RESEARCH ARTICLE

AMERICAN JOURNAL OF BOTANY

Genomewide variation provides insight into evolutionary relationships in a monkeyflower species complex (*Mimulus* sect. *Diplacus*)[1]

Madeline A. Chase, Sean Stankowski, and Matthew A. Streisfeld[2]

PREMISE OF THE STUDY: Evolutionary radiations provide excellent opportunities to study the origins of biodiversity, but rapid divergence and ongoing gene flow make inferring evolutionary relationships among taxa difficult. Consequently, combining morphological and genomic analyses will be necessary to clarify the evolutionary history of radiations. We used an integrative approach to shed light on relationships within a diverse radiation of monkeyflowers (*Mimulus* section *Diplacus*) with a controversial taxonomic history.

METHODS: We used genomewide single nucleotide polymorphism data and a combination of phylogenetic and population genomic analyses to infer the evolutionary relationships within the group. Tests for hybridization were performed to reveal sources of shared variation, and multivariate analyses of floral trait data were conducted to examine the correspondence between phenotypic and phylogenetic data.

KEY RESULTS: We identified four primary clades with evidence for some shared variation among them. We also detected evidence for recent gene flow between closely related subclades and populations. Strong discordance between floral trait and molecular data provides evidence for divergent and convergent phenotypic evolution.

CONCLUSIONS: *Mimulus* section *Diplacus* has all the hallmarks of a rapid radiation, including diverse taxa that are at different stages of divergence, extensive shared variation among taxa, and complex patterns of phenotypic evolution. Our findings will direct future evolutionary research and have important taxonomic implications that highlight the need for a new revision of section *Diplacus*.

KEY WORDS adaptive radiation; *Mimulus aurantiacus*; Phrymaceae; phylogenomics; RADseq

Evolutionary radiations provide excellent opportunities to study the processes that drive phenotypic divergence and speciation. However, because rapid divergence is a hallmark of radiations, past efforts to infer evolutionary relationships among their taxa often have been unsuccessful due to the low levels of sequence variation contained in the few genes typically used for phylogenetic analysis (Qiu et al., 1999; Wolfe et al., 2006; Jarvis et al., 2014). Advances in sequencing technology have overcome this limitation by greatly expanding the amount of the genome that can be queried. For example, reduced representation techniques, like restriction site associated DNA sequencing (RADseq), can be used to obtain data from thousands of genomic regions that can be combined in a single analysis (Miller et al., 2007; Baird et al., 2008). This approach has allowed relationships to be resolved in some radiations for the first time (Emerson et al., 2010; Heliconius Genome Consortium, 2012; Wagner et al., 2013; Eaton and Ree, 2013; Fontaine et al., 2015; McCluskey and Postlethwait, 2015; Wessinger et al., 2016; Pease et al., 2016).

Although genomewide phylogenies provide an excellent framework for understanding the history of radiations, a single bifurcating topology also may obscure important details about the divergence process (Mallet et al., 2015; Hahn and Nakhleh, 2016). One reason a bifurcating tree may be inappropriate is because the genomes of recently radiated taxa often are complex genealogical mosaics that have been shaped by a range of processes, including incomplete lineage sorting and introgressive hybridization (Maddison, 1997; Keller et al., 2013; Fontaine et al., 2015; Mallet et al., 2015; Pease et al., 2016). Although these processes once were considered to generate

[1] Manuscript received 12 June 2017; revision accepted 11 September 2017.
Institute of Ecology and Evolution, 335 Pacific Hall 5289, University of Oregon, Eugene, Oregon 97405 USA
[2] Author for correspondence (e-mail: mstreis@uoregon.edu)
https://doi.org/10.3732/ajb.1700234

noise that prevented the resolution of taxon-level relationships, recent studies have shown that they often are important sources of adaptive alleles that can drive speciation (Heliconius Genome Consortium, 2012; Keller et al., 2013; Fontaine et al., 2015; Lamichhaney et al., 2015; Pease et al., 2016; Wallbank et al., 2016). Thus, a holistic understanding of relationships within radiations requires integrated approaches using both tree-based and nontree-based analyses that can reveal patterns of divergence and sources of shared variation among taxa.

These new insights from genomic data also have sparked discussion about the nature of species, which has important implications for how we choose to delineate them. Although reproductive isolation always has been the cornerstone of the biological species concept (Mayr, 1995), we now know that speciation is a continuous process, and radiations will contain taxa at different stages of divergence. During this process, reproductive barriers can remain highly porous for long periods of time (Rieseberg et al., 1999; Turner et al., 2005; Harrison and Larson, 2014). Indeed, the genic view of speciation suggests that divergence occurs heterogeneously across the genome (Wu, 2001), such that the loci that underlie isolating traits become differentiated before the rest of the genome (Turner et al., 2005; Ellegren et al., 2012; Malinsky et al., 2015; Vijay et al., 2016). Therefore, even taxa at intermediate levels of divergence typically continue to share alleles, leading to a "gray zone" for which species concepts fail to reflect the realities of biological diversity (Mallet et al., 2015; Roux et al., 2016). These issues are paramount to the way that we consider patterns of taxonomic diversity in radiations, and they indicate the need for a more fluid, modern view of speciation that takes into account the continuous and multifaceted nature of the process.

In this study, we combine genomic and morphological data to shed light on evolutionary relationships within a recent radiation of monkeyflowers. *Mimulus* section *Diplacus* (Phrymaceae) is a monophyletic group of perennial shrubs distributed mainly in California (Beardsley et al., 2004). The phenotypically and ecologically diverse group (Fig. 1A) consists of at most 13 previously described taxa that are interfertile and continue to hybridize in narrow areas where their geographic ranges overlap. Although evolutionary studies have focused primarily on divergence between two parapatrically distributed taxa in San Diego county (Streisfeld and Kohn, 2005, 2007; Sobel and Streisfeld, 2015; Stankowski et al., 2015, 2017), little is known about the evolutionary history of divergence across the rest of the radiation. One reason for this is that the relationships among taxa remain unclear, as phylogenetic analyses have been limited to a handful of genes and included only some of the taxa (Beardsley et al., 2004; Stankowski and Streisfeld, 2015).

In addition to an incomplete understanding of evolutionary relationships, taxonomists have struggled to describe the extensive phenotypic diversity within *Diplacus*. As a consequence, there have been 12 different taxonomic revisions over the past century (Fig. 1B; Grant, 1924; Munz, 1935, 1959, 1973; McMinn, 1951; Pennell, 1951; Beeks, 1962; Thompson, 1993, 2005, 2012; Tulig, 2000; Tulig and Nesom, 2012). As few as two and as many as 13 species have been described, and many of the treatments also recognize additional subspecies or varieties. For example, the two most recent taxonomies were both published in 2012, but they differ dramatically in how they delimit the taxa. Thompson (2012) recognized two species, one of which included six varieties. By contrast, Tulig and Nesom (2012) split this same variation into 13 species, three of which were reported to be of hybrid origin. While much of the disagreement about the number and status of species results from the absence of intrinsic barriers to gene flow and the natural hybridization that occurs across their ranges (McMinn, 1951; Beeks, 1962; Streisfeld and Kohn, 2005), these taxonomic conclusions were based entirely on phenotypic data. Therefore, integrating genomic data with this phenotypic information will allow for an explicit evaluation of these taxonomic hypotheses.

In this study, we used a combination of phylogenetic and population genomic approaches to elucidate the evolutionary history and patterns of shared variation among taxa in section *Diplacus*. In addition, we combined phylogenomic and morphological data from a nearly complete sampling of taxa to explore patterns of phenotypic evolution across the group. In doing so, we provide a critical assessment of previously published taxonomic hypotheses in the light of new genomic analyses. This work will inform conservation and management practices, and it provides a framework for guiding future taxonomic treatments of this group. Finally, this work creates new opportunities for comparative evolutionary, ecological, and genomic studies of the history of divergence in this species complex.

MATERIALS AND METHODS

Study system—Members of *Mimulus* section *Diplacus* are perennial shrubs that vary most notably in floral characteristics (Fig. 1A). They occur throughout semi-arid regions of California, including most coastal sage scrub and inland chaparral communities, as well as some mountain peaks and deserts (Beeks, 1962). Hummingbirds and insects are their primary pollinators (Grant, 1994), and their preferences have been suggested to play an important role in the divergence of some taxa (Grant, 1993a, 1993b; Streisfeld and Kohn, 2007). Intrinsic crossing barriers appear to be absent among all taxa. The only exception is that crosses involving *Mimulus clevelandii* Brandegee frequently are unsuccessful (McMinn, 1951), which suggests that significant reproductive isolation exists between *M. clevelandii* and other members of the group. Consistent with this observation, all previous taxonomies have recognized *M. clevelandii* as a separate species.

By contrast, there has been little consensus about the ranks of other taxa (Fig. 1B). With the exception of Thompson (1993, 2005, 2012), who treated most taxa as varieties of the species *Mimulus aurantiacus* Curtis, all other treatments consistently recognized six species (*Mimulus aridus* Abrams, *Mimulus parviflorus* Greene, *Mimulus puniceus* Nutt., *Mimulus longiflorus* Nutt., *Mimulus grandiflorus* Groenland, and *M. aurantiacus*; Fig. 1B). Although *Mimulus stellatus* Kellogg is also treated consistently as a species, it has not been collected since 1940 (McMinn, 1951), and most taxonomists make no mention of it other than noting it was recognized as a species by Grant (1924). As a consequence, we did not consider *M. stellatus* further in this study. The remaining taxa have been more controversial. For example, *Mimulus calycinus* Eastw. and *Mimulus rutilus* A.L.Grant have been described either as separate species (McMinn, 1951; Beeks, 1962; Tulig, 2000; Tulig and Nesom, 2012) or as subspecies of *M. longiflorus* (Grant, 1924; Munz, 1935, 1959, 1973; Pennell, 1951). In addition, *M. linearis* Benth. has been described as a distinct species (Pennell, 1951; McMinn, 1951; Tulig, 2000), a subspecies of *M. longiflorus* (Grant, 1924; Munz, 1935), a subspecies of *M. grandiflorus* (Munz, 1959, 1973), and as a species of hybrid origin between *M. aurantiacus* and *M. calycinus* (Tulig

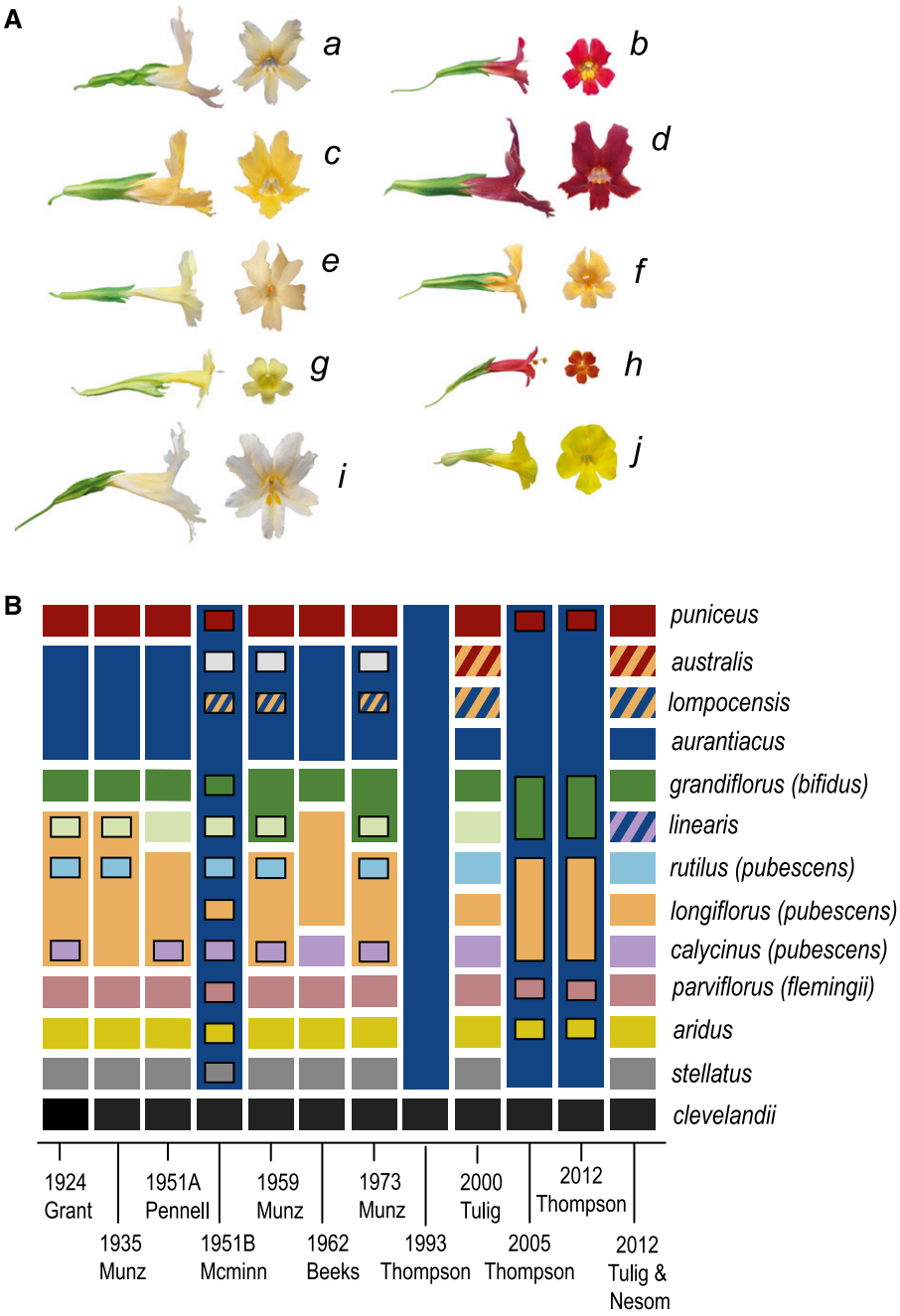

FIGURE 1 Morphological diversity and taxonomic history within *Mimulus* sect. *Diplacus*. (A) Representative photos in front and side view of some of the floral diversity present in *Mimulus* sect. *Diplacus*. Taxa included here are (a) *M. australis*, (b) *M. puniceus*, (c) *M. longiflorus*, (d) *M. rutilus*, (e) *M. calycinus*, (f) *M. aurantiacus*, (g) *M. aridus*, (h) *M. parviflorus*, (i) *M. grandiflorus,* and (j) *M. clevelandii*. (B) A summary of the 12 taxonomic revisions that have been published over the past century, beginning with Grant (1924). Across the different treatments, species status is represented by colored rectangles, and subspecies or variety status is represented by smaller rectangles with black outlines that occur within the colored rectangle for a species. The color of the box is associated with the name given by Tulig and Nesom (2012), presented to the right of the figure. Other names previously used to define taxa are included in parentheses. The location of the taxon names lines up with their treatment in each taxonomy. Hatched boxes indicate that a taxon is described as a hybrid species, with the color of the two lines representing the proposed progenitor species.

and Nesom, 2012). Different treatments also have recognized *Mimulus lompocensis* McMinn as both a subspecies of *M. aurantiacus* (Munz, 1959, 1973) and as a species of hybrid origin between *M. aurantiacus* and *M. longiflorus* (McMinn, 1951; Tulig, 2000; Tulig and Nesom, 2012). Finally, *Mimulus australis* McMinn ex Munz has been described as its own species (McMinn, 1951), a subspecies of *M. aurantiacus* (Munz, 1959, 1973), and most recently, as a hybrid species between *M. puniceus* and *M. longiflorus* (Tulig, 2000; Tulig and Nesom, 2012). Each of these taxa has also at some point been considered a synonym of another, less controversial taxon (Fig. 1B). Due to this extreme confusion over naming conventions, we choose to be as inclusive as possible with the taxonomy by addressing every previously described taxon without regard to species concepts. Therefore, unless otherwise noted, we refer to each taxon using its specific binomial epithet, according to Tulig and Nesom's (2012) treatment.

Taxonomic and population sampling—A recently published analysis of phylogenetic relationships included the eight most widely distributed taxa but avoided some of the more controversial groups (i.e., *M. lompocensis*, *M. linearis*, *M. rutilus*) (Stankowski and Streisfeld, 2015). Additionally, some taxa were sampled across a limited portion of their geographic range (i.e., *M. puniceus* and *M. australis*). We include samples of these taxa here to provide a more complete examination of the group. Thus, our analyses included individuals from 12 taxa from section *Diplacus* and one outgroup species (*Mimulus kelloggii* Curran, which is sister to *Diplacus* in section *Oenoe*; Beardsley et al., 2004).

Leaf tissue was collected either from the field or from field-collected seeds grown in the University of Oregon greenhouses. For ingroup taxa, samples included between one and 14 individuals across the taxon's geographic range, totaling 73 individuals (Fig. 2; Appendix S1, see the Supplemental Data with this article). One individual from the outgroup species *M. kelloggii* also was included. Samples were identified according to Tulig and Nesom (2012). Sixty-one of the 73 ingroup individuals were sequenced previously (Stankowski and Streisfeld, 2015); the new individuals included here are two *M. lompocensis*, four *M. rutilus*, one *M. parviflorus*, one *M. aridus*, and two additional *M. puniceus* and *M. australis* from the northern portion of their range. Forty-five of our samples come from locations that were previously visited by Tulig (2000) in a study of floral trait variation. Therefore,

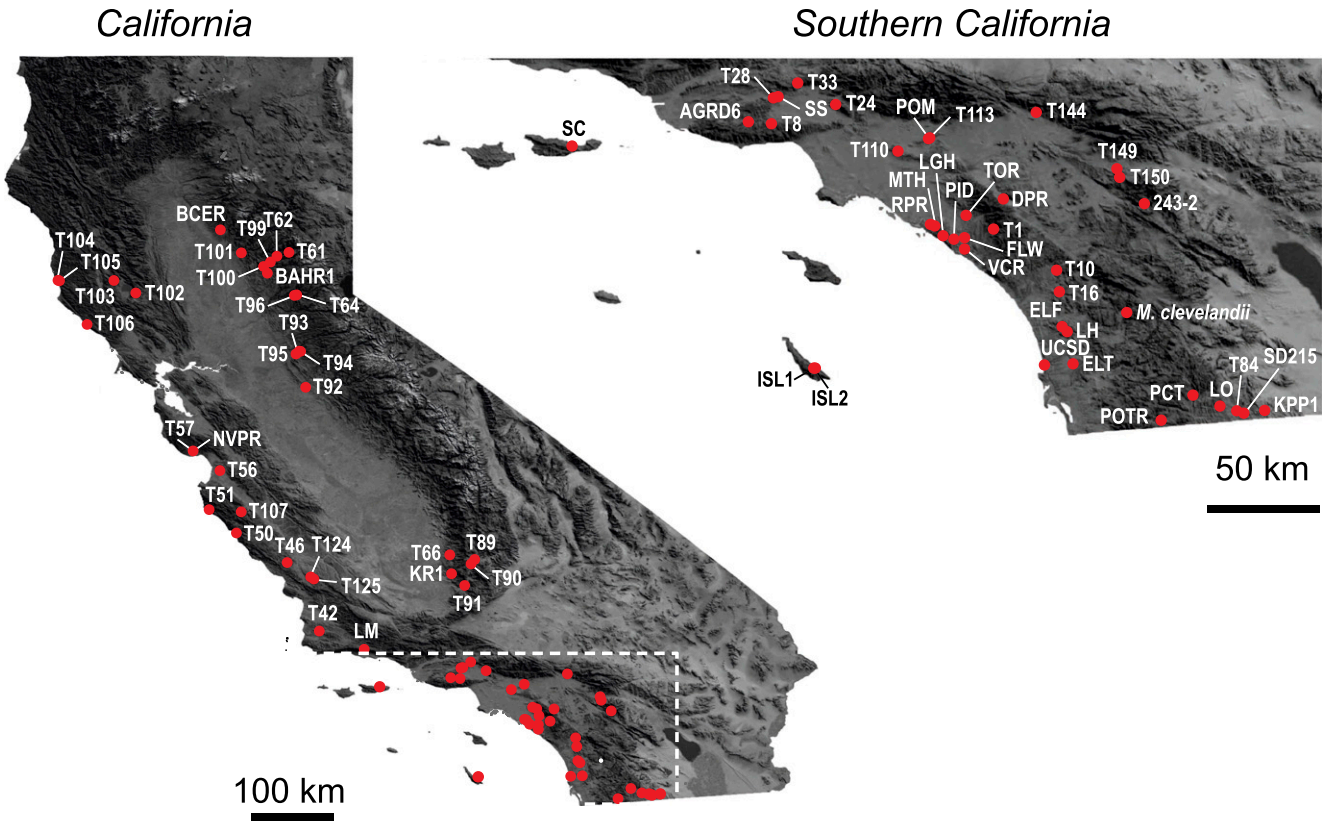

FIGURE 2 Geographic range and distribution of sampled individuals. Red dots represent the sampling locations used in this study. Population codes that begin with the letter "T" followed by a number indicate populations that were sampled previously for floral trait data by Tulig (2000). The region depicted by the inset in southern California is shown by the dashed line.

morphological data are available for these populations (described below). Although hybridization is known to occur between some taxa, we avoided sampling from zones of contact, because we did not want our analysis of broadly distributed taxa to be impacted by dynamics in narrow hybrid zones (except in the case of *M. rutilus*, which only occurs within populations described as *M. longiflorus*).

Analyses of evolutionary relationships—To generate genome-wide data to infer the evolutionary history of section *Diplacus*, we used Illumina sequencing of restriction-site-associated DNA tags (RADtags). DNA was isolated using either the modified CTAB extraction described by Sobel and Streisfeld (2015) or ZYMO Plant/Seed DNA miniprep kits. RAD libraries were then prepared using the PstI restriction enzyme, followed by single-end 100-bp Illumina HiSeq 2000 sequencing, according to methods described previously (Etter et al., 2011; Sobel and Streisfeld, 2015). We used the *process_radtags* module of the Stacks v. 1.35 package (Catchen et al., 2013) to remove reads with low quality or uncalled bases. Errors in the barcode and restriction site sequences were corrected before downstream analysis. Reads were aligned to an initial draft reference assembly from *M. puniceus* (described by Stankowski et al., 2017) using the *very-sensitive* option in bowtie2 (Langmead and Salzberg, 2012). Loci were constructed with the *ref_map.pl* script in Stacks v. 1.35 (Catchen et al., 2011, 2013). Single nucleotide polymorphisms (SNPs) were identified for our phylogenetic analysis using the *populations* module in Stacks v. 1.35, requiring that SNPs were present in at least 90% of the individuals and had a minimum minor allele frequency of 0.02 to exclude any SNPs found in a single heterozygote.

To infer relationships among samples, we used a maximum-likelihood method, implemented in the program RAxML v. 8.2.3 (Stamatakis, 2014). For each sample, we generated an alignment of all 24,699 polymorphic 95-bp RAD-tags, which included invariant sites (specified using the *–phylip_var_all flag* option in the *populations* module). Methods of phylogenetic reconstruction were developed for the use of sequence data that include invariant sites; therefore, using whole RAD-tags is more appropriate than including only polymorphic sites (Stamatakis, 2014). *RAxML* was run using the GTR+GAMMA model of nucleotide substitution. Support for each node was obtained by running 100 bootstrap replicates. Previous analyses using Bayesian, distance, and coalescent-based approaches yielded qualitatively similar results (Stankowski and Streisfeld, 2015).

Closely related populations often share high levels of sequence variation as a result of both incomplete lineage sorting (ILS) and ongoing hybridization (Lamichhaney et al., 2015; Mallet et al., 2015; Hahn and Nakhleh, 2016; Pease et al., 2016). Therefore, forcing individuals to conform to a bifurcating tree may obscure the complex evolutionary history of a group (Huson and Bryant, 2006). Thus, we also constructed a split network using the program SplitsTree v4 (Huson and Bryant, 2006). This method allowed us to visualize more complex signals in the data by adding splits that were not permitted in a bifurcating tree.

We then used the Bayesian clustering algorithm implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) as an alternative method

for inferring patterns of ancestry within *Diplacus*. Unlike phylogenetic methods, STRUCTURE reveals shared variation among inferred genetic groups, which could result from admixture or ancestral polymorphism. Because the phylogenetic analysis revealed four major clades, we conducted six replicate runs at $K = 4$, assuming the admixture model with correlated allele frequencies, 50,000 iterations of burn-in, and 200,000 iterations of sampling. Additional runs were added with subsets of the individuals to address hypotheses that emerged from the phylogenetic analysis (see below). *Mimulus clevelandii* and *M. kelloggii* were not included in these analyses. Due to computational limitations, we used a reduced data set of 6095 SNPs, generated by including one SNP per RAD-tag and a minimum minor allele frequency of 0.15. Results from each run were evaluated using Structure Harvester (Earl, 2012), and multiple runs were summarized in CLUMPP (Jakobsson and Rosenberg, 2007).

Tests for introgressive hybridization—Of the four primary clades identified in the phylogenetic analysis, one clade (Clade D) was especially diverse and contained up to six described taxa from southern California. Although three subclades are evident in the phylogenetic analysis, STRUCTURE revealed substantial levels of shared variation among the subclades. To investigate whether this shared variation reflects ancestral polymorphism or recent gene flow, we calculated Patterson's *D* statistic (Green et al., 2010). Patterson's *D* is calculated using four taxa with the relationship (((P$_1$, P$_2$), P$_3$), O) and provides a test for introgression between the donor population, P$_3$, and either of the two ingroup taxa, P$_1$ and P$_2$ (Green et al., 2010). The statistic is calculated as the ratio of SNPs that fits an ABBA pattern to the number of SNPs that fits a BABA pattern across the four taxa, where A is the ancestral allele and B is the derived allele. Under random sorting of ancestral variation, the number of SNPs fitting both patterns is expected to be equal; however, an excess of either pattern indicates introgression has occurred between the donor taxon and one of the ingroup taxa (Green et al., 2010). The taxa used in this data set include *M. longiflorus* (P1), *M. calycinus* (P2), *M. australis* and *M. puniceus* (P3), and *M. grandiflorus* (O). *M. australis* and *M. puniceus* were combined to form P3, because the two formed a single phylogenetic group (see results). Two *M. calycinus* individuals that grouped in Clade C and showed high levels of admixture (see results) were not included in this analysis. SNPs included in this data set were required to be present in 90% of the individuals included and to have a minimum minor allele frequency of 0.02. To reduce the effects of linkage, we included only a single SNP per RAD-tag. We did not require SNPs to be fixed within a taxon, and 16,920 polymorphic sites were included in the analysis. To assess whether *D* was significantly different from 0, we followed the approach of Eaton and Ree (2013) to calculate a *p*-value from the *Z*-score obtained from 1000 bootstrap replicates of the test statistic.

In addition to exploring the origins of shared variation revealed by our analyses, we tested Tulig's (2000) hypothesis that *M. australis* is of hybrid origin between *M. puniceus* and *M. longiflorus*. Tulig (2000) used multivariate analysis of floral traits (see below) to identify two groups that differed in flower size. The small-flowered group included *M. puniceus*, *M. aurantiacus*, and *M. parviflorus*, while the large-flowered group included *M. grandiflorus*, *M. longiflorus*, and *M. calycinus*. Populations of *M. australis* were intermediate in size and overlapped with populations of *M. lompocensis*, which McMinn (1951) previously suggested was of hybrid origin. Tulig (2000) took these patterns to be evidence that *M. australis* also is of hybrid origin. Tulig and Nesom (2012) further speculated that, based on their geographic range and phenotypic similarity, *M. longiflorus* and *M. puniceus* were likely to be the progenitors of modern day *M. australis*.

We used the F3 test (Reich et al., 2009) to ask whether there was genomic evidence that *M. australis* arose through hybridization between *M. longiflorus* and *M. puniceus*. The F3 test compares three populations, X, Y, and W, and evaluates whether Y is of mixed ancestry between X and W. The test is calculated by measuring the allele frequency difference between Y and either X or W, and taking the product of the two values. In this case, we combined all *M. australis* individuals as population Y, and *M. puniceus* and *M. longiflorus* were populations X and W, respectively. A negative value would support the hybrid origin of *M. australis*, and a non-negative value would refute it. The F3 statistic was calculated from the data set used to calculate Patterson's *D*, with 3204 sites polymorphic among these three taxa. We applied the same bootstrap approach as described above to determine the significance of the observed F3 statistic. The F3 and Patterson's *D* statistics were calculated using the program ADMIXTOOLS (Patterson et al., 2012).

Analyses of floral trait data—Previous studies used morphological characteristics—mainly floral traits—to delimit taxa in section *Diplacus* (Pennell, 1951; McMinn, 1951; Beeks, 1962; Munz, 1973; Tulig and Nesom, 2012; Thompson, 2012). However, given that 12 revisions have been published over the past century, a critical assessment of the taxonomic utility of floral traits is warranted. Indeed, the two most recent treatments differ considerably in how taxa are delimited (Thompson, 2012; Tulig and Nesom, 2012; Fig. 1B). Therefore, we used an existing morphometric data set that consisted of 18 floral traits that were measured on 1–30 plants from 45 of our collection sites (mean = 6 plants per site; SD = 4.17; Tulig, 2000; trait descriptions provided in Appendix S2) to ask how well each of these treatments performed at delineating taxa. We performed separate discriminant function analyses (DFA) with either Tulig and Nesom's (2012) taxonomy or Thompson's (2012) taxonomy as the grouping variable. If morphological characteristics alone can be used to delineate taxa, we would expect that one of the treatments would assign individuals to taxa more reliably than the other.

In addition, by combining floral trait data with phylogenetic and population genomic analyses, we now have the capacity to test whether trait variation can be used to reconstruct an accurate picture of evolutionary relationships. Specifically, if traits have strong phylogenetic signal, individuals within the same clade should be more phenotypically similar than individuals in different clades. However, this relationship may be obscured by the effects of convergent and divergent phenotypic evolution, which are common during radiations (Berner and Salzburger, 2015). For example, we would expect convergent evolution to result in phenotypic overlap among taxa from different clades, while divergence would cause pronounced phenotypic differences among taxa within clades. Therefore, to examine how trait variation is partitioned within and among clades, we summarized the multivariate trait data using a principal components analysis (PCA) and mapped the four phylogenetic clades in the bivariate space of the first two principal components. This approach separates samples based on the two largest sources of phenotypic variation across the entire data set. Therefore, if these trait data reflect the evolutionary history of divergence,

the first two principal components should correspond to the deepest evolutionary divisions in the group. However, convergent and divergent phenotypic evolution would prevent the accurate reconstruction of evolutionary history from these traits.

Finally, although it may be possible that the primary sources of floral trait variation fail to reflect the history of this group, there may be more subtle trait variation that does carry a phylogenetic signal. To test for such traits, we used DFA with phylogenetic clade as the grouping variable to examine how often individuals were assigned to the correct clade using the 18 floral traits. If this analysis reliably assigns individuals to the correct clade, then we can identify the traits that vary in accordance with the main evolutionary history of the group. All analyses of floral trait data were performed in R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Evolutionary relationships within section Diplacus—After quality filtering and aligning raw reads to the *M. puniceus* reference genome, an average of 69.8% of reads mapped uniquely when excluding *M. kelloggii*. The high percentage of reads aligning across taxa reflects the recent history of the group. In contrast, only 37.61% of the *M. kelloggii* reads aligned uniquely, as it is more distantly related to *M. puniceus*. The final data set for phylogenetic analysis included 24,699 loci (RAD-tags), totaling 2,346,405 bp, with 68,889 variable sites. Of these loci, 38.3% were missing from *M. kelloggii*. After further filtering for STRUCTURE analyses, we retained 6095 of the most informative SNPs.

Phylogenetic analysis revealed four highly supported (100% bootstrap support) clades (A–D; Fig. 3A), consistent with those previously identified by Stankowski and Streisfeld (2015). Clade A consists of all samples of *M. grandiflorus* and *M. linearis*, which are reciprocally monophyletic. Clade B includes all samples of *M. aridus* and *M. parviflorus*, which are reciprocally monophyletic. Clade C contains *M. aurantiacus* as a monophyletic group, but also includes one *M. lompocensis* individual and two individuals that phenotypically resemble *M. calycinus*. Clade D contains the highest number of described taxa, including *M. australis*, *M. puniceus*, *M. rutilus*, *M. longiflorus*, the remaining *M. calycinus* samples, and one *M. lompocensis* individual.

By including *M. kelloggii* as an outgroup, we were able to test the phylogenetic position of *M. clevelandii*. Although *M. clevelandii* frequently has been described as a separate species (Fig. 1B) and was used as the outgroup in a past analysis (Stankowski and Streisfeld, 2015), the only previous molecular phylogenetic analysis revealed that *M. clevelandii* grouped within the rest of the taxa (Beardsley et al., 2004). However, by rooting with *M. kelloggii*, we confirmed that *M. clevelandii* indeed is sister to the remaining taxa.

In addition to the phylogenetic tree, we illustrated relationships through a split network (Appendix S3). This analysis highlights the deep division that separates Clades A and B from Clades C and D, and it reveals the complex nature of the relationships among taxa, especially within the rapidly radiating Clades C and D.

Prevalence of shared variation between and within clades—As an alternative to phylogenetic analysis, we used STRUCTURE to infer patterns of admixture among the individuals. The analysis at $K = 4$ revealed clusters of individuals that largely agreed with the clades recovered in the phylogenetic analysis. However, it also revealed shared variation among Clades B, C, and D that was not apparent from the bifurcating tree (Fig. 3C). For example, we detected extensive admixture in the two *M. lompocensis* individuals and the two *M. calycinus* individuals that group within Clade C. The intermediate STRUCTURE scores of these *M. calycinus* individuals suggest that they are hybrids, so they were excluded from other analyses. In addition, consistent with previous evidence of introgressive hybridization (Stankowski and Streisfeld, 2015), *M. puniceus* and *M. australis* from Clade D show some mixed ancestry with individuals in Clade B.

We performed an additional STRUCTURE analysis to test for divergence and admixture in Clade D. The STRUCTURE analysis at $K = 3$ recapitulated the three highly supported subclades from the phylogeny (one that includes both *M. puniceus* and *M. australis*, one that only includes *M. calycinus*, and one that includes *M. longiflorus* and *M. rutilus*). However, it also revealed extensive shared variation across all of Clade D (Fig. 3C). For example, southern populations of *M. calycinus* share some ancestry with individuals of *M. longiflorus*, and the four southern populations of *M. longiflorus* and *M. rutilus* share variation with individuals of *M. puniceus* and *M. australis.*

This shared variation may be due to retained ancestral polymorphisms or recent gene flow. To determine whether gene flow can explain some amount of shared variation among taxa, we performed an ABBA–BABA test within clade D. Because *M. puniceus* and *M. australis* are indistinguishable based on STRUCTURE analyses, we treated them as one taxon for this test. Moreover, since this test requires sister ingroup taxa, we used *M. calycinus* and *M. longiflorus* as the ingroups. Patterson's *D* for the test was 0.1672 (Table 1), which reflects a 16.7% excess of BABA sites over ABBA sites and suggests likely introgression between the *M. australis* and *M. puniceus* lineage and the *M. longiflorus* lineage. Bootstrap analysis revealed that the value of Patterson's *D* was highly significant (Table 1, $P < 0.00001$).

To address Tulig and Nesom's (2012) claim that *M. australis* is a hybrid species, we also tested whether *M. australis* individuals are significantly admixed between *M. puniceus* and *M. longiflorus*. The result from the F3 test, designed to measure whether population Y is admixed between populations X and W, was 0.024408, which is a significantly positive value (Table 1, $P < 0.00001$). This result suggests that *M. australis* is not the product of hybridization between *M. puniceus* and *M. longiflorus*, as proposed by Tulig and Nesom (2012). Although it remains possible that *M. australis* arose due to hybridization between other taxa, neither the split network nor the STRUCTURE analysis provides substantial evidence for admixture that would support this conclusion.

Patterns of phenotypic variation—Both discriminant function analyses reliably assigned individuals to the set of taxa described by each taxonomic treatment. Tulig and Nesom's (2012) treatment and Thompson's (2012) treatment each correctly assigned 97.06% of the individuals into taxa (Fig. 4A, B). Based on Tulig and Nesom's (2012) treatment, the traits that loaded most highly on discriminant function 1 (DF1) were pedicel width (PDWD), corolla height (CRHT), and the length of the short filament (FSLN; Table 2). By contrast, width of the throat opening (THRO), calyx height (CAHT), and length of the lower central petal lobe (LLCL) loaded most strongly on discriminant function 2 (DF2). Based on Thompson's (2012) treatment, the traits that explained most of the variation on both DF1 and DF2 were pedicel width (PDWD), width of the throat

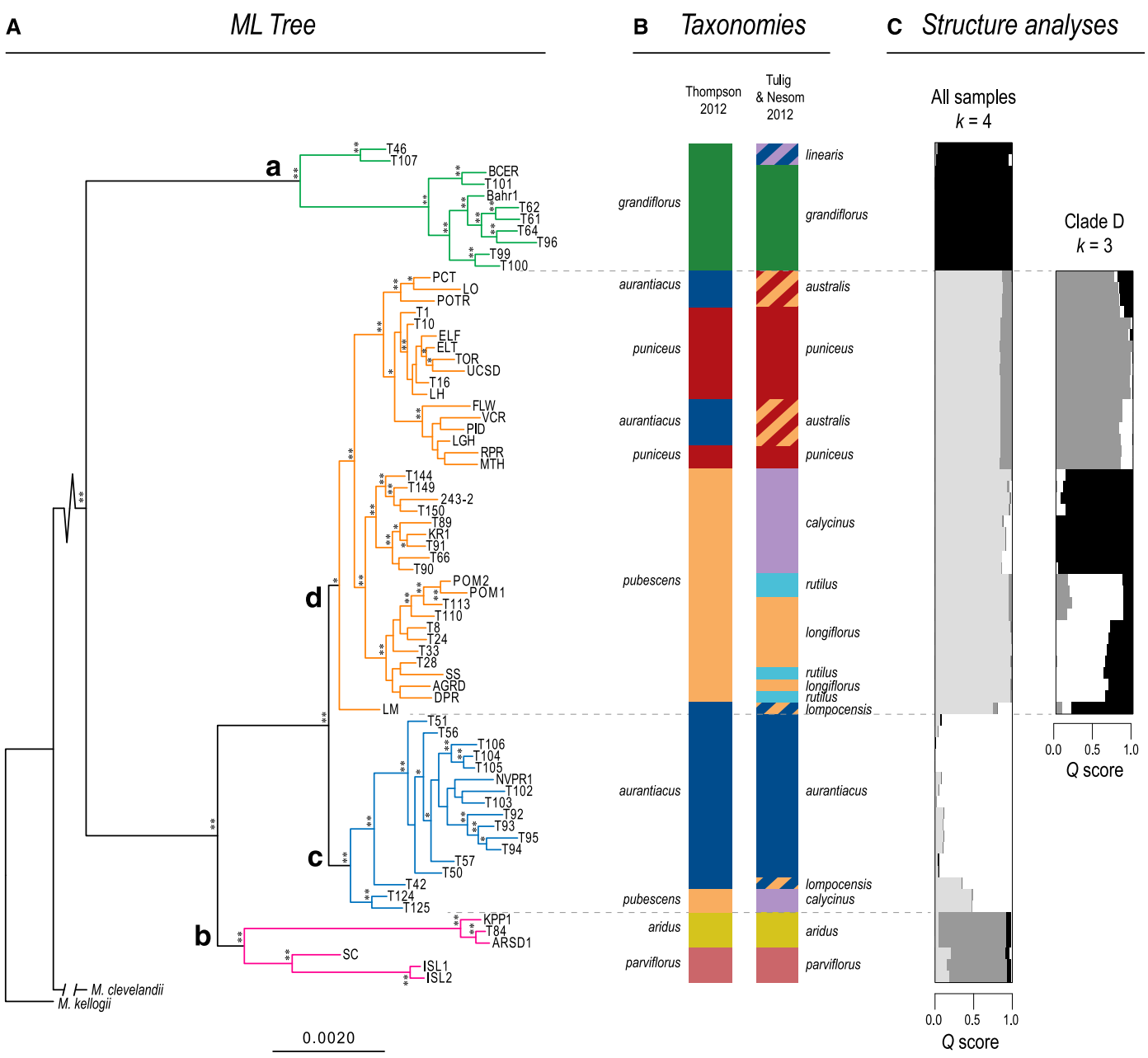

FIGURE 3 Evolutionary relationships within *Mimulus* sect. *Diplacus*. (A) Maximum-likelihood tree illustrating the relationships among samples. Nodes with 100% bootstrap support are represented with two stars; nodes with >90% bootstrap support are represented with one star. We identified four highly supported clades (a–d), which are labeled on the tree, with branches colored according to clade. (B) Taxonomic identity of samples that is based on the two most recent taxonomies. Colors in the rectangles correspond with Fig. 1. (C) STRUCTURE analyses that show ancestry scores (*Q*) for all samples at $K = 4$ (left), and only for Clade D at $K = 3$ (right). Individuals line up with tips of the phylogeny. Dashed lines separate the four clades.

opening (THRO), and length of the lower central petal lobe (LLCL). Thus, these traits appear to be most important in separating taxa. Notably, both taxonomies performed equally well at separating taxa based on this floral trait variation. Consequently, given the substantial differences between the taxonomic treatments, this analysis provides little guidance as to which taxonomy more accurately describes the diversity.

To test whether floral trait variation can reconstruct evolutionary history, we performed a PCA with individuals colored by phylogenetic clade and performed a DFA with phylogenetic clade as the grouping variable. The first two principal components explained 75.8% of the variation among the 18 floral traits (PC1: 60.4%; PC2: 15.4%). However, rather than revealing a series of discrete groups, the samples were distributed along a continuum of phenotypic variation. In addition, there was almost no discrete clustering of samples from the same clade in PC space. Rather, individuals from different clades broadly overlapped one another. The only exception was Clade B, which was distinct from the other three clades and formed two clusters corresponding to *M. parviflorus* and *M. aridus*. This analysis indicates that the largest sources of phenotypic

TABLE 1. Results from Patterson's *D* and F3 tests.

Test	Comparison	Test statistic	*Z* score	*P* value	BABA sites	ABBA sites	Total sites
D	P1: *M. longiflorus* P2: *M. calycinus* P3: *M. australis/puniceus* O: *M. grandiflorus*	0.1672	7.578	<0.00001	196	140	16920
F3	X: *M. puniceus* Y: *M. australis* W: *M. longiflorus*	0.0244	6.135	<0.00001	N/A	N/A	3204

variation present in the data set do not separate the samples into distinct groups that correspond to the deep evolutionary divisions revealed by the phylogenetic and population genomic analyses. Alternatively, substantial phenotypic overlap exists among the individuals from Clades A and D, and among individuals from Clades C and D, indicating convergence on similar phenotypes across clades. Moreover, the distinctness of the two taxa in Clade B, and the well-studied differences in floral traits between the closely related *M. puniceus* and *M. australis* (Streisfeld and Kohn, 2005; Stankowski et al., 2015), reveal a complex history of phenotypic evolution in this group that involves both convergent evolution between clades and divergent evolution within clades.

Although the most conspicuous traits do not carry a phylogenetic signal, more subtle characters might distinguish the major clades from one another. To test for such traits, we conducted a discriminant function analysis using phylogenetic clade as the grouping variable. In contrast to the PCA, the individuals within each clade were largely separated from each other across discriminant space and were correctly assigned to clade 94.12% of the time (Fig. 4D). Clade B is once again distinct from all other groups, but it no longer forms two separate clusters. Clades A and D are differentiated more clearly in discriminant space than in PC space, with only minor overlap between them. The greatest overlap occurred between Clades C and D, but there was less overlap evident than in the PCA. Pedicel width (PDWD) and the width of the throat opening (THRO) loaded most heavily on these canonical axes (Table 3), suggesting that these traits carried the strongest phylogenetic signal based on the clustering of clades.

DISCUSSION

Evolutionary relationships in radiations can be complex, and their resolution often requires detailed sampling and integrated analyses. By combining phylogenetic, population genomic, and phenotypic analysis, we show that these monkeyflowers exhibit the hallmarks of a rapid radiation, including a range of diverse taxa at different stages of divergence, extensive shared variation across the group, and evidence for divergent and convergent phenotypic evolution. Our results also have taxonomic implications, and we discuss how they might inform a future revision.

Patterns of divergence and shared variation across the radiation—Our analysis of genomic data provides some of the first insight into the evolutionary history of this diverse group of monkeyflowers. Specifically, we show that the taxa are closely related but are at different stages of divergence, which creates exciting opportunities for comparative studies across the speciation continuum. For example, in the early stages of speciation, the genomes of taxa are thought to be largely undifferentiated as a result of their very recent history (Rundle and Nosil, 2005; Nosil, 2012). This is the case between *M. puniceus* and *M. australis* from clade D, which have divergent floral phenotypes as a consequence of pollinator-mediated selection (Streisfeld and Kohn, 2007; Handelman and Kohn, 2014; Sobel and Streisfeld, 2015), but do not form separate monophyletic groups in our phylogenetic analysis. In contrast, another pair of ecologically divergent taxa from Clade D, *M. calycinus* and *M. longiflorus*, form shallow monophyletic sister clades, suggesting they are at an intermediate stage of speciation (Beeks, 1962; Grant, 1993a, 1993b). A much more distantly related pair of taxa, *M. parviflorus* from Clade B and *M. longiflorus* from Clade D, are able to co-occur in sympatry on Santa Cruz Island off the coast of California despite hybridization between them (Wells, 1980; M. Chase, personal observation). Future comparative, ecological, and genomic studies in these and other taxa will examine how the factors that generate and maintain diversity change with progress toward speciation.

While our phylogenetic analysis provides insight into the patterns of divergence between taxa, our population genomic analyses reveal a complex pattern of shared variation among taxa. Although incomplete lineage sorting probably accounts for most of the shared variation within and between clades, our analyses indicate that some is due to introgressive hybridization. Hybridization is a relatively common phenomenon in radiations, and in some cases, it can be so extensive that relationships cannot be illustrated accurately with a tree (Malinsky et al., 2017). In *Mimulus* section *Diplacus*, many studies have noted hybridization between taxa in areas where their ranges overlap. Although this mixing has been a major cause of taxonomic conflict in this group (McMinn, 1951; Beeks, 1962; Thompson, 1993, 2005, 2012; Tulig, 2000), our data indicate that hybridization does not have a major effect on the core structure of clades and taxa. Rather, hybridization probably occurs in areas that coincide with transitions between different environments, which is consistent with the observations that floral trait differences between the taxa are stable over large geographic areas and that hybrid zones are narrow in comparison.

Evidence for divergent and convergent phenotypic evolution—By examining floral trait variation in combination with phylogenetic analyses, we show striking phenotypic similarity between comparatively distantly related taxa, and remarkable dissimilarity between very closely related taxa. This pattern of convergent and divergent evolution has been observed in many rapidly diverging groups (Muschick et al., 2012; Heliconius Genome Consortium, 2012; Mahler et al., 2013) and is common in adaptive radiations (Schluter, 2000; Berner and Salzburger, 2015).

Multiple processes can cause the patterns of phenotypic evolution we observe. Divergent phenotypic evolution is thought to occur most commonly when populations adapt to contrasting environments, which can cause ecological isolating barriers to evolve

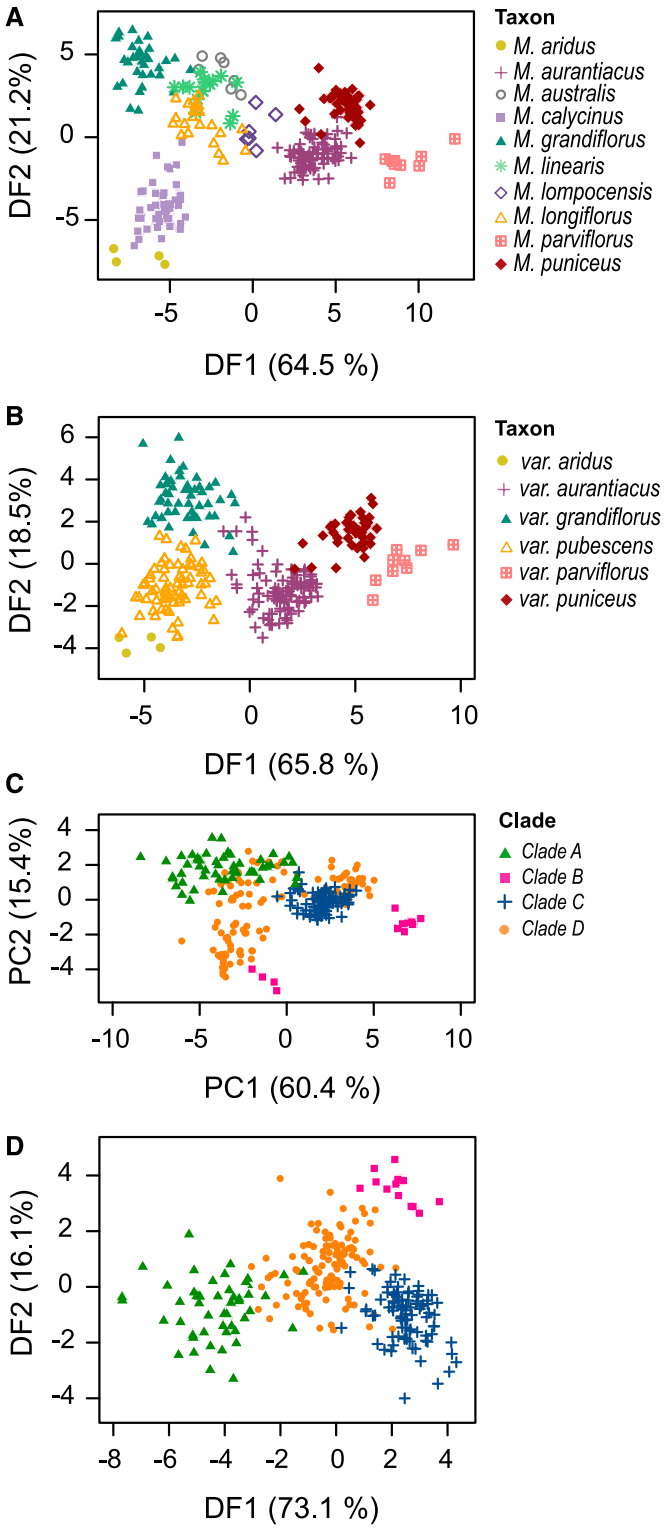

FIGURE 4 Phenotypic variation within section *Diplacus*. (A, B) Discriminant function analyses on 18 floral traits, based on individuals from the 45 populations that were previously phenotyped by Tulig (2000). Individuals within each plot are grouped according to the taxonomy of (A) Tulig and Nesom (2012) or (B) Thompson (2012). (C) Principal component analysis on floral traits, with individuals colored according to phylogenetic clade, as identified in Fig. 3. (D) Discriminant function analysis on floral traits using phylogenetic clade as the grouping variable. Individuals are colored by clade. Based on these 18 traits, individuals were correctly assigned to Clade A 94% of the time, Clade B 100% of the time, Clade C 93.3% of the time, and Clade D 90.8% of the time.

(Rundle and Nosil, 2005; Nosil, 2012). Previous work indicates that this is the case between *M. puniceus* and *M. australis*, which have low levels of genomic differentiation despite selection on flower color and other floral traits (Streisfeld and Kohn, 2005; Handelman and Kohn, 2014; Stankowski et al., 2015). However, in other cases, sister taxa are geographically isolated from each other. Thus, phenotypic divergence may be the result of neutral processes rather than adaptation (Schluter, 2009). For instance, *M. aridus* and *M. parviflorus* have entirely distinct ranges, but they differ in flower color (Fig. 1) and are completely separated from each other in the DFA of floral traits (Fig. 4). Given their allopatric distributions, drift or selection may have played a role in their phenotypic divergence. Thus, further study is required to determine the evolutionary forces responsible for phenotypic divergence across different taxa.

Convergent phenotypic evolution also may arise through various processes. Shared features among clades could result from independent origins of a trait through new mutations, the sharing of ancestral polymorphisms, or through introgressive hybridization. The latter two possibilities may be especially common in systems marked by rapid diversification (Hahn and Nakhleh, 2016), and they signal the need for caution when interpreting phenotypic evolution in the context of a phylogeny. Although our phylogeny reflects the demographic history of divergence, there are likely regions of the genome with discordant evolutionary histories, some of which may underlie adaptive traits. Indeed, previous work in this system has provided evidence that a mutation causing red flowers was shared between Clade B and *M. puniceus* through historical introgression (Stankowski and Streisfeld, 2015). Future analyses, aided by an improved, chromosome-level genome assembly, will allow us to reveal the underlying genomic features responsible for the patterns of divergent and convergent phenotypic evolution we observe. In addition, these data will provide new opportunities to document the evolutionary history of ecologically important phenotypic transitions associated with adaptive divergence in a recent radiation.

Taxonomic implications and recommendations for formal revision—Although our primary focus was to infer the evolutionary relationships in this group, this work has important taxonomic implications and highlights the need for a new revision of section *Diplacus*. Over the last century, all 12 of the published treatments disagree to some extent on the appropriate number and rank of the taxa described. The disagreement is most apparent in the two most recent treatments that were published in 2012 (Tulig and Nesom, 2012; Thompson, 2012), as our results indicate that neither one is better at describing the floral trait variation analyzed here. This uncertainty is especially problematic for managers and conservationists, as well as for evolutionary biologists, who are left without a clear conceptual framework for how to appropriately refer to the diversity in the group. While we do not provide a formal revision here, we present recommendations for future changes that are based on the integration of genomic and phenotypic analyses that emerge from this study.

TABLE 2. Loadings for the first two discriminant function axes using the taxonomy of Tulig and Nesom (2012) or Thompson (2012) as the grouping variable. Descriptions of trait name abbreviations can be found in Appendix S2.

	Tulig and Nesom (2012)		Thompson (2012)	
Trait	DF1	DF2	DF1	DF2
CRLN	−0.05332177	0.05981519	−0.098544301	0.049198411
CULN	0.11349505	−0.027188252	−0.064752232	−0.178416174
CLLN	−0.11456936	−0.16278508	0.012548931	−0.061930311
BLLN	−0.09347819	0.196527236	−0.042122811	0.017305656
UCOS	0.08753006	−0.074278674	−0.107440042	−0.083783893
INFL	−0.07794273	−0.104483587	−0.133694509	−0.01175704
UCIS	−0.0581908	−0.067652583	−0.036843668	0.004904862
WLCL	−0.08329381	−0.044438801	0.000161317	−0.026132869
LLCL	0.09515379	0.383080129	0.461992819	0.447229467
THRO	−0.26309451	0.646242479	−0.384743181	0.683290511
CRHT	−0.56351185	0.213665212	0.145023746	−0.096769739
CAHT	0.09380583	−0.598988778	−0.229591581	−0.239920631
PDLN	0.07142001	0.040301165	0.100065766	0.149577015
PDWD	−1.461039	0.01673164	−0.44245656	−2.063862501
CTN	−0.27308312	−0.329314029	−0.209082137	−0.092024316
FLLN	0.3154864	−0.000242331	0.333242237	−0.148756483
FSLN	0.35834455	0.092175164	0.131968689	0.153662132
STLN	−0.02811806	−0.00905903	0.080392938	0.116118715

Five of the taxa that we examined have faced frequent revision, including *M. calycinus*, *M. rutilus*, *M. linearis*, *M. lompocensis*, and *M. australis*. Both *M. calycinus and M. rutilus* have been described previously as subspecies of *M. longiflorus*, and *M. calycinus* recently has been grouped together with *M. longiflorus* to form *M. aurantiacus* var. *pubescens* (Thompson, 2005, 2012). Although the genomic and phenotypic data clearly separate *M. calycinus* from *M. longiflorus*, *M. rutilus* is not genetically distinct from *M. longiflorus*, even though they differ considerably in flower color (Fig. 1). Thus, based on these results, we would recommend that *M. calycinus* be treated as a distinct entity. However, given that red-flowered *M. rutilus* is found growing only within otherwise yellow-flowered populations of *M. longiflorus*, the genomic data suggest that *M. rutilis* should be recognized more appropriately as a simple flower color polymorphism that is restricted to a few geographic areas.

TABLE 3. Loadings for the first two principal components and first two discriminant functions using phylogenetic clade as the grouping variable. Descriptions of trait name abbreviations can be found in Appendix S2.

Trait	PC1	PC2	Clade DF1	Clade DF2
CRLN	0.87484	0.38394	−0.007534267	−0.23149174
CULN	0.64359	0.63356	0.133768577	0.03993585
CLLN	0.67048	0.68768	0.082474462	−0.03988584
BLLN	0.91132	−0.32478	−0.049643515	−0.0069482
UCOS	0.90306	−0.31785	0.112837875	−0.21298351
INFL	0.87773	−0.36545	0.065978965	−0.42316548
UCIS	0.83717	−0.42061	−0.129622483	0.32149581
WLCL	0.75442	−0.09692	0.050158241	−0.07255096
LLCL	0.85984	−0.38537	−0.412127261	0.40931716
THRO	0.87132	−0.34805	−0.791635928	0.09593173
CRHT	0.83665	−0.22986	0.026632214	−0.18093956
CAHT	0.71113	0.39742	0.203823105	−0.16578586
PDLN	−0.48729	−0.07971	−0.082918263	−0.07242255
PDWD	0.84532	0.0959	1.750621312	1.4659284
CTN	0.77697	0.53037	−0.019122676	0.20307343
FLLN	−0.67735	0.16934	0.121460739	0.44573151
FSLN	−0.74221	0.11235	0.083141571	−0.39595641
STLN	0.52842	0.6334	−0.132464288	0.16109519

Mimulus linearis has had many proposed evolutionary histories, including being a subspecies of either *M. longiflorus* or *M. grandiflorus*, as well as being a species of hybrid origin between *M. aurantiacus* and *M. calycinus*. Our data reveal that even though *M. linearis* and *M. grandiflorus* are geographically distinct from each other, they emerge as sister taxa in both the phylogeny and the split network, and there is little shared variation between *M. linearis* and taxa from other clades. Therefore, it remains unclear whether a future taxonomic revision should consider *M. linearis* to be its own entity or a form of *M. grandiflorus*, as proposed previously (Munz, 1959, 1973; Thompson, 2005, 2012).

Mimulus lompocensis has been described as a hybrid species between *M. aurantiacus* and *M. longiflorus* by several authors (McMinn, 1951; Tulig, 2000; Tulig and Nesom, 2012). The two individuals included in this study grouped in different clades in the tree and split network (Clades C and D), and they showed high levels of admixture in the STRUCTURE analysis. While these results are consistent with a history of hybridization, it will be necessary to determine whether *M. lompocensis* is ecologically distinct from its presumed progenitors (Gross and Rieseberg, 2004) before concluding that this admixture reflects a stable taxon of hybrid origin (as done by Tulig and Nesom, 2012) rather than a product of recent natural hybridization.

Finally, *M. australis* has been described as a subspecies of *M. aurantiacus*, its own species, a species of hybrid origin, or in some treatments, *M. australis* has not been described at all (Grant, 1924; Munz, 1935; Pennell, 1951; Beeks, 1962; Thompson, 1993, 2005, 2012). Based on the genomic data analyzed in the current study, *M. australis* is not distinguishable from *M. puniceus*, and the two are interdigitated in the phylogeny. In addition, populations described as *M. australis* show no evidence of being hybrids between *M. puniceus* and *M. longiflorus* (Table 1), as proposed previously by Tulig and Nesom (2012). Nevertheless, partial reproductive isolation has evolved between western red-flowered populations and eastern yellow-flowered populations (Sobel and Streisfeld, 2015). Moreover, multiple floral and vegetative traits are differentiated along this same geographical transition (Stankowski et al., 2015; J. M. Sobel et al., Binghamton University, unpublished manuscript), indicating an early stage of ecological divergence between the taxa. Therefore, based on these data, we would not recommend that *M. puniceus* and *M. australis* be defined as distinct entities. However, even though no previous description of the red-flowered *M. puniceus* exists that also would include the yellow-flowered *M. australis*, we suggest that future revisions incorporate these genomic and ecological patterns into a description that recognizes this divergence in the form of "ecotypes" of the consistently recognized *M. puniceus* (Streisfeld et al., 2013).

In addition to delimiting taxa, a new treatment also must consider the appropriate taxonomic rank for each entity. The difficulty of assigning ranks at or below the species level for this group has been recognized for a long time, as demonstrated by McMinn (1951, p. 34) who wrote, "…since complete agreement has not been reached by botanists as to the status of species, subspecies, and varieties, I have chosen to treat all these field entities (taxa) simply as binomials. Inasmuch as binomials to most botanists indicate species, I have endeavored not to use the word species when writing of these various entities. I must point out, however, that if sterility and geographical distribution tests were the main criteria applied in delimiting species and subspecies, then the field entities … probably would be classified as two taxonomic species, eleven subspecies, and numerous hybrids."

Although McMinn (1951) ends by considering the biological species concept as one way to delimit taxa, this statement foreshadowed the need for integrative taxonomic approaches that considered the different stages of divergence present among taxa in radiations. In most of the previous treatments of this group, the rank employed appears arbitrary and often was not justified by the authors. However, given the interfertility, natural hybridization, and shared genomic variation present among taxa, we support the view by McMinn (1951), and more recently by Thompson (2012), who treated the taxa (with the exception of *M. clevelandii*) as intraspecific subspecies or varieties of *M. aurantiacus*. This view, which acknowledges the reproductive continuity and close relationships among these taxa, emphasizes our need to understand how and why so much diversity arose and has been maintained within this group.

ACKNOWLEDGEMENTS

The authors thank Joshua Bahr for assistance with data collection, James Sobel for providing the *M. kelloggii* sample, and Stacey Smith for discussions on the taxonomic implications of this work. The authors thank two anonymous reviewers and the associate editor for their helpful comments on this manuscript. This project was supported by the University of Oregon and by National Science Foundation grants: DEB-1258199 and DEB-1311686.

DATA ACCESSIBILITY

The *M. puniceus* draft genome assembly has been archived at the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.7j3rq. All data sets and processed short sequence reads from all samples used in phylogenetic and population genomic analyses are deposited at the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5bd62.

LITERATURE CITED

Baird, N. A., P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. A. Lewis, E. U. Selker, W. A. Cresko, and E. A. Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One* 3: e3376.

Beardsley, P. M., S. E. Schoenig, J. B. Whittall, and R. G. Olmstead. 2004. Patterns of evolution in western north american mimulus (Phrymaceae). *American Journal of Botany* 91: 474–489.

Beeks, R. M. 1962. Variation and hybridization in southern California populations of *Diplacus* (Scrophulariaceae). *Aliso* 5: 83–122.

Berner, D., and W. Salzburger. 2015. The genomics of organismal diversification illuminated by adaptive radiations. *Trends in Genetics* 31: 491–499.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. Stacks: An analysis tool set for population genomics. *Molecular Ecology* 22: 3124–3140.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko, and J. H. Postlethwait. 2011. Stacks: Building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes. Genetics* 1: 171–182.

Earl, D. A. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361.

Eaton, D. A., and R. H. Ree. 2013. Inferring phylogeny and introgression using RADseq data: An example from flowering plants (Pedicularis: Orobanchaceae). *Systematic Biology* 62: 689–706.

Ellegren, H., L. Smeds, R. Burri, P. I. Olason, N. Backström, T. Kawakami, A. Künstner, et al. 2012. The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature* 491: 756–760. doi:10.138/nature11584

Emerson, K. J., C. R. Merz, J. M. Catchen, P. A. Hohenlohe, W. A. Cresko, W. E. Bradshaw, and C. M. Holzapfel. 2010. Resolving postglacial phylogeography using high-throughput sequencing. *Proceedings of the National Academy of Sciences, USA* 107: 16196–16200.

Etter, P. D., S. Bassham, P. A. Hohenlohe, E. A. Johnson, and W. A. Cresko. 2011. SNP discovery and genotyping for evolutionary genetics using RAD sequencing. *Molecular Methods for Evolutionary Genetics* 772: 157–178.

Fontaine, M. C., J. B. Pease, A. Steele, R. M. Waterhouse, D. E. Neafsey, I. V. Sharakhov, X. Jiang, et al. 2015. Extensive introgression in a malaria vector species complex revealed by phylogenomics. *Science* 347: 1258524.

Grant, A. L. 1924. A monograph of the genus *Mimulus*. *Annals of the Missouri Botanical Garden* 11: 99–388.

Grant, V. 1993a. Effect of hybridization and selection on floral isolation. *Proceedings of the National Academy of Sciences, USA* 90: 990–993.

Grant, V. 1993b. Origin of floral isolation between ornithophilous and sphingophilous plant species. *Proceedings of the National Academy of Sciences, USA* 90: 7729–7733.

Grant, V. 1994. Modes and origins of mechanical and ethological isolation in angiosperms. *Proceedings of the National Academy of Sciences, USA* 91: 3–10.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson. 2010. A draft sequence of the Neandertal genome. *Science* 328: 710–722.

Gross, B. L., and L. H. Rieseberg. 2004. The ecological genetics of homoploid hybrid speciation. *Journal of Heredity* 96: 241–252.

Hahn, M. W., and L. Nakhleh. 2016. Irrational exuberance for resolved species trees. *Evolution* 70: 7–17.

Handelman, C., and J. R. Kohn. 2014. Hummingbird color preference within a natural hybrid population of *Mimulus aurantiacus* (Phrymaceae). *Plant Species Biology* 29: 65–72.

Harrison, R. G., and E. L. Larson. 2014. Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity* 105 (S1): 795–809.

Heliconius Genome Consortium. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature* 487: 94–98.

Huson, D. H., and D. Bryant. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254–267.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801–1806.

Jarvis, E. D., S. Mirarab, A. J. Aberer, B. Li, P. Houde, C. Li, S. Y. Ho, et al. 2014. Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science* 346: 1320–1331.

Keller, I., C. E. Wagner, L. Greuter, S. Mwaiko, O. M. Selz, A. Sivasundar, S. Wittwer, and O. Seehausen. 2013. Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. *Molecular Ecology* 22: 2848–2863.

Lamichhaney, S., J. Berglund, M. S. Almén, K. Maqbool, M. Grabherr, A. Martinez-Barrio, M. Promerová, et al. 2015. Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature* 518: 371–375.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9: 357–359.

Maddison, W. P. 1997. Gene trees in species trees. *Systematic Biology* 46: 523–536.

Mahler, D. L., T. Ingram, L. J. Revell, and J. B. Losos. 2013. Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science* 341: 292–295.

Malinsky, M., R. J. Challis, A. M. Tyers, S. Schiffels, Y. Terai, B. P. Ngatunga, E. A. Miska, et al. 2015. Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake. *Science* 350: 1493–1498.

Malinsky, M., H. Svardal, A. M. Tyers, E. A. Miska, M. J. Genner, G. F. Turner, and R. Durbin. 2017. Whole genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. *bioRxiv* 143859. doi.org/10.1101/143859

Mallet, J., N. Besansky, and M. W. Hahn. 2015. How reticulated are species? *BioEssays* 38: 140–149.

Mayr, E. 1995. Species, classification, and evolution: Biodiversity and evolution. National Science Museum Foundation, Tokyo, Japan.

McCluskey, B. M., and J. H. Postlethwait. 2015. Phylogeny of zebrafish, a "model species," within *Danio*, a "model genus". *Molecular Biology and Evolution* 32: 635–652.

McMinn, H. E. 1951. Studies in the genus *Diplacus*, Scrophulariaceae. *Madroño* 11: 33–128.

Miller, M. R., J. P. Dunham, A. Amores, W. A. Cresko, and E. A. Johnson. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Research* 17: 240–248.

Munz, P.A., 1935. Manual of southern California botany. Claremont Colleges, Claremont, California, USA.

Munz, P. A., 1959. A California flora. University of California Press, Berkeley, California, USA.

Munz, P. A. 1973. A California flora and supplement. University of California Press, Berkeley, USA. 605–624.

Muschick, M., A. Indermaur, and W. Salzburger. 2012. Convergent evolution within an adaptive radiation of cichlid fishes. *Current Biology* 22: 2362–2368.

Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford, UK.

Patterson, N., P. Moorjani, Y. Luo, S. Mallick, N. Rohland, Y. Zhan, T. Genschoreck, T. Webster, and D. Reich. 2012. Ancient admixture in human history. *Genetics* 192: 1065–1093.

Pease, J. B., D. C. Haak, M. W. Hahn, and L. C. Moyle. 2016. Phylogenomics reveals three sources of adaptive variation during a rapid radiation. *PLoS Biology* 14: e1002379.

Pennell, F.W., 1951. *Mimulus*: Illustrated flora of the Pacific states, vol. 3, 688–731.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Qiu, Y.-L., J. Lee, F. Bernasconi-Quadroni, and D. E. Soltis. 1999. The earliest angiosperms: Evidence from mitochondrial, plastid and nuclear genomes. *Nature* 402: 404–407.

Reich, D., K. Thangaraj, N. Patterson, A. L. Price, and L. Singh. 2009. Reconstructing Indian population history. *Nature* 461: 489–494.

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

Roux, C., C. Fraisse, J. Romiguier, Y. Anciaux, N. Galtier, and N. Bierne. 2016. Shedding light on the grey zone of speciation along a continuum of genomic divergence. *PLoS Biology* 14: e2000234.

Rundle, H. D., and P. Nosil, P. 2005. Ecological speciation. *Ecology Letters* 8: 336–352.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford, UK.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science* 323: 737–741.

Sobel, J. M., and M. A. Streisfeld. 2015. Strong premating isolation exclusively drives insipient speciation in *Mimulus aurantiacus. Evolution* 69: 447–461.

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Stankowski, S., J. M. Sobel, and M. A. Streisfeld. 2015. The geography of divergence with gene flow facilitates multitrait adaptation and the evolution of pollinator isolation in *Mimulus aurantiacus*. *Evolution* 69: 3054–3068.

Stankowski, S., J. M Sobel, and M. A. Streisfeld. 2017. Geographic cline analysis as a tool for studying genome-wide variation: A case study of pollinator-mediated divergence in a monkeyflower. *Molecular Ecology* 26: 107–122.

Stankowski, S., and M. A. Streisfeld. 2015. Introgressive hybridization facilitates adaptive divergence in a recent radiation of monkeyflowers. *Proceedings of the Royal Society, B, Biological Sciences* 282: 20151666.

Streisfeld, M. A., and J. R. Kohn. 2005. Contrasting patterns of floral and molecular variation across a cline in *Mimulus aurantiacus*. *Evolution* 59: 2548–2559.

Streisfeld, M. A., and J. R. Kohn. 2007. Environment and pollinator-mediated selection on parapatric floral races of *Mimulus aurantiacus. Journal of Evolutionary Biology* 20: 122–132.

Streisfeld, M. A., W. N. Young, and J. M. Sobel. 2013. Divergent selection drives genetic differentiation in an R2R3-Myb transcription factor that contributes to incipient speciation in *Mimulus aurantiacus. PLOS Genetics* 9: e1003385.

Thompson, D. M. 1993. *Mimulus*. *In* J. C. Hickman [ed.], The Jepson manual: Higher plants of California, 1037–1047. University of California Press, Berkeley, California, USA.

Thompson, D. M. 2005. Systematics of *Mimulus* subgenus *Schizoplacus* (Scrophulariaceae). *Systematic Botany Monographs* 75: 1–213.

Thompson, D. M. 2012. *Mimulus*. *In* B. G. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, and T. J. Rosatti [eds.], The Jepson manual: Vascular plants of California, 2nd ed. University of California Press, Berkeley, California, USA.

Tulig, M. 2000. Morphological variation in *Mimulus* section *Diplacus* (Scrophulariaceae). Ph.D. dissertation, California State Polytechnic University, Pomona, California, USA.

Tulig, M. C. and G. L. Nesom. 2012. Taxonomic overview of *Diplacus* sect. *Diplacus (Phrymaceae). Phytoneuron* 45: 1–20.

Turner, T. L., M. W. Hahn, and S. V. Nuzhdin. 2005. Genomic islands of speciation in *Anopheles gambiae. PLoS Biology* 3: e285.

Vijay, N., C. M. Bossu, J. W. Poelstra, M. H. Weissensteiner, A. Suh, A. P. Kryukov, and J. B. Wolf. 2016. Evolution of heterogeneous genome differentiation across multiple contact zones in a crow species complex. *Nature Communications* 7: 13195.

Wagner, C. E., I. Keller, S. Wittwer, O. M. Selz, S. Mwaiko, L. Greuter, A. Sivasundar, and O. Seehausen. 2013. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Molecular Ecology* 22: 787–798.

Wallbank, R. W., S. W. Baxter, C. Pardo-Diaz, J. J. Hanly, S. H. Martin, J. Mallet, K. K. Dasmahapatra, et al. 2016. Evolutionary novelty in a butterfly wing pattern through enhancer shuffling. *PLoS Biology* 14: e1002353.

Wells, H. 1980. A distance coefficient as a hybridization index: An example using *Mimulus longiflorus* and *M. flemingii* (Scrophulariaceae) from Santa Cruz Island, California. *Taxon* 29: 53–65.

Wessinger, C. A., C. C. Freeman, M. E. Mort, M. D. Rausher, and L. C. Hileman. 2016. Multiplexed shotgun genotyping resolves species relationships within the North American genus Penstemon. *American Journal of Botany* 103: 912–922.

Wolfe, A. D., C. P. Randle, S. L. Datwyler, J. J. Morawetz, N. Arguedas, and J. Diaz. 2006. Phylogeny, taxonomic affinities, and biogeography of *Penstemon* (Plantaginaceae) based on ITS and cpDNA sequence data. *American Journal of Botany* 93: 1699–1713.

Wu, C. I. 2001. The genic view of the process of speciation. *Journal of Evolutionary Biology* 14: 851–865.