

**Mosaic hybrid zone structure in two species of columbine (*Aquilegia*)**

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## Abstract

Herbarium specimens provide an extensive, but often underutilized, resource for morphological analysis. Potentially, they can be used to describe the broad structure of hybrid zones, leading to insights about evolutionary dynamics during secondary contact and the nature of reproductive isolation between taxa. We demonstrate the use of herbarium collections to describe the structure of a hybrid zone between two North American columbines, *Aquilegia formosa* and *A. flavescens*. Hybrid indices were calculated from floral morphology measurements and mapped according to specimen geolocations, revealing a 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 ecological divergence maintaining the structure of the hybrid zone.

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

## Introduction

Describing the structure of hybrid zones has important consequences for our understanding of diversification. A detailed knowledge of hybrid zone structure can serve as the basis for models that allow dissection of the evolutionary forces at play during secondary contact (Barton and Hewitt 1985; Szymura and Barton 1986), and aid in identifying the phenotypes and genomic regions underlying reproductive isolation (Rieseberg et al. 1999; Carneiro et al. 2014; Sung et al. 2018). Importantly, interpreting hybrid zone structure can teach us about the selective forces maintaining reproductive barriers between taxa (Harrison 1986; Mallet and Barton 1989; Nosil et al. 2005). Hybrid zones that present as a monotonic cline between two parental taxa are typically modelled as a balance between gene flow and selection against hybrids, i.e. the ‘tension-zone’ model (Barton and Hewitt 1985), and are often associated with intrinsic reproductive barriers (e.g. Turner and Harr 2014). Alternatively, hybridization can occur at habitat boundaries within a broad habitat mosaic, i.e. the ‘mosaic’ model (Harrison 1986); such hybrid zones are thought to be maintained by divergent ecological selection (e.g. Ravinet et al. 2015). 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 similar or context-specific 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 novel 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 in particular offers several advantages in this enterprise. They can be easily measured from herbarium collections in some taxa, and there is a natural bias for collectors to collect plants in flowering condition (Daru et al. 2018). Moreover, 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). Separate from their role in 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). While careful experiments are necessary to address these alternative hypotheses, documenting floral phenotypes patterns in areas of sympatry is a prerequisite step in understanding the fate of trait divergence during secondary contact in recently-diverged taxa.

The columbines (*Aquilegia*) are well-known for showing impressive floral diversity despite broad similarity at the genomic level and extensive natural hybridization (Hodges and Arnold 1994; Filiault et al. 2018). In the present work, we show results from an analysis of herbarium collections in an effort to describe the structure of hybrid zone between two species of columbine in western North America, *A. flavescens* S.Watson and *A. formosa* Fisch. ex DC. We combine this with phenotypic analyses of population samples from 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. Last, we analyzed phenotypes along a local elevational gradient in a previously identified hybrid population in British Columbia, Canada (Groh et al. 2018), to examine evidence for divergent ecological selection between parental phenotypes as an explanation for the observed mosaic hybrid zone structure.

## **Materials and Methods**

### *Herbarium data collection*

We used a set of 151 floral phenotypes extracted from herbarium specimens of *Aquilegia formosa* and *A. flavescens*, collected as part of a previous study (Groh et al. 2018), supplemented with an additional 107 herbarium 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 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 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 et al. 2018). Details of herbarium specimens are found in supplemental data associated with this article.

### *Construction of the hybrid index*

We used a linear discriminant function based on a training set of herbarium specimen phenotypes to calculate hybrid index values. 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, and any specimens which were labelled as putative hybrids based on a preliminary visual assessment. Linear discriminant analysis (LDA) was implemented using the R package MASS (Venables and Ripley 2002). Discriminant scores for all specimens were then predicted with equal prior probability for each species. Note that this hybrid index for specimens not in the training set is therefore not influenced by their initial identification status. 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 fully 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. Indeed, we found that smaller flowers of either species 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. In a principal components analysis (PCA) of all individuals with

complete data from the herbarium data set and across two sampling seasons (522 individuals in total), PC1 separated the two species, and PC2 captured variation in overall flower size. As we found significant correlations between our predicted discriminant scores and size values, we implemented a size correction procedure by taking residuals from a regression of discriminant scores onto PC2, and added these to discriminant scores predicted by within-population regression lines 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 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 hybrid index could be predicted by 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). 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 hybrid indices. To formally test for an association of reduced floral divergence and sympatry, we plotted the linearly-

transformed hybrid index against the log-transformed minimum distance to a record of the other species, and performed within-species linear regressions.

### *Sampling of natural populations*

As herbarium specimen collections generally capture only a single individual from one locality, and collectors may be biased towards collecting aberrant plants, we collected population samples from contact regions to corroborate the patterns revealed through the analysis of herbarium specimen phenotypes. In addition to populations previously sampled (Groh et al. 2018), we sampled two further *A. flavescens*-like populations in regions of range contact with *A. formosa*. Similarly, we included two additional populations of plants resembling *A. formosa*: one introgressed population and one apparently pure population. The two *A. flavescens*-like populations were located at Mission Ridge ski resort in the Wenatchee Mountains of Washington State, USA, and on Cheops Mountain within Glacier National Park in the Columbia Mountains of BC. The *A. formosa*-type populations include one “pure” population from Manning Park, BC, and an introgressed population along the Pavilion-Clinton Highway, approximately 20 km away from a previously characterized hybrid population in the Marble Range, BC (Groh et al. 2018). All sampled populations are listed with details in Table 1.

At each site, we 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 (Datacolor™, 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 red to green reflectance was centered around zero by log-transformation. We measured floral characters from pressed specimens and calculated discriminant scores for individuals from sampled populations under an equal prior probability for each species. Size-correction was performed as described above by displacing individual points from predicted population discriminant scores at the mean overall size by the value of their residuals from their respective population regression line. Size-adjusted discriminant scores of sampled populations were then transformed by the sigmoid function.

#### *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 Idaho, from 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, that any association between the genetic bases for altitudinal adaptation and floral morphology could reveal a cline in species-type floral morphology across elevation. We revisited the Marble Range hybrid population and sampled plants along an elevational cline across adjacent drainages descending from the hybrid population. Size-corrected hybrid index values were regressed against collection elevation to test for an association between elevation and species-type floral morphology.

## Results

### *Patterns of introgression*

#### Herbarium specimens

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). The reasonably linear relationship suggests that the effect is not purely driven by strongly intermediate early-generation hybrids which occurring in contact zones. Rather, introgressive effects of hybridization appear to permeate within each species beyond areas of immediate proximity, likely through continued backcrossing.

The broad region of range overlap can be divided into several more or less disjunct contact zones. The most striking of these occurs in central Idaho and southwestern Montana, where the phenotypes of the two species homogenize to a considerable extent. Macbride and Payson (1917) noted this over a century ago, and even wrote that botanists had previously

recognized that these two species seem to merge in this territory and become virtually indistinguishable. A second contact zone occurs in the Wenatchee Mountains of central Washington. 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. A third contact zone occurs where the two species come into contact in the Columbia Mountains and Rocky Mountains of southeastern BC. Here, the two species can often be found in close proximity, along with a variety of hybrids (Jamie Fenneman, personal communication, 2018). While collections of *Aquilegia* from this area are less extensive, samples with intermediate morphology are clearly represented.

### Natural Populations

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 in between 1200 m and 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). While we observed a similar phenotype in one individual in the Mt. Kobau (pure *A. flavescens*) population, both the frequency and intensity of elevated red reflectance exceeded what we observed in the Mt. Kobau population. While pink variants of pure *A. flavescens* do evidently occur (Whittemore,

1997), the floral intermediacy of this population together with its close proximity to *A. formosa* are consistent with a history of introgression from the latter species. Indeed, *A. formosa* has been found in an adjacent valley on the other side of Cheops Mountain alongside other plants of intermediate phenotype (Jamie Fenneman, personal communication, 2018).

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 habitat more typical of *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).

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 show intermediacy (Fig 3B). While we did not obtain morphological data from this population, we visually observed some morphological affinity to *A. flavescens*.

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

*Aquilegia flavescens* var. *miniana* refers to a variety of this species with pink colored sepals, which are typically yellow. The original description of this entity 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. 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 the most extreme *A. flavescens*-like phenotypes out of all specimens collected from the type locality. However, the sample of type specimens exhibits 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 implied.

#### *Clinal variation across a local elevational hybrid zone*

In the Marble Range hybrid population, we found evidence for clinal variation in the floral morphology hybrid index across an elevational transect. Moreover, the transition between phenotypes varies in the expected direction according to the altitudinal norms of the species, with *A. flavescens*-like phenotypes at high elevations transition into *A. formosa*-like phenotypes in the valleys. While a common garden experiment would be needed to confirm this pattern is not driven by plasticity, there is no clear reason to suspect variation in floral shape that is independent of overall floral size to show a plastic response to elevation. It is nonetheless also possible that this pattern could reflect a neutral decaying cline if the hybrid zone formed recently. Future genetic work could directly address the hypothesis of selection 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, *Aquilegia* have evidently undergone a dramatic altitudinal range expansion following the last glacial retreat, necessitating the potential for seed dispersal over a local scale. Second, we observed hummingbirds visiting *Aquilegia* flowers in the vicinity, which are highly capable pollen vectors over the spatial scale in question.

## Discussion

We have characterized the hybrid zone between *A. formosa* and *A. flavescens* as conforming to a mosaic model. In several disjunct regions, the ranges of these species interdigitate, and hybridization is evidently very common in such areas, such that intermediate phenotypes can virtually replace the typical forms of the species, as in central Idaho (Fig. 1; MacBride and Payson, 1917). Furthermore, intermediacy is predicted by proximity to the alternative species (Fig. 2). This type of pattern is consistent with a “divergence and refusion” type of plant speciation cycle (Grant 1971). Yet, in regions where the species co-occur, parental phenotypes appear to retain an association with their respective parental habitats. This was first observed by Payson (1918), who 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. While our herbarium geolocation data are generally too coarse to allow examination of fine-scale habitat association in local contact zones, our biogeographic analysis supports a basic pattern of habitat-association; the easternmost records of *A. flavescens*-type populations originate predominantly from high elevation areas. Furthermore, 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. These patterns are consistent with a scenario of ecological divergence to different climatic niches in allopatry followed by range expansion of either or both species, bringing them into secondary contact in regions with complex elevational landscapes. This is necessarily the case in southeastern British Columbia, where *Aquilegia* populations have been present only since the Pleistocene glacial retreat. This pattern of habitat association, particularly in the Marble Range population where the association is maintained within a plausible dispersal distance for *Aquilegia* pollinators, 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).

In spite of evidence for habitat association in the mosaic hybrid zone, the sheer prevalence of hybrid phenotypes, particularly in central Idaho, begs an explanation. It is immediately clear that prezygotic isolation is not strong enough to prevent somewhat frequent heterospecific pollen transfer. In *Aquilegia*, closely-related species are highly interfertile in artificial crosses (Taylor 1967), so prezygotic isolation in this species pair is likely governed primarily by ecogeographic separation and pollinator isolation. Explanations for the formation of hybrid populations must concern these two isolations mechanisms. Concerning the former, two factors potentially contribute. First, sharp transitions between the elevational niches characteristic of these species in the steep mountainous areas where they come into contact can bring them within pollinator dispersal distance of one another. Second, while most commonly found in low areas along creek sides, *A. formosa* appears able to commonly colonize

high-elevation habitats (Groh et al. 2018), potentially bridging elevational gaps between populations of the two species in contact zones. Concerning the latter, it might be expected that differences in floral morphology and color should confer pollinator isolation in these taxa, as has been carefully demonstrated in other hybridizing *Aquilegia* (Grant 1952; Fulton and Hodges 1999). We have no data on the potential action of pollinator isolation in this system, but it is worth noting that both species 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 species, suggesting that pollinator-isolation may be absent or weak. Where hybrid offspring do occur, the establishment of populations of hybrids indicates weak post-zygotic isolation. This could reflect the availability of intermediate habitats in the region of overlap (Anderson 1948), which seems rather likely given the highly heterogeneous landscapes in which the two species come into contact. Indeed, selection against hybrids is evidently not strong enough to drive reinforcement, which would generate the opposite pattern to that observed here – an increase in floral divergence in sympatry (Grossenbacher and Whittall 2011; Hopkins 2013).

We have shown that the use of herbarium specimens can be effectively used to investigate hybrid zone structure. Our analysis of herbarium collections revealed that *Aquilegia formosa* and *A. flavescens* frequently hybridize in contact zones, which is complemented by evidence of introgression in populations from contact zones identified in our biogeographic analysis. These include the type specimens of *A. flavescens* var. *miniana*, which evidently refers to the *A. flavescens* × *A. formosa* hybrid, as implied by the original authors. We find evidence of a habitat-associated mosaic hybrid zone structure in our biogeographic analysis and in a species



morphology cline across a local elevational hybrid zone, consistent with a role of ecological selection in the divergence and isolation of these taxa.

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## References

- Anderson, E. 1948. Hybridization of the habitat. *Evolution*. **2**(1): 1–9.
- Barton, N.H., and Hewitt, G.M. 1985. Analysis of Hybrid Zones. *Annu. Rev. Ecol. Evol. Syst.* **16**: 113–148.
- 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.
- Carneiro, M., Albert, F.W., Afonso, S., Pereira, R.J., Burbano, H., Campos, R., Melo-Ferreira, J., Blanco-Aguiar, J.A., Villafuerte, R., Nachman, M.W., Good, J.M., and Ferrand, N. 2014. The genomic architecture of population divergence between subspecies of the European Rabbit. *PLoS Genet.* **10**(8). doi:10.1371/journal.pgen.1003519.

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

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

378 Filiault, D.L., Ballerini, E.S., Mandakova, T., Akoz, G., Derieg, N.J., Schmutz, J., Jenkins, J.,  
379 Grimwood, J., Shu, S., Hayes, R.D., Hellsten, U., Barry, K., Yan, J., Mihaltcheva, S.,  
380 Karafiatova, M., Nizhynska, V., Kramer, E.M., Lysak, M.A., Hodges, S.A., and Nordborg, M.  
381 2018. The *Aquilegia* genome provides insight into adaptive radiation and reveals an  
382 extraordinarily polymorphic chromosome with a unique history. *Elife*: 1–31.  
383 doi:10.7554/eLife.36426

384 Fulton, M., and Hodges, S.A. 1999. Floral isolation between *Aquilegia formosa* and *Aquilegia*  
385 *pubescens*. *Proc. R. Soc. B Biol. Sci.* **266**: 2247–2252.

386 Grant, V. 1949. Pollination Systems as Isolating Mechanisms in Angiosperms. **3**(1): 82–97.

387 Grant, V. 1952. Isolation and hybridization between *Aquilegia formosa* and *A. pubescens*. *El*  
388 *Aliso* **2**(4): 341–360.

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

390 Grant, V. 1994. Historical development of ornithophily in the western North American flora.  
391 *Evolution*. **91**: 10407–10411.

392 Groh, J.S., Percy, D.M., Björk, C.R., and Cronk, Q.C.B. 2018. On the origin of orphan hybrids  
393 between *Aquilegia formosa* and *Aquilegia flavescens*. *AoB Plants* **11**(1): 1–15.

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**(3): 337–349. doi:10.1038/hdy.1986.55.

Hijmans, R.J. 2016. geosphere: Spherical geometry.

Hodges, S.A., and Arnold, M.L. 1994. Columbines: a geographically widespread species flock Author. *Proc. Natl. Acad. Sci. USA* **91**(11): 5129–5132.

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

Kahle, D., and Wickham, H. 2013. ggmap: Spatial Visualization with ggplot2. *R J.* **5**(1): 144–161. Available from <https://journal.r-project.org/archive/2013/RJ-2013-014/index.html>.

Mallet, J., and Barton, N.H. 1989. Strong natural selection in a sarning-color hybrid zone. *Evolution.* **43**: 421–431.

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. *Philos. Trans. R. Soc., B* **374**(1763), 20170403. doi:<https://doi.org/10.1098/rstb.2017.0403>

Milano, E.R., Kenney, A.M., and Juenger, T.E. 2016. Adaptive differentiation in floral traits in the

416 presence of high gene flow in scarlet gilia (*Ipomopsis aggregata*). Mol. Ecol. **25**(23): 5862–  
417 5875. doi:10.1111/mec.13752.

418 van der Niet, T., Johnson, S.D., and Linder, H.P. 2006. Macroevolutionary Data Suggest a Role  
419 for Reinforcement in Pollination System Shifts. Evolution. **60**(8): 1596–1601.  
420 doi:10.1554/05-705.1.

421 Nosil, P., Vines, T.H., and Funk, D.J. 2005. Perspective: Reproductive Isolation Caused By Natural  
422 Selection Against Immigrants From Divergent Habitats. Evolution. **59**(4): 705.  
423 doi:10.1554/04-428.

424 Ravinet, M., Hynes, R., Poole, R., Cross, T.F., McGinnity, P., Harrod, C., and Prodöhl, P.A. 2015.  
425 Where the lake meets the sea: Strong reproductive isolation is associated with adaptive  
426 divergence between lake resident and anadromous three-spined sticklebacks. PLoS One  
427 **10**(4): 1–22. doi:10.1371/journal.pone.0122825.

428 Rieseberg, L.H., Whitton, J., and Gardner, K. 1999. Hybrid zones and the genetic architecture of  
429 a barrier to gene flow between two sunflower species. Genetics **152**(2): 713–727.

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

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

438 Suarez-Gonzalez, A., Lexer, C., and Cronk, Q.C.B. 2018. Adaptive introgression: a plant  
 439 perspective. *Biol. Lett.* **14**(3). doi:10.1098/rsbl.2017.0688.  
 440 Sung, C.J., Bell, K.L., Nice, C.C., and Martin, N.H. 2018. Integrating Bayesian genomic cline  
 441 analyses and association mapping of morphological and ecological traits to dissect  
 442 reproductive isolation and introgression in a Louisiana Iris hybrid zone. *Mol. Ecol.* **27**(4):  
 443 959–978. doi:10.1111/mec.14481.  
 444 Szymura, J.M., and Barton, N.H. 1986. Genetic analysis of a hybrid zone between the fire-bellied  
 445 toads, *Bombina bombina* and *B. variegata*, near cracow in southern Poland. *Evolution.*  
 446 **40**(6): 1141–1159. doi:10.2307/2408943.  
 447 Taylor, R.J. 1967. Interspecific hybridization and its evolutionary significance in the genus  
 448 *Aquilegia*. *Brittonia* **19**: 374–390.  
 449 Turner, L.M., and Harr, B. 2014. Genome-wide mapping in a house mouse hybrid zone reveals  
 450 hybrid sterility loci and Dobzhansky-Muller interactions. *Elife* **3**: 1–25.  
 451 doi:10.7554/eLife.02504.  
 452 Venables, W.N., and Ripley, B.D. 2002. *Modern Applied Statistics with S-Plus*. Springer, New  
 453 York.  
 454 Whittall, J.B., and Hodges, S.A. 2007. Pollinator shifts drive increasingly long nectar spurs in  
 455 columbine flowers. *Nature* **447**: 1–6. doi:10.1038/nature05857.  
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## Tables

Table 1. Sampled natural populations of *Aquilegia*

Population	Identification	Location	Lat., Long.
Mt. Kobau	<i>A. flavescens</i>	Southern British Columbia (BC), Canada	49.113°, -119.674°
Mission Ridge	introgressed <i>A. flavescens</i>	Wenatchee Mts, Washington State, USA	47.284°, -120.408°
Cheops Mtn.	introgressed <i>A. flavescens</i>	Glacier National Park, BC, Canada	51.266°, -117.546°
Porcupine Ridge	hybrid	Marble Range, BC	51.109°, -120.831°
Pavilion-Clinton Rd.	introgressed <i>A. formosa</i>	Near Marble Range, BC	50.970°, -121.780°
Clearwater	<i>A. formosa</i>	Central BC	51.729°, -120.030°
Manning Park	<i>A. formosa</i>	Southern BC	49.054°, -120.908°
Robert's Lake	<i>A. formosa</i>	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*.

Coloured ovals circumscribe contact zones referred to in text. Top to bottom, pink: southeastern British Columbia, blue: Wenatchee Mts., 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. Red: *Aquilegia formosa*, yellow: *A. flavescens*.

Figure 3. (A) Floral morphology hybrid index values for populations of *Aquilegia formosa*, *A. flavescens*, hybrids 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*. Left to right, yellow = Mt. Kobau “pure” *A. flavescens*; orange: Mission Ridge introgressed *A. flavescens*; purple = type specimens of *A. flavescens* var. *miniana* from central Idaho; green = Cheops Mtn. introgressed *A. flavescens*; pink = Porcupine Ridge hybrids; gray = Clearwater “pure” *A. formosa*; brown = Manning park “pure” *A. formosa*; blue-green = Robert’s Lake “pure” *A. formosa*. (B) Sepal reflectance of natural populations of *A. flavescens*, *A. formosa*, hybrids, and introgressed populations. Colors are the same as above with the addition of turquoise = Pavilion-Clinton Highway introgressed *A. formosa* (left to right, Mt. Kobau,

490 Mission Ridge, Cheops Mtn., Porcupine Ridge, Pavilion-Clinton Highway, Manning Park,  
491 Clearwater).

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493 Figure 4. Hybrid index values along an elevational transect through a hybrid population in the  
494 Marble Range of British Columbia show evidence of habitat association. Lower values of the  
495 hybrid index indicate closer resemblance to typical *A. flavescens*, while higher values indicate  
496 closer resemblance to typical *A. formosa*.