each plant, we first photographed the outer face of the sepal whorl for a single flower 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). The ratio of mean red to green reflectance was centered around zero by log-transformation. We measured floral characters on pressed specimens and calculated discriminant scores for individuals from sampled populations under an equal prior probability for each species. Size-correction was then performed as described above (method 1).

*Type specimen analysis of* Aquilegia flavescens *var.* miniana *J. F. Macbr. & Payson*

We accessed digital images of all 13 type specimens of *A. flavescens* var*. miniana* collected by Macbride and Payson in central Idahofrom JSTOR Global Plants (<https://plants.jstor.org>). These include the holotype (GH), eight isotypes from the same location (RM, MO × 2, US, E, CM, CAS, NY), and four paratypes (RM × 3, CM) from different locations. A hybrid index value was calculated for each plant according to the procedure described above.

*Cline analysis*

In our previous characterization of a hybrid population in the Marble Range, we observed hybrids in a high-elevation habitat, but an absence of pure *A. flavescens,* a high-elevation specialist. We therefore predicted that if alleles from *A. flavescens* were maintained by selection at high elevation in the hybrid zone, then any association between the genetic bases for floral morphology and adaptation to high altitude could result in a cline in species-type floral morphology with elevation. We revisited the Marble Range hybrid population and sampled plants along an elevational cline across adjacent drainages descending from the hybrid population. To test for an association of the hybrid index with elevation, we first calculated predicted discriminant scores for transect specimens using the herbarium specimen training data set described above, then rescaled these to range between zero and one, using the range of herbarium specimen discriminant scores. Thus, the hybrid index values in this analysis are comparable to the values calculated for herbarium specimens. We fit a linear model using the lm function in R to run a regression of the hybrid index against collection elevation, while accounting for size as a covariate.

**Results**

*Patterns of introgression*

Herbarium specimens

The linear discriminant function predicted original species determinations of all herbarium specimens with 89% accuracy. The spatial distribution of hybrid index values (Fig. 1) demonstrates that many intermediate floral phenotypes originate from areas where the ranges of *A. formosa* and *A. flavescens* overlap. Moreover, within each species, hybrid index values are strongly predicted by spatial proximity to records of the alternative species (Fig. 2, adjusted r2 = 0.74, note that the main effect of distance is non-significant as the distance\*species slopes are in opposing directions). The broad region of overlap appears to divide into several more or less disjunct contact zones. The most striking of these occurs in central Idaho and southwestern Montana, where a large proportion of sampled phenotypes are strongly intermediate (Fig. 1). A second contact zone occurs in the Wenatchee Mountains of central Washington, where *A. flavescens* occurs disjunct from the main body of its distribution. A third contact zone occurs where the two species come into contact in the Columbia Mountains and Rocky Mountains of southeastern BC.

Natural populations

The *Aquilegia* population at Mission Ridge lies in the contact zone in the Wenatchee Mountains of Washington State. The plants in this population occurred on a ski run consisting of dry scree with sparse herbaceous vegetation, a typical habitat for *A. flavescens.*  This population showed evidence of introgression from *A. formosa*, on the basis of intermediate hybrid index scores (Fig. 3A) and a higher frequency of individuals with elevated red reflectance in the sepals (Fig. 3B). Herbarium specimens of both species have been collected in close proximity to the sampling site, and these collections also show intermediate hybrid index values (Fig. 1). Note that we observed a single pink-flowered individual in the Mt. Kobau (pure *A. flavescens*) population, indicating that pink floral mutants can occur. However, this was the only pink-flowered individual observed in our entire survey of the Mt. Kobau population, which was much larger than the Mission Ridge population.

The *Aquilegia* population at Cheops Mountain lies within the contact zone in southeastern BC. The plants at this site were distributed along the western edge of a waterfall from 1200 m to 1350 m in elevation. Hybrid index values from this population show greater affinity to *A. flavescens,* but with noticeable variation in the direction of *A. formosa* (Fig. 3A). We observed elevated values of red reflectance in the sepals of this population, with some individuals having completely pink or nearly red sepals (Fig. 3B). Furthermore, within the same sampling season, *A. formosa* was observed in an adjacent valley on the other side of Cheops Mountain alongside other plants of intermediate phenotype (Jamie Fenneman, personal communication, 2018), indicating the potential for recent local hybridization.

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.* We obtained colorimetric data from an *A. formosa* population along the Pavilion-Clinton Highway approximately 20 km away from the hybrid zone. Color values of this population indicate intermediacy (Fig 3B). While we did not obtain morphological data from this population, while sampling we observed several specimens showing morphological affinity to *A. flavescens*.

Type specimens of *A. flavescens* var. *miniana*

We found that the specimens annotated by Whittemore (MO), on which his judgement that this name refers to typical *A. flavescens* was evidently based*,* have more extreme *A. flavescens-*like phenotypes relative to other specimens collected from the type localities (hybrid index < 0.2 for both). Overall, the sample of type specimens exhibits variation in the direction of *A. formosa* (to a similar extent as the introgressed populations at Cheops Mountain and Mission Ridge).

Clinal variation across a local elevational hybrid zone

In the Marble Range hybrid population, we found a significant association between the hybrid index and elevation while accounting for covariation in floral size (adjusted r2 = 0.49,p < 0.01). The direction of the relationship is as predicted by the altitudinal norms of the species, with *A. flavescens-*like phenotypes occurring at high elevations and *A. formosa*-like phenotypes in the valleys (Fig. 4). The range of hybrid indices present indicate that the plants at high elevation are roughly intermediate between the two species, while the plants at lower elevation bear strong resemblance to typical *A. formosa.*

**Discussion**

*Models of introgression*

Hybridization appears to be a frequent outcome in the broad region of sympatry between *A. flavescens* and *A. formosa* in western North America*.* This fact has been understood for over a century (Macbride and Payson 1917), and our results illustrate this picture clearly.

We argue that the spatial pattern of hybridization between these species represents a mosaic, in that they overlap and hybridize in several disjunct regions. This mosaic structure, which we have characterized at a coarse scale, is recapitulated at finer spatial scales. This is seen in the Marble Range, where a local hybrid population occurs in the midst of the range of *A. formosa*. As the ranges of these species interdigitate in a highly topographically complex landscape, we suspect that many hybrid populations form in a discontinuous fashion throughout the broad region of sympatry.

The geographically widespread gene flow in the *Aquilegia* species examined here also belongs to a type of introgressive hybridization that Heiser (1973) distinguished as ‘dispersed introgression’. While some parapatric species may form only a narrow hybrid zone at the immediate contact zone, as Heiser pointed out, plant systems often display highly dispersed hybridity. A classic example is that of hybridization between *Purshia stansburyana* (cliff rose) and *Purshia tridentata* (bitterbrush) (Stutz and Thomas 1964). In this case the distribution of individual plants showing evidence of hybridity extends much further north that the distribution of the more localized and southern species (*P. stansburyana*).

Stutz and Thomas (1964) argued that this pattern resulted from stepwise introgression, in other words, continued backcrossing beyond immediate contact zones. Our data suggest a similar scenario in *Aquilegia;* within *A. flavescens,* the reasonably linear relationship between the hybrid index and distance to *A. formosa* (Fig. 2) suggests that this association is not purely driven by strongly intermediate early-generation hybrids in immediate contact zones. Rather, introgressive effects of hybridization appear to permeate beyond areas of immediate proximity, likely through continued backcrossing. For *A. formosa,* the relationshippossibly reflects a larger influence of misclassification, as pink-flowered *A. flavescens* individuals are likely to be identified as *A. formosa* (Fig. 2)*.* An alternative explanation to the dispersed pattern of introgression is the persistence of hybrids in zones of historical sympatry. We previously presented evidence for this phenomenon in this species pair(Groh et al. 2019), which has also been observed in oaks (Maze 1968) and poplar (Chhatre et al. 2018; Cronk and Suarez-Gonzalez 2018).

*Ecological considerations on hybridization*

The sheer prevalence of hybrid phenotypes, particularly in central Idaho where the two forms virtually homogenize, suggests that reproductive isolation is overall weak in sympatry. Prezygotic isolation, in form of ecogeographic separation or pollinator isolation, is evidently not strong enough to prevent frequent heterospecific pollen transfer. Concerning the former, sharp transitions between the typical habitats of these species and the presence of intermediate habitats in the steep mountainous areas where they meet can bring them within pollinator dispersal distance. Concerning the latter, it might be expected that differences in floral morphology and color could confer pollinator isolation in these taxa, as has been carefully demonstrated in other hybridizing *Aquilegia* (Grant 1952; Fulton and Hodges 1999). While data on the strength of pollinator isolation between these species is lacking, we note that both are characterized as ‘hummingbird-pollinated’ on the basis of floral morphology (Grant 1994; Whittall and Hodges 2007), and we have observed frequent visits by bees to both, suggesting that the species do not differ qualitatively in their pollination systems. Intrinsic post-zygotic isolation is also weak among closely related *Aquilegia* species (Prazmo 1965; Taylor 1967). Indeed, hybrid unfitness is apparently not strong enough to have driven reinforcement, which could generate the opposite pattern to that observed here – an increase in floral divergence in sympatry (Grossenbacher and Whittall 2011; Hopkins 2013).

Yet, while we see a preponderance of phenotypic intermediacy, parental phenotypes appear to retain an association with their respective parental habitats in sympatry, suggesting that immigrant inviability may play a role in restricting gene flow (Nosil et al. 2005). Payson (1918) first noted that in central Idaho, *A. flavescens* can be found in higher areas, *A. formosa* in the valley bottoms, and intermediate forms in between these two altitudes. We also observe that the easternmost records of *A. flavescens*-type populations in our data setoriginate only from high elevation areas (e.g. Interior Plateau of BC, Wenatchee Mountains of WA). Finally, our finding of a cline in species-type floral morphology along an elevational gradient in the Marble Range hybrid population provides stronger evidence for habitat association.

Although selection is one possible explanation for this pattern there are other possibilities. It could conceivably be driven by plasticity. A common garden experiment would be needed to confirm that this pattern is not driven by plasticity, but there is no clear reason to expect variation in floral shape (independent of overall floral size) to show a plastic response to elevation. Alternatively, it is also possible that this pattern could reflect a neutral decaying cline, if the hybrid zone has formed recently, or if there is spatially restricted gene flow.

If gene flow if sufficient to homogenize the cline, then selection is a likely candidate for maintaining it. Future genetic work could directly address the hypothesis of selection maintaining the cline by controlling for neutral genetic structure across the cline, but several observations suggest that gene flow may not be strongly restricted at such a local scale. First, *A. formosa* has evidently undergone a dramatic latitudinal range expansion following the last glacial retreat and can colonize high-elevation habitats (Groh et al. 2019), implying seed dispersal over this scale is possible. Second, we observed hummingbirds visiting *Aquilegia* flowersin the vicinity, which are capable pollen vectors over these distances. This pattern of habitat association is consistent with selection against at least some immigrant alleles as a primary factor in the maintaining the structure of the hybrid zone in the elevational habitat mosaic of western North America (Nosil et al. 2005; Suarez-Gonzalez et al. 2018).

*A note on taxonomic practice*

Given a longstanding acknowledgement that these two species homogenize in regions of sympatry (Macbride and Payson 1917), and the most recent taxonomic treatment of *Aquilegia* 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), it is remarkable that in our entire data set of herbarium specimens, not a single specimen was originally directly identified as a hybrid. Instead there is an evident tendency to shoehorn even the most intermediate specimens into one or other of the parental species.

The original description of *A. flavescens* var. *miniana* conveys the authors’ impression that it represented introgression from *A. formosa* (Macbride and Payson 1917). However, 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. Our analysis revealed that Whittemore’s assessment was based on two of the most extremely *A. flavescens*-like type specimens and that the type specimens show clear variation in the direction of *A. formosa* (Fig. 3A)*,* suggesting that this variety accurately describes the product of hybridization, as originally implied.

*Concluding remarks*

We have shown that herbarium collections can be leveraged to investigate hybrid zone structure. Our analysis of herbarium collections revealed that *A. flavescens* and *A. formosa* frequently hybridize in contact zones, which is complemented by evidence of introgression in populations from identified contact zones. These include the type specimens of *A. flavescens* var. *miniana*, which evidently refers to the product of introgression from *A. formosa*, as implied by the original authors. We report evidence for a habitat-associated mosaic hybrid zone structure at broad and fine spatial scales, consistent with a role of divergent ecological selection in the divergence and isolation of these taxa.

**Data accessibility**

All data sets and R scripts used in this paper can be found at https://github.com/jgroh/mosaic.

**Acknowledgements**

We are grateful to the staff of several herbaria: to Linda Jennings and Erin Manton for facilitating access to UBC herbarium specimens, and to 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 for assistance with field surveys. We also thank Curtis Björk and Jamie Fenneman for drawing our attention to the Mission Ridge and Cheops Mtn. populations, respectively.

**References**

Barton, N.H., and Hewitt, G.M. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Evol. Syst. **16**: 113–148. doi:10.1146/annurev.es.16.110185.000553.

Bergman, T.J., and Beehner, J.C. 2008. A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). Biol. J. Linn. Soc. **94**(2): 231–240. doi:10.1111/j.1095-8312.2008.00981.x.

Chhatre, V.E., Evans, L.M., DiFazio, S.P, and Keller, S.R. 2018. Adaptive introgression and maintenance of a trispecies hybrid complex in range-edge populations of *Populus*. Mol. Ecol. **27**(23): 4820–4838. doi:10.1111/mec.14820.

Crepet, W.L., and Niklas, K.J. 2009. Darwin’s second “abominable mystery”: Why are there so many angiosperm species? Am. J. Bot. **96**(1): 366–381. doi:10.3732/ajb.0800126.

Cronk, Q.C., and Suarez-Gonzales, A. 2018. The role of interspecific hybridization in adaptive potential at range margins. Mol. Ecol. **27**(23): 4653–4656. doi: 10.1111/mec.14927.

Daru, B.H., Park, D.S., Primack, R.B., Willis, C.G., Barrington, D.S., Whitfeld, T.J.S., Seidler, T.G., Sweeney, P.W., Foster, D.R., Ellison, A.M., and Davis, C.C. 2018. Widespread sampling biases in herbaria revealed from large-scale digitization. New Phytol. **217**(2): 939–955. doi:10.1111/nph.14855.

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

Fulton, M., and Hodges, S.A. 1999. Floral isolation between *Aquilegia formosa* and *Aquilegia pubescens*. Proc. R. Soc. B, **266**(1435): 2247–2252. doi:10.1098/rspb.1999.0915.

Grant, V. 1949. Pollination Systems as Isolating Mechanisms in Angiosperms. Evolution, **3**(1): 82–97. doi:10.1111/j.1558-5646.1949.tb00007.x.

Grant, V. 1952. Isolation and hybridization between *Aquilegia formosa* and *A. pubescens*. Aliso, **2**(4): 341–360. Available from https://scholarship.claremont.edu/aliso/vol2/iss4/3.

Grant, V. 1971. Plant Speciation. Columbia University Press, New York.

Grant, V. 1994. Historical development of ornithophily in the western North American flora. Proc. Natl. Acad. Sci. U. S. A. **91**(22): 10407–10411. doi:10.1073/pnas.91.22.10407.

Groh, J.S., Percy, D.M., Björk, C.R., and Cronk, Q.C.B. 2019. On the origin of orphan hybrids between *Aquilegia formosa* and *Aquilegia flavescens.* [Online]. AoB Plants, **11**(1): ply071. doi:10.1093/aobpla/ply071.

Grossenbacher, D.L., and Stanton, M.L. 2014. Pollinator-mediated competition influences selection for flower-color displacement in sympatric monkeyflowers. Am. J. Bot. **101**(11): 1915–1924. doi:10.3732/ajb.1400204.

Grossenbacher, D.L., and Whittall, J.B. 2011. Increased floral divergence in sympatric monkeyflowers. Evolution, **65**(9): 2712–2718. doi:10.1111/j.1558-5646.2011.01306.x.

Harrison, R.G. 1986. Pattern and process in a narrow hybrid zone. Heredity, **56**: 337–349. doi:10.1038/hdy.1986.55.

Heiser, C.B. 1973. Introgression re-examined. Bot. Rev. **39**: 347–366. doi:10.1007/BF02859160.

Hijmans, R.J. 2017. geosphere: Spherical trigonometry. R package version 1.5–7. https://CRAN.R-project.org/package=geosphere.

Hodges, S.A., and Arnold, M.L. 1994. Columbines: a geographically widespread species flock. Proc. Natl. Acad. Sci. U. S. A. **91**(11): 5129–5132. doi:10.1073/pnas.91.11.5129.

Hopkins, R. 2013. Reinforcement in plants. New Phytol. **197**(4): 1095–1103. doi:10.1111/nph.12119.

Kahle, D., and Wickham, H. 2013. ggmap: Spatial visualization with ggplot2. R J. **5**(1): 144–161. doi:10.32614/RJ-2013-014. https://CRAN.R-project.org/package=ggmap.

Macbride, J.F., and Payson, E.B. 1917. New or otherwise interesting plants from Idaho. Contributions from the Gray Herbarium of Harvard University, **49**: 60–72. Available from https://www.jstor.org/stable/41763936.

Mallet, J., and Barton, N.H. 1989. Strong natural selection in a warning-color hybrid zone. Evolution, **43**(2): 421–431. doi:10.1111/j.1558-5646.1989.tb04237.x.

Maze, J. 1968. Past hybridization between *Quercus macrocarpa* and *Quercus* gambelii. Brittonia, **20**(4): 321–333. doi:10.2307/2805689.

McAllister, C.A., McKain, M.R., Li, M., Bookout, B., and Kellogg, E.A. 2018. Specimen-based analysis of morphology and the environment in ecologically dominant grasses: the power of the herbarium. Phil. Trans. R. Soc. B, **374**(1763): 20170403. doi:10.1098/rstb.2017.0403.

Milano, E.R., Kenney, A.M., and Juenger, T.E. 2016. Adaptive differentiation in floral traits in the presence of high gene flow in scarlet gilia (*Ipomopsis aggregata*). Mol. Ecol. **25**(23): 5862–5875. doi:10.1111/mec.13752.

Namkoong, G. 1966. Statistical analysis of introgression. Biometrics, **22**(3): 488–502. doi:10.2307/2528184.

van der Niet, T., Johnson, S.D., and Linder, H.P. 2006. Macroevolutionary Data Suggest a Role for Reinforcement in Pollination System Shifts. Evolution, **60**(8): 1596–1601. doi:10.1111/j.0014-3820.2006.tb00504.x.

Nosil, P., Vines, T.H., and Funk, D.J. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution, **59**(4): 705–719. doi:10.1111/j.0014-3820.2005.tb01747.x.

Payson, E.B. 1918. The North American species of *Aquilegia.* Contrib. U. S. Natl. Herb. **20**: 133–157. Available from https://repository.si.edu/handle/10088/26985.

Prazmo, W. 1965. Cytogenic studies on the genus *Aquilegia.* IV. Fertiilty relationships among the *Aquilegia* species. Acta Soc. Bot. Pol. **34**(4): 667–685. doi:10.5586/asbp.1965.046.

R Core Team. 2018. R: A language and environment for statistical

computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rand, D.M., and Harrison, R.G. 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. Evolution, **43**(2): 432–449. doi:10.1111/j.1558-5646.1989.tb04238.x.

Rieseberg, L.H., Whitton, J., and Gardner, K. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. Genetics, **152**(2): 713–727. Available from https://www.genetics.org/content/152/2/713.

Riquet, F., Liautard-Haag, C., Woodall, L., Bouza, C., Louisy, P., Hamer, B., Otero-Ferrer, F., Aublanc, P., Béduneau, V., Briard, O., El Ayari, T., Hochscheid, S., Belkhir, K., Arnaud-Haond, S., Gagnaire, P.A., and Bierne, N. 2019. Parallel pattern of differentiation at a genomic island shared between clinal and mosaic hybrid zones in a complex of cryptic seahorse lineages. Evolution, **73**(4): 817–835. doi:10.1111/evo.13696.

Runquist, R.D.B., and Moeller, D.A. 2014. Floral and mating system divergence in secondary sympatry: testing an alternative hypothesis to reinforcement in *Clarkia.* Ann. Bot. **113**(2): 223–235. doi:10.1093/aob/mct218.

Schemske, D.W., and Bradshaw, H.D. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. U. S. A. **96**(21): 11910–11915. doi:10.1073/pnas.96.21.11910.

Stutz, H.C., and Thomas, L.K. 1964. Hybridization and introgression in *Cowania* and *Purshia*. Evolution, **18**(2): 183–195. doi:10.1111/j.1558-5646.1964.tb01590.x.

Suarez-Gonzalez, A., Lexer, C., and Cronk, Q.C.B. 2018. Adaptive introgression: a plant perspective. [Online]. Biol. Lett. **14**(3): 20170688. doi: 10.1098/rsbl.2017.0688.

Sung, C-J., Bell, K.L., Nice, C.C., and Martin, N.H. 2018. Integrating Bayesian genomic cline analyses and association mapping of morphological and ecological traits to dissect reproductive isolation and introgression in a Louisiana Iris hybrid zone. Mol. Ecol. **27**(4): 959–978. doi:10.1111/mec.14481.

Szymura, J.M., and Barton, N.H. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads , *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution, **40**(6): 1141–1159. doi:10.1111/j.1558-5646.1986.tb05740.x.

Tavares, H., Whibley, A., Field, D.L., Bradley, D., Couchman, M., Copsey, L., Elleouet, J., Burrus, M., Andalo, C., Li, M., Li, Q., Xue, Y., Rebocho, A.B., Barton, N.H., and Coen, E. 2018. Selection and gene flow shape genomic islands that control floral guides. Proc. Natl. Acad. Sci. U. S. A. **115**(43): 11006–11011. doi:10.1073/pnas.1801832115.

Taylor, R.J. 1967. Interspecific hybridization and its evolutionary significance in the genus *Aquilegia*. Brittonia **19**(4): 374–390. doi:10.2307/2805535.

Turner, L.M., and Harr, B. 2014. Genome-wide mapping in a house mouse hybrid zone reveals hybrid sterility loci and Dobzhansky-Muller interactions. [Online]. eLife, **3**: e02504. doi:10.7554/eLife.02504.

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

Vines, T.H., Köhler, S.C., Thiel, M., Ghira, I., Sands, T.R., MacCallum, C.J., Barton, N.H., and Nürnberger, B. 2003. The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata.* Evolution, **57**(8): 1876–1888. doi:10.1111/j.0014-3820.2003.tb00595.x.

Whittall, J.B., and Hodges, S.A. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. **447**: 706–709. doi:10.1038/nature05857.

Whittemore, A.T. 1997. *Aquilegia. In* Flora of North America North of Mexico. *Edited by*

Flora of North America Editorial Committee, eds. 1993+. New York and Oxford. Available from http://beta.floranorthamerica.org/Aquilegia.

**Tables**

Table 1.Sampled natural populations and type specimens of *A. flavescens* var. *miniana*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Population** | **Identification** | **Mean Hybrid Index** | **Location** | **Lat., Long.** |
| 1 | Mt. Kobau | *A. flavescens* | 0.04 | Southern British Columbia (BC), Canada | 49.113°,  -119.674° |
| 2 | Mission Ridge | introgressed *A. flavescens* | 0.27 | Wenatchee Mts,  WA, USA | 47.284°,  -120.408° |
| 3 | *A. flavescens* var*. miniana* type specimens | introgressed *A. flavescens* | 0.28 | Central Idaho, USA | ----- |
| 4 | Cheops Mtn. | introgressed *A. flavescens* | 0.32 | Glacier National Park, BC, Canada | 51.266°,  -117.546° |
| 5 | Porcupine Ridge | hybrid | 0.42 | Marble Range, BC | 51.109°,  -120.831° |
| 6 | Pavilion-Clinton Rd. | introgressed *A. formosa* | ----- | Near Marble Range, BC | 50.970°,  -121.780° |
| 7 | Robert’s Lake | *A. formosa* | 0.96 | Vancouver Island, BC | 50.225°,  -125.55° |

**Figure Captions**

Figure 1.The spatial distribution of a floral morphology hybrid index reveals broad areas of phenotypic intermediacy in contact zones between Aquilegia flavescens and A. formosa.Colored ovals circumscribe contact zones referred to in text. Top to bottom, pink: southeastern BC, blue: Wenatchee Mts., WA, green: central Idaho. Inset: representative flowers of (A) A. formosa, (B) A. flavescens and (C) introgressed A. flavescens.

Figure 2.Geographic proximity to the alternative species predicts the hybrid index, indicative of introgressive hybridization in contact zones. Circles: Aquilegia formosa, squares: A. flavescens. Solid lines give least-squares regression lines. Dotted lines indicate 95% confidence bands.

Figure 3. (A) Floral morphology hybrid index values for populations of Aquilegia formosa, A. flavescens, and introgressed populations. Data are from natural populations sampled in the Pacific Northwest in the summers of 2017 and 2018 and type specimens of A. flavescens var. miniana. Horizontal axis labels refer to population numbers in Table 1. (B) Sepal reflectance of natural populations of A. flavescens, A. formosa, and introgressed populations.

Figure 4**.** Hybrid index values along an elevational transect through a hybrid population in the Marble Range, British Columbia show evidence of habitat association. Lower values of the hybrid index indicate closer resemblance to typical *A. flavescens*, while higher values indicate closer resemblance to typical *A. formosa.* Shaded area represents 95% confidence band.