When good species have porous boundaries: weak reproductive isolation and extensive gene

flow between *Mimulus glaucescens* and *M. guttatus* in northern California.

C. T. Ivey[1][*], N. M. Habecker[1], J. P. Bergmann[1], J. Ewald[1], and J. M. Coughlan[2]

[1]Department of Biological Sciences, California State University, Chico, CA, 95929-0515

[2]Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect St., New

Haven, CT, 06520

*correspondence: ctivey@csuchico.edu

Running title: Good species with porous boundaries

14   ACKNOWLEDGEMENTS

We acknowledge the indigenous native peoples of California as the traditional

stewards of the ecosystems studied for this project. K. M. Kay, J. M. Sobel, and the A. L.

Sweigart lab commented on the manuscript; L. S. Kimsey of the University of California,

Davis Bohart Museum identified floral visitors; L. Bergmann, C. Bruns, T. Devine, J.

Habecker, M. Patterson, and A. Pokrzywinski helped in the field and greenhouse; K. A. Blee,

K. F. Gorman, C. Hatfield, J. M. Sobel, D. G. Miller, and K. A. Schierenbeck provided

advice and suggestions; and the Big Chico Creek Ecological Reserve, California Botanical

Society, California Native Plant Society, Chico State Center for Water and the Environment,

Friends of the Chico State Herbarium, Northern California Botanists, and the Vesta Holt

Field Research Award provided financial support.

AUTHOR CONTRIBUTIONS

28 CTI conceived and designed experiments, collected and analysed data; NMH, JPB, and JE

29 designed experiments and collected data; JMC analysed data; all authors contributed to

30 writing the manuscript.

DATA ACCESSIBILITY

32 Data used to characterize reproductive isolation available on Figshare:

33 https://figshare.com/articles/dataset/Monkeyflower_reproductive_boundaries_-

34 _glaucescens_and_guttatus/20407368

Abstract:

Barriers to reproduction are often how progress in speciation is measured.

Nonetheless, a key unresolved question is the extent to which reproductive barriers diminish

gene flow in incipient species in nature. The Sierra Nevada foothill endemic *Mimulus*

glaucescens and the widespread *M. guttatus* are considered to be distinct species based on

contrasting vegetative traits, but barriers to reproduction are not readily apparent, although

these species are not known to hybridize in nature. To explore boundaries between taxa, we

examined 15 potential reproductive barriers between species in a Northern California area of

broad sympatry. Most barriers, with the exception of ecogeographic isolation, were weak, and

total isolation for each species was estimated to be incomplete. Population genomic analyses

of range-wide and broadly sympatric accessions revealed that gene flow between these taxa is

common across the range, and rampant within areas of sympatry. Thus, despite fairly strong

ecological differentiation - which may be involved in maintenance of vegetative differences -

ecological isolation is a weak barrier to gene flow in this system. This work underscores the

value of combining classical measures of reproductive isolation with estimates of natural

gene flow for studies of speciation in natural communities.

Keywords: speciation continuum, reproductive isolation, *Mimulus*, introgression, gene flow,

ecotype

 INTRODUCTION

Species boundaries are often considered to be maintained by reproductive barriers that

limit interbreeding (Mayr 1942). The effectiveness of these barriers at limiting gene flow

typically varies (Lowry et al. 2008a; Widmer et al. 2009; Baack et al. 2015), and in many

cases boundaries are established only through multiple barriers in combination, no one of

which is sufficient to preclude all gene flow (Ramsey et al. 2003; Kay 2006; Ostevik et al.

2016; Christie and Strauss 2019). Species boundaries also can be maintained with few

barriers to reproduction if opposing selection is sufficiently strong (Via 2009; DiVittorio et

al. 2020). However, interbreeding can itself alter the strength of reproductive barriers, either

positively or negatively (Servedio and Kirkpatrick 1997; Runemark et al. 2019). Moreover,

the pace and sequence of barrier evolution appears to be quite variable (Widmer et al. 2009;

Baack et al. 2015; Coughlan and Matute 2020), and in some cases results in apparently stable

partial isolation, in which some gene flow is routine (Stankowski and Ravinet 2021).

As a consequence, some authors have suggested that boundaries between species or

populations are best regarded as falling along a continuum, with panmixia and complete

isolation at the extremes, and the strength of reproductive barriers between two populations

defining their position on the continuum (Merill et al. 2011; Powell et al. 2013; Feder et al.

2014; Harrison and Larson 2014; Christie and Strauss 2019; Stankowski and Ravinet 2021).

In this context, estimates of reproductive isolation between species or populations might be

expected to inform predictions about genetic divergence as well as the likelihood of ongoing

gene exchange. Estimates of barrier strength, however, are not always reliable indicators of

gene flow. Sambatti et al. (2012), for example, estimated reproductive isolation between

Helianthus annuus and *H. petiolaris* to exceed 0.999, based on multiple potential barriers, but

nonetheless found extensive introgression between species, based on sampling multiple

nuclear loci. De La Torre et al. (2014) similarly found extensive admixture between two

Picea species based on genome-wide SNP panels, but also found evidence that adaptation to

different climatic conditions helped maintain species integrity. Finally, although prezygotic

reproductive isolation has been estimated to exceed 0.99 between the recently diverged

Mimulus guttatus and *M. nasutus* (Martin and Willis 2006), introgression appears common

between these taxa (Brandvain et al. 2014; Kenney and Sweigart 2016). Thus, interpretation

of estimates of reproductive isolation with respect to species boundaries benefits from more

direct estimates of gene flow and introgression based on genomic sampling.

104   Progress in understanding the evolution of reproductive isolation and patterns of

speciation has often come from studies of groups of related taxa (Coyne and Orr 1989; Moyle

et al. 2004; Owens and Rieseberg 2013; Christie and Strauss 2018; Coughlan and Matute

2020). One such group includes the diverse wildflower genus *Mimulus* (Wu et al. 2008;

Twyford et al. 2015; Nelson et al. 2021; note that some taxa were placed into other genera by

Barker et al. [2012], but we herein follow Lowry et al. [2019] in the use of *Mimulus* given the

lack of a well-resolved phylogeny). Comparative studies involving *Mimulus* have suggested

habitat divergence, mating system differentiation, or shifts in floral traits associated with

pollination may be common during speciation (Grossenbacher and Whittall 2011;

Grossenbacher et al. 2014; Sobel et al. 2014). As divergence proceeds, however, post-

pollination barriers appear to increase in strength (Ferris et al. 2014; Coughlan et al. 2020;

Sandstedt et al. 2021; Joffard et al. 2021). Introgression has also been a salient feature during

speciation in *Mimulus* (Brandvain et al. 2014; Kenny and Sweigart 2016; Nelson et al. 2021).

Within the adaptive radiation of *Mimulus* section *Erythranthe*, for example, extensive

ancestral and recent introgression has been documented, even between taxa with strong

reproductive barriers, which underscores the importance of natural selection in boundary

maintenance (Nelson et al. 2021).

Many of the *Mimulus* taxa studied to date have relatively conspicuous contrasts in

distribution, habitat, floral traits, or mating system that result in barriers to reproduction. In

contrast, the taxa studied herein, *M. glaucescens* and *M. guttatus*, have few apparent

constraints on gene exchange (see below). Taxonomists, however, have treated them as

distinct species based on marked contrasts in vegetative morphology (Fig. 1; Baldwin et al.

2012; Barker et al. 2012; Nesom 2012), and natural hybrids are not commonly observed

(Vickery 1964). Here, we characterize 15 potential reproductive barriers and use whole-

genome resequencing to estimate barrier strength and compare patterns of reproductive

isolation with patterns of population genetic structure, divergence, and introgression to better

understand boundaries between these taxa. Our results contribute to understanding how

reproductive barriers relate to introgression and illuminate the speciation process in recently

diverged taxa.

134   METHODS

<u>Study system</u>

The genus *Mimulus* (family Phrymaceae) is a diverse group of at least 180 plant

species (Grant 1924; Beardsley et al. 2004; Barker et al. 2012) that has been a focus for

studies of evolution and speciation (Schemske and Bradshaw 1999; Martin and Willis 2007;

Lowry et al. 2008b; Wu et al. 2008; Sobel 2014; Twyford et al. 2015; Nelson et al. 2021;

Sandstedt et al. 2021). Some taxa within section *Simiolus*, which includes *M. guttatus*, have

been taxonomically problematic (Munz 1959; Hickman 1993; Nesom 2012) and likely

represent multiple recently evolved lineages (Beardsley et al. 2004; Sweigart and Willis

2003; Lowry et al. 2008b; Barker et al. 2012). The similar *M. glaucescens*, however, has been

considered distinct from *M. guttatus* based on vegetative characters (Baldwin et al. 2012;

Nesom 2012). Nesom (2012) described *M. glaucescens* as "clearly defined" in a recent

treatment of the section. *Mimulus glaucescens* has peduncular bracts that are perfoliate to

connate, glabrous, distinctly glaucous, and with entire margins, whereas the peduncular

bracts of *M. guttatus* are generally distinct, bearing trichomes that are often glandular, lacking

in glaucousness, and with margins that are often dentate (Fig. 1; Baldwin et al. 2012).

Phylogenies suggest the two are closely related, and possibly sister taxa (Beardsley et al.

2004; Lowry et al. 2019). Both species are mixed-mating herbs, flower during the spring,

occur in similar habitats, and are $n = 14$ (Vickery 1978; Ritland and Ritland 1989; Baldwin et

al. 2012). *Mimulus glaucescens* is endemic to a few counties within northern California,

whereas the broad western North American range of *M. guttatus* wholly overlaps that of *M.*

glaucescens (Baldwin et al. 2012; Nesom 2012). Within the range of *M. glaucescens*, *M.*

guttatus is found on non-serpentine soils (C. T. Ivey, pers. obs.), although it occurs on

serpentine elsewhere (Selby and Willis 2018; Sianta & Kay 2021). In contrast, *M.*

glaucescens occurs on both serpentine and non-serpentine soils (Nesom 2012). Populations in

the current study were from non-serpentine sites unless otherwise noted. Previous work using

few maternal lines reported reduced hybrid seed set and F_1 fertility between *M. glaucescens*

and *M. guttatus* (Vickery 1964). Otherwise, little appears to be known about what limits

hybridization between these taxa.

<u>Pre-pollination barriers</u>

To characterize the importance of pre-pollination barriers to species boundaries,

geographical distribution, local habitat, flowering phenology, floral visitor behavior, and

floral morphology were compared between species. Habitat associated with each species was

modelled using 2512 georeferenced collection records from the Global Biodiversity

Information Facility, which were filtered after download (GBIF; 2194 *M. guttatus* records*:*

https://doi.org/10.15468/dl.ywy9s3 and 318 *M. glaucescens:*

https://doi.org/10.15468/dl.r93avz; see Supplemental Methods and Results) and

environmental data including primary and secondary geological substrates, elevation, and 19

bioclimatic variables (https://www.worldclim.org/), all standardized to a resolution of 2.5

minutes. Species distribution models were constructed using MaxEnt by sampling locations

of collection records as well as 1000 randomly located background samples from across the

geographic extent to create pseudo-absence data (Coughlan et al. 2021). Models were

computed separately for each species, and reproductive isolation was inferred based on the

number of shared and unshared pixels in the map following equation RI_{4C} from Sobel and

Chen (2014) (see Supplemental Methods and Results).

In addition, local-scale habitat characteristics were measured for each species within a

6 km^2 region of sympatry along Butte Creek within Butte county California. Within each of

15 populations (*M. glaucescens* = 9; *M. guttatus* = 6), slope, percent shade cover, percent

vegetative cover, percent bare ground, percent debris, and percent standing water was

measured for each of 8-10 randomly chosen plants (*sensu* Li et al. 2018). Scores of the first

two principal components of the combined data were compared between species using a

mixed-model ANOVA with species as the fixed effect and population as the random effect.

Flowering phenology was compared between species within a flowering season for

four populations of each species occurring within a 2 km^2 region along Butte Creek (Table

S1). The number of flowering plants was recorded once each week from mid-March (when

no plants were flowering) until early August (after all flowering had stopped). Flowering

plant numbers were compared between species using a repeated measures model in which

plant species, day of observation, and their interaction were fixed effects, and population pair

was a random effect. The day of observation was the repeated effect with population as the

subject.

The behavior of floral visitors was compared between species using experimental

mixed-species arrays placed near natural populations of each species (Table S1). Each array

consisted of 18 plants of each species (36 total) in 9 cm^2 pots arranged into a 6 x 6 grid at 0.5

m spacing. Each plant in the array had a single first-day flower open during observations.

Arrays were observed between 0900 and 1500 for 1 hr intervals (total 67 hr) during which the

visitor taxon and sequence of visited plants was recorded. The likelihood of floral visitors

moving conspecifically (response = 0) vs. heterospecifically (1) was compared for visitors

foraging on each species using a generalized linear mixed model with a binomial error

distribution. Fixed effects included the plant species from which the visitor moved, site

location, and their interaction. Random effects included visitor taxon (Table S2) and the

observation.

First-day floral morphology was compared between species using greenhouse-grown

plants reared from open-pollinated seeds collected in four populations of each species (Table

S1). Corolla width at the widest point, corolla tube width at distal opening, anther length,

pistil length, and herkogamy were measured to the closest mm for 120 first-day flowers (*M.*

glaucescens n = 73; *M. guttatus* n = 47), and scores of the first two principal components

based on family mean values were compared between species using a mixed-model ANOVA

with species as the fixed effect and population as the random effect (see Supplemental

Methods and Results).

Post-pollination barriers

To characterize the importance of post-pollination barriers to species boundaries,

pollen-pistil interactions, seed number, seed germination, and several measures of post-

germination performance were measured following controlled hand-pollinations of

emasculated flowers within species (both within- and between-populations) and between

species. Hand-pollinations were performed on plants reared in the greenhouse from open-

pollinated seeds collected from at least two populations of each species within the Butte

Creek watershed (Table S1).

To evaluate pollen-pistil interactions, pollen adhesion, pollen germination, and pollen-

tube growth rate (PTGR) were compared among types of hand-pollinations (*sensu* Kay

2006). The success of pollen adhesion was compared using a modification of Zinkl et al.

(1999). A known number of pollen grains was applied to first-day stigmas (*M. glaucescens n*

= 42; *M. guttatus n* = 53), which were then vortexed in a phosphate-Tween-20 solution,

centrifuged for 60 sec at 7200 rpm, and fixed in 9:1 ethanol:glacial acetic acid for 24 hr

before staining with basic fuchsin and viewing at 100x to record number of adhered pollen

grains. Pollen germination was observed in styles (*M. glaucescens n* = 61; *M. guttatus n* = 56)

that were excised 6-12 hr following hand-pollination, fixed and stained in decolorized 0.1%

aniline blue before viewing at 100x under epifluorescence. The number of pollen tubes in

styles and number of pollen grains on stigmas was recorded. Pollen tube growth rate was

observed in styles (*M. glaucescens n* = 25; *M. guttatus n* = 42) that were excised five minutes

after hand-pollination and placed onto enriched 1% agar. Style length was measured to the

nearest 0.1 mm using a dissection microscope fitted with an ocular micrometer and the time

in min for pollen tubes to emerge from the style was recorded (see Supplemental Methods

and Results).

Seed number was recorded for individual fruits resulting from hand-pollinations of

emasculated flowers (*M. glaucescens n* = 119; *M. guttatus n* = 185) using digital images

following Ivey and Carr (2012). Sixty fruits from each species were randomly chosen, and 20

seeds from each were scored for germination (number of seedlings) two weeks after sowing

into 100 cm^2 pots. One randomly chosen seedling from each fruit was grown to maturity, and

the number of days until the first flower opened (DFF) was scored to indicate development.

In addition, ovule number and pollen viability were scored from the first open flower

following Carr and Dudash (1997). After 59 days, total number of flowers was recorded, and

all above-ground biomass was dried to constant mass at 55º C before weighing to nearest mg.

Post-pollination traits were compared using mixed-model ANOVA that included

hand-pollination category (within-population, between-population conspecific, and

heterospecific), the species of pollen recipient, and their interaction as fixed effects, whereas

random effects included the population identities of pollen donor and pollen recipient.

Additional details about models used for each trait can be found in Supplemental Methods

and Results.

<u>Reproductive isolation</u>

Reproductive isolation was estimated for each of the potential barriers above where

possible, following Sobel and Chen (2014). To provide context for interpreting estimates of

isolation between species, estimates of isolation were calculated between populations within

each species as well (Table S3). Total reproductive isolation was estimated from all barriers

using equation RI_{4E} from Sobel and Chen (2014). Stability of the estimates was evaluated by

constructing 95% confidence intervals for individual barriers as well as total isolation using

10,000 bootstrap replicates except in the case of ecogeographic isolation in which we used

1,000 bootstraps for computational efficiency (*sensu* Ostevik et al. 2016; Christie and Strauss

2019).

<u>Genomic analyses</u>

We paired previously published whole-genome re-sequencing data (Brandvain et al.

2014, Puzey et al. 2018, Coughlan et al. 2020, Coughlan et al. 2021) with new whole-genome

re-sequencing data described herein to assess taxonomic relationships, patterns of population

genetic structure, and the presence of introgression between *M. guttatus* and *M. glaucescens*

(See Table S4 for sample details). In total, we leverage data from 75 accessions representing

10 named species across the *M. guttatus* species complex plus a single accession of *M.*

dentilobus as an outgroup. For the newly described whole-genome re-sequencing data, we

followed protocols described elsewhere for sample preparation, sequencing, and preparation

of VCF files (e.g. Coughlan et al. 2020, Coughlan et al. 2021; see Supplemental Methods for

details).

Phylogenetic relationships: To assess phylogenetic relationships among named

members of the *M. guttatus* species complex we inferred a Maximum Likelihood (ML)

phylogeny as well as 1,000 ultrafast bootstraps using IQtree V2.0.3 (Nguyen et al. 2015) with

a TVM+F+R4 model, which IQtree identified as the best fitting model based on both AIC

and BIC. For this analysis, we filtered the VCF file to include sites in which 72 of the 75

accessions were genotyped (~95% of accessions), which resulted in 195,771 sites, 146,697 of

which were parsimony informative. We also performed principal components analyses on

genome-wide data using PCANGSD (Korneliussen et al. 2014) for all members of the

complex (Fig. S1) as well as just *M. guttatus* and *M. glaucescens,* to evaluate whether named

species correspond to genetically distinct clusters. We furthered this analysis by performing

K-means clustering analysis using both PCANGSD (in which K is identified as the number of

significant PCs +1) and more traditional K-means clustering analyses using NGSAdmix

(Skotte et al. 2013), in which we determined the best K by performing 10 replicate runs at Ks

1-7, then using CLUMPAK (Kopelman et al. 2015) to identify the best value of K. To

perform both PCANGSD and NGSADMIX analyses with only *M. glaucescens* and *M.*

guttatus, we generated a genotype likelihood file to include sites which contained data for 31

(out of a total of 34) individuals, a minimum mapping quality of 30, and a minimum base

quality of 20, which resulted in a final dataset including 13,827,721 sites. In both cases, K=3

was optimal, so we restricted subsequent analyses to the results from PCANGSD.

Divergence, differentiation, and diversity: We used *Pixy* to calculate divergence,

differentiation, and diversity (D_{XY}, F_{ST}, and π, respectively; Korunes & Samuk 2021) on 4-

fold degenerate synonymous sites for all 11 taxa included herein (Northern and Southern *M.*

guttatus clades were treated as distinct taxa for these analyses). For this analysis, the VCF

described above was curated to include only 4-fold degenerate synonymous sites (from

Brandvain et al. 2014, Coughlan et al. 2020), for the 14 main scaffolds of the *M. guttatus*

reference genome (corresponding to the 14 chromosomes in this group). We used the default

filtering expressions in *Pixy* (DP>=10, RGQ>=20 for invariant sites; DP>=10, GQ>=20,

RGQ>=20 for variant sites), and calculated D_{XY}, F_{ST}, and π in 500KB windows before

averaging all windows for genome-wide values (Table S5).

Evidence for introgression: We used Dsuite to assess Patterson's D (i.e. ABBA-

BABA statistics; Malinsky et al. 2021) for all possible trios of all individual genomes of

Northern *M. guttatus* (see Results for distinction of Northern *M. guttatus* versus Southern *M.*

guttatus) and *M. glaucescens*, given the ML phylogeny produced using IQtree. Our aims in

this analysis were first, to assess evidence for introgression between Northern *M. guttatus* and

M. glaucescens. Second, if significant introgression was inferred, we sought to compare the

magnitude of introgression between sympatric accessions of *M. glaucescens* and *M. guttatus,*

vs. allopatric accessions. Greater introgression between sympatric samples would be

indicative of ongoing gene flow, as opposed to historic gene flow or more complex patterns

of species bifurcation. To assess introgression we used a standard Jack-knifing procedure on

all Patterson's D statistics, with Bonferroni correction for multiple comparisons to determine

if any pairs of *M. guttatus* and *M. glaucescens* genomes exhibited significant introgression.

To assess whether introgression was stronger between sympatric accessions, we constructed a

linear mixed model with f_G (the proportion of the genome that has experienced introgression;

Malinksy *et al.* 2021) as the response variable, distribution type (whether *M. guttatus* sample

was sympatric or allopatric) as fixed effect, and the individual identities of P1 in each trio as

random effects. We assessed significance of the fixed effect (i.e. distribution type) with an

ANOVA using a Wald's χ^2.

325
326
327   RESULTS

<u>Species distribution modeling</u>

The predicted distribution of *M. glaucescens* was centered on an area spanning the

boundary of Butte and Tehama counties, whereas *M. guttatus* distribution was predicted to

occur more broadly across the study area (Fig. 1A). The first principal component of the 22

modelled environmental variables explained over half of the variation observed and was

positively associated with elevation and bioclimatic variables indicative of precipitation

during dry and warm seasons, and negatively associated with bioclimatic variables explaining

variation in temperature. PC 2 explained nearly 20% of the observed variation and was

positively associated with temperature range variables and negatively associated with several

precipitation variables (Fig. 1C). *Mimulus guttatus* and *M. glaucescens* differed in their mean

position along PC1 ($t = -5.93$, $df = 246.5$, $P < 0.001$) for predicted occurrence points, but not

PC2 ($t = -0.407$, $df = 279.8$, $P = 0.68$).

<u>Reproductive isolation</u>

With the exception of ecogeographic isolation, the barriers to reproduction measured

provided modest or no contribution to reproductive isolation between species (Fig. 2,

Supplementary Methods and Results). For both species, the confidence intervals of

reproductive isolation overlapped zero for a majority of potential barriers (Table S3), and two

barriers for each species were significantly negative, indicating that hybrid matings

outperformed conspecific ones for these barriers. Total isolation was estimated to be

incomplete for both species, but higher for *M. glaucescens* (Fig. 2, *M. glaucescens* total RI

95% CI = 0.350-0.913; *M. guttatus* = 0.024-0.755), suggesting asymmetry in the strength of

isolation. Other than ecogeographic isolation, flowering phenology (both pre- and post-

zygotic) was the only barrier that was significantly positive for both species (Fig. 2).

Between-population estimates of reproductive isolation within each species were also weak

or overlapping zero, and broadly similar to between-species estimates (Table S3).

Genomic analyses

The complex phylogenetic history within *Simiolus* section of *Mimulus* reported

elsewhere (Swegart et al. 2008; Brandvain et al. 2014; Twyford & Friedman 2015; Coughlan

2020; Coughlan 2021) was re-confirmed (Fig. 3A). The widespread taxon *M. guttatus*

included deep genetic structure distinguishing a Northern and Southern clade, as has been

reported elsewhere (Brandvain et al. 2014; Twyford & Friedman 2015), whereas most other

named taxa included in the phylogeny represented monophyletic clades sister to one or both

of the two *M. guttatus* clades. This was also the case for *M. glaucescens*, which emerged as

monophyletic and sister to the Northern *M. guttatus* clade (Fig. 3A), although that monophyly

was not reciprocal for Northern *M. guttatus* (similar patterns appear elsewhere within the

phylogeny: Fig. 3A). Moreover, *M. glaucescens* appears to be one of the most recently

diverged taxa, with both a shorter branch length and relatively lower F_{ST} and D_{XY} compared

to other species pairs in the complex (Fig. 3C).

The first principal component of the genome-wide data within *M. glaucescens* and *M.*

guttatus mirrored the phylogenetic structure for Northern and Southern *M. guttatus* clades, in

that the accessions for the two clades clustered on different ends of that axis (Fig. 3B). The

second principal component, however, revealed distinct ancestry between *M. glaucescens*

from *M. guttatus*, with two Northern *M. guttatus* accessions clustering with all *M.*

glaucescens genomes. These two accessions had a majority (78% and 87%) of genomic

ancestry characteristic of *M. glaucescens* (Fig. 3D), and were from two populations within

the Butte Creek canyon area of local sympatry (Table S1). Although the ancestry percentages

in these two accessions were high and were responsible for breaking reciprocal monophyly

between Northern *M. glaucescens* and *M. guttatus* (Fig. 3A), *M. glaucescens*-like ancestry

was relatively common across *M. guttatus* accessions (mean *M. glaucescens*-like ancestry

was 24% and 10% for the Northern and Southern clade of *M. guttatus*, respectively; Fig. 3D).

The reverse, however, was not observed; *M. guttatus*-like ancestry was far lower within *M.*

glaucescens accessions (chiefly within sympatric accessions), consistent with the asymmetry

in reproductive isolation observed (Fig. 2).

Several lines of evidence suggested recent or ongoing introgression between *M.*

glaucescens and *M. guttatus*. *Mimulus glaucescens* and *M. guttatus* shared unusually low

differentiation and divergence compared to other pairs of taxa sampled (Fig. 3C, Table S5;

$D_{XY} = 0.039$ vs 0.043 for Northern vs. Southern *M. guttatus* clades, respectively). This result,

however, varied with distribution; differentiation and divergence were higher between

allopatric accessions of Northern *M. guttatus* and *M. glaucescens* vs. sympatric accessions

(allopatric: $D_{XY} = 0.0399$, $F_{ST} = 0.1090$; sympatric: $D_{XY} = 0.0351$, $F_{ST} = 0.0037$), suggesting a

history of introgression in sympatry. Similarly, Patterson's D revealed significantly higher

introgression between sympatric accessions than allopatric accessions of *M. glaucescens* and

Northern *M. guttatus* (Fig. 4). Overall, introgression appeared to be common between both

clades of *M. guttatus* and *M. glaucescens*, particularly for Northern *M. guttatus* in close

proximity to *M. glaucescens*.

397   DISCUSSION

Speciation presupposes robust barriers to gene flow (Mayr 1942; Coyne and Orr

2004), thus well-defined species with less than robust isolation provoke consternation. The

taxa considered herein, *Mimulus guttatus* and *M. glaucescens*, have distinctive vegetative

morphology (Fig. 1B) that has led generations of taxonomists (Greene 1885; Grant 1924;

Hickman 1993; Baldwin et al. 2012; Barker et al. 2012; Nesom 2012) to place them into

separate species, despite nomenclatural instability in close relatives (e.g., Barker et al. 2012;

Nesom 2012), and even within *M. guttatus* (Grant 1924; Nesom 2012). The morphological

traits distinguishing *M. glaucescens* from *M. guttatus* are not reported as intermediate forms

in the field (C.T. Ivey, personal observation) or herbaria (Nesom 2012, but see Vickery

1964). Despite divergent phenotypes, our estimates of reproductive isolation between these

taxa were low (Fig. 2), with only a couple of barriers estimated to be stronger between

species than between populations within a species (Table S3). Genomic evidence underscored

the idea that species boundaries were porous. Allopatric accessions had greater genetic

divergence, but regardless of distribution, weak genetic differentiation and significant

introgression was observed between *M. glaucescens* and *M. guttatus* (Fig. 4). Overall, *M.*

glaucescens should perhaps be regarded as an incipient species or an ecotype of *M. guttatus*,

despite over a century of stable taxonomic status as a distinct species.

The notion that reproductively isolated or otherwise genetically distinct taxa could

experience routine gene flow was historically controversial but has been widely reported

more recently (Mebert 2008; Fitzpatrick et al. 2008; Martin et al. 2013; Harrison and Larson

2014; Gao et al. 2020; Jiao and Yang 2020). Persistent gene flow despite significant

reproductive barriers has been observed in diverse taxa (Fitzpatrick et al. 2008; Larson et al.

2014; Richards et al. 2018; Schield et al. 2019), including other monkeyflowers (Brandvain et

al. 2014; Kenny and Sweigart 2016; Nelson et al. 2021; Kiang and Hamrick 1978; Martin and

Willis 2007; Case and Willis 2008; Fishman et al. 2014; Kenny and Sweigart 2016;

Schemske and Bradshaw 1999; Ramsey et al. 2003). Speciation has been shown to proceed in

the face of gene flow in some cases (Martin et al. 2013; Harrison and Larson 2014). Our

results are thus consistent with an emerging understanding of speciation that admits that gene

flow can be a routine occurrence during speciation, particularly during its early stages.

Of course, reproductive barriers limit introgression for many taxa. Prezygotic barriers

are often considered to evolve more rapidly (Widmer et al. 2009; Baack et al. 2015; Christie

et al. 2022), but postzygotic reproductive isolation can also be important (DiVittorio et al.

2020; Coughlan and Matutue 2020; Sandstedt et al. 2021). Regardless, the environment often

mediates isolation, as with immigrant inviability (Nosil et al. 2005; Richards and Ortiz-

Barrientos 2016) or selection against ecologically-unfit hybrids (Jacquemyn et al. 2018;

Ferris and Willis 2018). For *M. glaucescens* and *M. guttatus*, the strongest barrier was

ecogeographic, with climate and elevation as potential agents of ecological differentiation

(Fig. 1). Ecogeographic isolation is often strong in plants (Lowry et al. 2008a, 2008b; Sobel

2014; Li et al. 2018; Christie & Strauss 2018; Vargas et al. 2020; Sianta and Kay 2021),

although divergence in habitat is often associated with genetically based differences in

reproductive biology, such as flowering time (Silvertown et al. 2005; Antonovics 2006;

Bomblies 2010; Sianta and Kay 2021) or pollination (Grossenbacher and Whittall 2011;

Ostevik et al. 2012). Otherwise, assortative mating within taxa may be limited, with free

genomic exchange, except for loci explicitly involved in local adaptation (e.g., Hendrick et al.

2016). In addition to ecogeographic isolation, we found significantly positive, though weak,

isolation based on divergence in flowering time (Fig. 2), which is consistent with the idea that

habitat and reproductive divergence may co-occur during speciation. Despite weak isolation,

the distinctive genetic coherence of *M. glaucescens* was supported based on the phylogenetic

analysis (Fig. 3) as well as estimates of differentiation; for example, D_{XY} between Northern

M. guttatus and *M. glauscens* was higher than π (nucleotide diversity) within Northern *M.*

guttatus (Fig. 3; Table S4).

The factors maintaining vegetative differences between *M. glaucescens* and *M.*

guttatus (Fig. 1) remain unclear. To our knowledge the genetic basis for expression of these

traits (peduncular bract fusion and glaucousness, trichomes) in *M. glaucescens* has not been

characterized, but large-effect QTL for glaucousness and trichome density reported from

other studies (Bennet et al. 2012; Holeski et al. 2010; Hendrick et al. 2016) suggest that

segregating phenotypes might not be intermediate. If so, hybrid or introgressed individuals

may be difficult to discern in the field based on vegetative phenotype alone. Glaucousness

has been found to facilitate drought tolerance (Xue et al. 2017) and defense against insect

herbivores (Eigenbrode and Espelie 1995). Similarly, transitions in trichome density have

been shown to be influenced by natural selection within other monkeyflower taxa (Hendrick

et al. 2016). The extent to which these traits contribute to the divergence observed in habitat

distribution between *M. glaucescens* and *M. guttatus* remains to be tested.

The pattern of distribution observed – asymmetric range sizes, broadly sympatric, but

with environmentally based geographic divergence – was similar to that reported for other

pairs of *Mimulus* sister taxa (Grossenbacher et al. 2014; Sobel 2014) and indeed, pairs of

sister taxa more generally within the California Floristic Province (Anacker and Strauss

2014). This pattern is not inconsistent with models of budding (or peripatric) speciation

(Grossenbacher et al. 2014). The genomic analyses are also consistent with the idea that

budding speciation is common throughout the species complex; many of the taxa included in

the phylogeny, including *M. glaucescens*, were sister to one of the main *M. guttatus* clades

(Fig. 3A). Similarly, *M. guttatus* ancestral variation was substantially represented within each

of the putatively derived taxa (Fig. 3A; Fig. S1; Table S5).

In conclusion, we show that the recently diverged, but well differentiated taxa, *M.*

glaucescens and *M. guttatus*, have surprisingly low reproductive isolation and abundant gene

flow, in contrast to what is suggested by their taxonomy and morphology. This study

contributes to a growing understanding of the complex evolutionary patterns involved in the

formation of species boundaries during speciation (Martin et al. 2013; Feder et al. 2014;

Harrison and Larson 2014; Jiao and Yang 2020; Stankowski and Ravinet 2021; Coughlan et

al. 2021) and the contribution of reproductive barriers toward delineation of lineages (Baack

et al. 2015; Ostevik et al. 2016; Christie and Strauss 2019; Runemark et al. 2019). More

fundamentally, these results underscore that integration of classical ecological studies of

reproductive isolation with genomic approaches will be instrumental for continued progress

in disentangling this fundamental and intriguing evolutionary process.

484   REFERENCES

485
486   Anacker BL, Strauss SY. 2014. The geography and ecology of plant speciation: range overlap
487   and niche divergence in sister species. Proceedings of the Royal Society B 281: 20132980.
488
489   Antonovics J. 2006. Evolution in closely adjacent plant populations X: long-term persistence
490   of prereproductive isolation at a mine boundary. Heredity 97: 33-37.
491
492   Baack E, Melo MC, Rieseberg LH, Ortiz-Barrientos D. 2015. The origins of reproductive
493   isolation in plants. New Phytologist 207: 968-984.
494
495   Baldwin BG, Goldman DH, Keil DJ, Patterson R, Rosatti TJ, Wilken DH (eds.). 2012. The
496   Jepson manual: vascular plants of California, second edition. University of California Press,
497   Berkeley, CA.
498
499   Barker WR, Nesom GL, Beardsley PM, Fraga NS. 2012. A taxonomic conspectus of
500   Phrymaceae: a narrowed circumscription for *Mimulus*, new and resurrected genera, and new
501   names and combinations. Phytoneuron 39: 1-60.
502
Beardsley PM, Schoenig SE, Whittall JB, Olmstead RG. 2004. Patterns of evolution in
western North American *Mimulus* (Phrymaceae). American Journal of Botany 91: 474-489.

Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, Reyonolds M,
Schnurbusch T, Langridge P. 2012. Identification of novel quantitative trait loci for days to
ear emergence and flag leaf glaucouseness in a bread wheat (*Triticum aestivum* L.)
population adapted to southern Australian conditions. Theoretical and Applied Genetics 124:
697-711.

Bomblies K. 2010. Doomed lovers: mechanisms of isolation and incompatibility in plants.
Annual Review of Plant Biology 61: 109-124.

Brandvain Y, Kenney AM, Flagel L, Coop G, Sweigart AL. 2014. Speciation and
introgression between *Mimulus nasutus* and *Mimulus guttatus*. PLOS Genetics 106:
e1004410.

Carr DE, Dudash MR. 1997. The effects of five generations of enforced selfing on potential
male and female function in *Mimulus guttatus*. Evolution 51: 1797-1807.

Case AL, Willis JH. 2008. Hybrid male sterility in *Mimulus* (Phrymaceae) is associated with
a geographically restricted mitochondrial rearrangement. Evolution 62: 1026-1039.

Christie K, Strauss SY. 2018. Along the speciation continuum: quantifying intrinsic and
extrinsic isolating barriers across five million years of evolutionary divergence in California
jewelflowers. Evolution 72: 1063-1079.

Christie K, Strauss SY. 2019. Reproductive isolation and the maintenance of species
boundaries in two serpentine endemic jewelflowers. Evolution 73: 1375-1391.

Christie K, Fraser LS, Lowry DB. 2022. The strength of reproductive isolating barriers in
seed plants: insights from studies quantifying premating and postmating reproductive barriers
over the past 15 years. Evolution https://doi.org/10.1111/evo.14565

Coughlan JM, Matute DR. 2020. The importance of intrinsic postzygotic barriers throughout
the speciation process. Philosophical Transactions of the Royal Society B 375: 20190533.

Coughlan JM, Brown MW, Willis JH. 2020. Patterns of hybrid seed inviability in the
Mimulus guttatus sp. complex reveal a potential role of parental conflict in reproductive
isolation. Current Biology 30: 83-93.

Coughlan JM, Brown MW, Willis JH. 2021. The genetic architecture and evolution of life-
history divergence among perennials in the *Mimulus guttatus* species complex. Proceedings
of the Royal Society B 288: 20210077.

Coyne JA, Orr HA. 1989. Patterns of speciation in *Drosophila*. Evolution 43: 362-381.

Coyne JA, Orr HA. 2004. Speciation. Sinauer Associates, Sunderland, MA.
550

551   DiVittorio CT, Singhal S, Roddy AB, Zapata F, Ackerly DD, Baldwin BG, Brodersen CR,
552   Búrquez A, Fine PVA, Flores MP, Solis E, Morales-Villavicencio J, Morales-Arce D, Kyhos
553   DW. 2020. Natural selection maintains species despite frequent hybridization in the desert
554   shrub *Encelia*. Proceedings of the National Academy of Sciences USA 117: 33373-33383.
555

556   De La Torre AR, Roberts DR, Aitken SN. 2014. Genome-wide admixture and ecological
557   niche modelling reveal the maintenance of species boundaries despite long history of
558   interspecific gene flow. Molecular Ecology 23: 2046-2059.
559

560   Eigenbrode SD, Espelie KE. 1995. Effects of plant epicuticular lipids on insect herbivores.
561   Annual Review of Entomology 40: 171-194.
562

563   Feder JL, Nosil P, Wacholder AC, Egan SP, Berlocher SH, Flaxman SM. 2014. Genome-
564   wide congealing and rapid transitions across the speciation continuum during speciation with
565   gene flow. Journal of Heredity 105: 810-820.
566

567   Ferris KG, Willis JH. 2018. Differential adaptation to a harsh granite outcrop habitat between
568   sympatric *Mimulus* species. Evolution 72: 1225-1241.
569

570   Ferris KG, Sexton JP, Willis JH. 2014. Speciation on a local geographic scale: the evolution
571   of a rare rock outcrop specialist in *Mimulus*. Philosophical Transactions of the Royal Society
572   B 369: 20140001.
573

574   Fishman L, Sweigart AL, Kenny AM, Campbell S. 2014. Major quantitative trait loci control
575   divergence in critical photoperiod for flowering between selfing and outcrossing species of
576   monkeyflower (*Mimulus*). New Phytologist 201: 1498-1507.
577

578   Fitzpatrick BM, Placyk Jr. JS, Niemiller ML, Casper GS, Burghardt GM. 2008.
579   Distinctiveness in the face of gene flow: hybridization between specialist and generalist
580   gartersnakes. Molecular Ecology 17: 4107-4117.
581

582   Gao Y, Harris AJ, Li H, Gao X. 2020. Hybrid speciation and introgression both underlie the
583   genetic structures and evolutionary relationships of three morphologically distinct species of
584   *Lilium* (Liliaceae) forming a hybrid zone along an elevational gradient. Frontiers in Plant
585   Science 11: 576407.
586

587   Grant AL. 1924. A monograph of the genus *Mimulus*. Annals of the Missouri Botanical
588   Garden 11: 99-389.
589

590   Greene EL. 1885. Studies in the botany of California and parts adjacent. Bulletin of the
591   California Academy of Sciences 1: 66-127.
592

593   Grossenbacher DL, Whittall JB. 2011. Increased floral divergence in sympatric
594   monkeyflowers. Evolution 65: 2712-2718.
595
596   Grossenbacher DL, Veloz SD, Sexton JP. 2014. Niche and range size patterns suggest that
597   speciation begins in small, ecologically diverged populations in North American
598   monkeyflowers (*Mimulus* spp.). Evolution 68: 1270-1280.
599
600   Harrison RG, Larson EL. 2014. Hybridization, introgression, and the nature of species
601   boundaries. Journal of Heredity 105: 795-809.
602
603   Hendrick MF, Finseth FR, Mathiasson ME, Palmer KA, Broder EM, Breigenzer P, Fishman
604   L. 2016. The genetics of extreme microgeographic adaptation: an integrated approach
605   identifies a major gene underlying leaf trichome divergence in Yellowstone *Mimulus*
606   *guttatus*. Molecular Ecology 25: 5647-5662.
607
608   Hickman JC (ed.). 1993. The Jepson Manual. University of California Press, Berkeley, CA.
609
610   Holeski LM, Chase-Alone R, Kelly JK. 2010. The genetics of phenotypic plasticity in plant
611   defense: trichome production in *Mimulus guttatus*. American Naturalist 175: 391-400.
612
613   Ivey CT, Carr DE. 2012. Tests for the joint evolution of mating system and drought escape in
614   *Mimulus*. Annals of Botany 109: 583-598.
615
616   Jacquemyn H, De Kort H, Broeck AV, Brys R. 2018. Immigrant and extrinsic hybrid seed
617   inviability contribute to reproductive isolation between forest and dune ecotypes of *Epipactis*
618   *helleborine* (Orchidaceae). Oikos 127: 73-84.
619
620   Jiao X, Yang Z. 2021. Defining species when there is gene flow. Systematic Biology 70: 108-
621   119.
622
623   Joffard N, Silva WTAF, Twyford AD. 2021. The role of postzygotic isolation in *Mimulus*
624   speciation. Evolution 75: 756-758.
625
626   Kay KM. 2006. Reproductive isolation between two closely related hummingbird-pollinated
627   neotropical gingers. Evolution 60: 538-552.
628
629   Kenney AM, Sweigart AL. 2016. Reproductive isolation and introgression between
630   sympatric *Mimulus* species. Molecular Ecology 25: 2499-2517.
631
632   Kiang YT, Hamrick JL. 1978. Reproductive isolation in the *Mimulus guttatus-M. nasutus*
633   complex. American Midland Naturalist 100: 269-276.
634

635 Kopelman NM, Mayzel, J, Jakobsson M, Rosenberg NA, Mayrose I. 2015. CLUMPAK: a
636 program for identifying clustering modes and packaging population structure inferences
637 across *K*. Molecular Ecology Resources 15: 1179-1191.
638
639 Korneliussen TS, Albrechtsen A, Nielsen R. 2014. ANGSD: analysis of next generation
640 sequencing data. BMC Bioinformatics 15: 356.
641
642 Korunes KL, Samuk K. 2021. PIXY: Unbiased estimation of nucleotide diversity and
643 divergence in the presence of missing data. Molecular Ecology Resources 21: 1359-1368.
644
645 Larson EL, White TA, Ross CL, Harrison RG. 2014. Gene flow and the maintenance of
646 species boundaries. Molecular Ecology 23: 1668-1678.
647
648 Li Q, Grossenbacher DL, Angert AL. 2018. The effect of range overlap on ecological niche
649 divergence depends on spatial scale in monkeyflowers. Evolution 72: 2100-2113.
650
651 Lowry DB, Modliszewski JL, Wright KM, Wu CA, Willis JH. 2008a. The strength and
652 genetic basis of reproductive isolating barriers in flowering plants. Philosophical
653 Transactions of the Royal Society B 363: 3009-3021.
654
655 Lowry DB, Rockwood RC, Willis JH. 2008b. Ecological reproductive isolation of coast and
656 inland races of *Mimulus guttatus*. Evolution 62: 2196-2214.
657
Lowry DB, Sobel JM, Angert AL, Ashman T-L, Baker RL, Blackman BK, Brandvain Y,
Byers KJRP, Cooley AM, Coughlan JM, Dudash MR, Fenster CB, Ferris KG, Fishman L,
Friedman J, Grossenbacher DL, Holeski LM, Ivey CT, Kay KM, Koelling VA, Kooyers NJ,
Murren CJ, Muir CD, Nelson TC, Peterson ML, Puzey JR, Rotter MC, Seemann JR, Sexton
JP, Sheth SN, Streisfeld MA, Sweigart AL, Twyford AD, Vallejo-Marín M, Willis JH,
Wright KM, Wu CA, Yuan Y-W. 2019. The case for the continued use of the genus name
Mimulus for all monkeyflowers. Taxon 68: 617-623.
665
666 Malinsky M, Matschiner M, Svardal H. 2021. Dsuite-Fast *D*-statistics and related admixture
667 evidence from VCF files. Molecular Ecology Resources 21: 584-595.
668
669 Martin NH, Willis JH. 2007. Ecological divergence associated with mating system causes
670 nearly complete reproductive isolation between sympatric *Mimulus* species. Evolution 61:
671 68-82.
672
673 Martin SH, Dasmahapatra KK, Nadeau NJ, Salazar C, Walters JR, Simpson F, Blaxter M,
674 Manica A, Mallet J, Jiggins CD. 2013. Genome-wide evidence for speciation with gene flow
675 in *Heliconius* butterflies. Genome Research 23: 1817-1828.
676
677 Mayr E. 1942. Systematics and the origin of species. Columbia University Press, New York,
678 NY.
679

Mebert K. 2008. Good species despite massive hybridization: genetic research on the contact zone between the watersnakes *Nerodia sipedon* and *N. fasciata* in the Carolinas, USA. Molecular Ecology 17: 1918-1929.

Merrill RM, Gompert Z, Dembeck LM, Kronforst MR, McMillan WO, Jiggins CD. 2011. Mate preference across the speciation continuum in a clade of mimetic butterflies. Evolution 65: 1489-1500.

Moyle LC, Olson MS, Tiffin P. 2004. Patterns of reproductive isolation in three angiosperm genera. Evolution 58: 1195-1208.

Munz PA. 1959. A California flora. University of California Press, Berkeley, CA.

Nelson TC, Stathos AM, Vanderpool DD, Finseth FR, Yuan Y-W, Fishman L. 2021. Ancient and recent introgression shape the evolutionary history of pollinator adaptation and speciation in a model monkeyflower radiation (*Mimulus* section Erythranthe). PLOS Genetics 17: 1009095.

Nesom GL. 2012. Taxonomy of *Erythranthe* sect. Simiola (Phrymaceae) in the USA and Mexico. Phytoneuron 40:1-123.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–74.

Nosil P, Vines TH, Funk DJ. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution 59: 705-719.

Ostevik KL, Moyers BT, Owens GL, Rieseberg LH. 2012. Parallel ecological speciation in plants? International Journal of Ecology 2012: 939862.

Ostevik KL, Andrew RL, Otto SP, Rieseberg LH. 2016. Multiple reproductive barriers separate recently diverged sunflower ecotypes. Evolution 70: 2322-2335.

Owens GL, Rieseberg LH. 2013. Hybrid incompatibility is acquired faster in annual than in perennial species of sunflower and tarweed. Evolution 68: 893-900.

Powell TH, Hood GR, Murphy MO, Heilveil JS, Berlocher SH, Nosil P, Feder JL. 2013. Genetic divergence along the speciation continuum: the transition from host race to species in *Rhagoletis* (Diptera: Tephritidae). Evolution 67: 2561-2576.

Puzey JR, Willis JH, Kelly JK. 2017. Population structure and local selection yield high genomic variation in *Mimulus guttatus*. Molecular Ecology 26: 519-535.

Ramsey J, Bradshaw Jr., HD, Schemske DW. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). Evolution 57: 1520-1534.

728 Richards TJ, Ortiz-Barrientos D. 2016. Immigrant inviability produces a strong barrier to
729 gene flow between parapatric ecotypes of *Senecio lautus*. Evolution 70: 1239-1248.
730
731 Richards EJ, Poelstra JW, Martin CH. 2018. Don't throw out the sympatric speciation with
732 the crater lake water: fine-scale investigation of introgression provides equivocal support for
733 causal role of secondary gene flow in one of the clearest examples of sympatric speciation.
734 Evolution Letters 2: 524-540.
735
736 Ritland C, Ritland K. 1989. Variation of sex allocation among eight taxa of the *Mimulus*
737 guttatus species complex (Scrophulariaceae). American Journal of Botany 76: 1731-1739.
738
739 Runemark A, Vallejo-Marin M, Meier JI. 2019. Eukaryote hybrid genomes. PLOS Genetics
740 15: e1008404.
741
742 Sambatti JBM, Strasburg JL, Ortiz-Barrientos D, Baack EJ, Rieseberg LH. 2012. Reconciling
743 extremely strong barriers with high levels of gene exchange in annual sunflowers. Evolution
744 66: 1459-1473.
745
746 Sandstedt GD, Wu CA, Sweigart AL. 2021. Evolution of multiple postzygotic barriers
747 between species of the *Mimulus tilingiii* complex. Evolution 75: 600-613.
748
749 Schemske DW, Bradshaw Jr. HD. 1999. Pollinator preference and the evolution of floral
750 traits in monkeyflowers (*Mimulus*). Proceedings of the National Academy of Sciences 96:
751 11910-11915.
752
753 Schield DR, Perry BW, Adams RH, Card DC, Jezkova T, Pasquesi GIM, Nikolakis ZL, Row
754 K, Meik JM, Smith CF, Mackessy SP, Castoe TA. 2019. Allopatric divergence and secondary
755 contact with gene flow: a recurring theme in rattlesnake speciation. Biological Journal of the
756 Linnean Society 128: 149-169.
757
758 Selby JP, Willis JH. 2018. Major QTL controls adaptation to serpentine soils in *Mimulus*
759 *guttatus*. Molecular Ecology 27: 5073-5087.
760
761 Servedio MR, Kirkpatrick M. 1997. The effects of gene flow on reinforcement. Evolution 51:
762 1764-1772.
763
764 Sianta SA, Kay KM. 2021. Parallel evolution of phenological isolation across the speciation
765 continuum in serpentine-adapted annual wildflowers. Proceedings of the Royal Society B
766 288: 20203076.
767
768 Silvertown J, Servaes C, Biss P, Macleod D. 2005. Reinforcement of reproductive isolation
769 between adjacent populations in the Park Grass Experiment. Heredity 95: 198-205.
770

771 Skotte L, Korneliussen TS, Albrechtsen A. 2013. Estimating individual admixture
772 proportions from next generation sequencing data. Genetics 195: 693-702.
773
774 Sobel JM. 2014. Ecogeographic isolation and speciation in the genus *Mimulus*. American
775 Naturalist 184: 565-579.
776
777 Sobel JM, Chen GF. 2014. Unification of methods for estimating the strength of reproductive
778 isolation. Evolution 68: 1511-1522.
779
780 Stankowski S, Ravinet M. 2021. Defining the speciation continuum. Evolution 75: 1256-
781 1273.
782
783 Sweigart AL, Willis JH. 2003. Patterns of nucleotide diversity in two species of *Mimulus* are
784 affected by mating system and asymmetric introgression. Evolution 57: 2490-2506.
785
786 Sweigart AL, Martin NH, Willis JH. 2008. Patterns of nucleotide variation and reproductive
787 isolation between a *Mimulus* allotetraploid and its progenitor species. Molecular Ecology 17:
788 2089-2100.
789
790 Twyford AD, Friedman J. 2015. Adaptive divergence in the monkey flower *Mimulus guttatus*
791 is maintained by a chromosomal inversion. Evolution 69: 1476-1486.
792
793 Twyford AD, Streisfeld MA, Lowry DB, Friedman J. 2015. Genomic studies on the nature of
794 species: adaptation and speciation in *Mimulus*. Molecular Ecology 24: 2601-2609.
795
796 Vargas OM, Goldston B, Grossenbacher DL, Kay KM. 2020. Patterns of speciation are
797 similar across mountainous and lowland regions for a neotropical plant radiation (Costaceae:
798 *Costus*). Evolution 74: 2644-2661.
799
800 Via S. 2009. Natural selection in action during speciation. Proceedings of the National
801 Academy of Sciences USA. 106: 9939-9946.
802
803 Vickery Jr. RK. 1964. Barriers to gene exchange between members of the *Mimulus guttatus*
804 complex (Scrophulariaceae). Evolution 18: 52-69.
805
806 Vickery Jr. RK. 1978. Case studies in the evolution of species complexes in *Mimulus*
807 Evolutionary Biology 11: 405-507.
808
809 Widmer A, Lexer C, Cozzolino S. 2009. Evolution of reproductive isolation in plants.
810 Heredity 102: 31-38.
811
Wu CA, Lowry DB, Cooley AM, Wright KM, Lee YW, Willis JH. 2008. *Mimulus* is an
emerging model system for the integration of ecological and genomic studies. Heredity 100:
220-230.

Xue D, Zhang X, Lu X, Chen G, Chen ZH. 2017. Molecular and evolutionary mechanisms of

cuticular wax for plant drought tolerance. Frontiers in Plant Science 8: 621.

Zinkl GM, Zwiebel BI, Grier DG, Preuss D. 1999. Polen-stigma adhesion in Arabidopsis: a

species-specific interaction mediated by lipophilic molecules in the pollen exine.

Development 126: 5431-5440.

Figure captions:

Figure 1: Geographic distributions of *Mimulus glaucescens* and *M. guttatus*. (A) State of

California with focal counties in gray; inset: predicted local ranges of *M. guttatus* (blue) and

M. glaucescens (yellow) based on maxent modeling. Points represent collection records upon

which the models are based. (B) *M. glaucescens* (left) and *M. guttatus* (right), showing

distinct vegetative morphology, (C) PCA based on 19 Bioclim variables, elevation, and two

soil substrate variables used to build the maxent models.

Fig. 2: Reproductive isolation for *M. glaucescens* (A) and *M. guttatus* (B). Distributions

based on 10,000 bootstrapped values (ecogeographic is based on 1000). Shadings represent

types of barriers pale gray = premating barriers, dark gray = postmating, prezygotic barriers,

black = postzygotic barriers. Asterisks indicate estimates with 95% CI that do not overlap 0

(Table S5).

Figure 3. (A) Maximum-likelihood phylogeny of sampled *Mimulus* taxa based on 75 whole-

genome sequences, with *M. dentilobus* (not shown) as an outgroup. Number of accessions is

shown at tips next to each taxon name. (B) First two principal components of genomic

variation in *Mimulus guttatus* (Southern and Northern clades represented in black and pale

gray, respectively) and *M. glaucescens* (dark gray). (C) Divergence (D_{XY}) in genomic

variation in pairs of accessions of *Mimulus*, arranged in increasing order of D_{XY}. Mean D_{XY}

between selected taxa indicated by arrows (see also Table S4). NMG = Northern *M. guttatus*;

SMG = Southern *M. guttatus*; MT = *M. tillingii*; Mgl = *M. glaucescens*. (D) Proportion of

genome associated with ancestry of *M. glaucescens* (dark gray), Northern *M. guttatus* (light

gray), and Southern *M. guttatus* (black) taxa for accessions sampled in study (see also Table

S3), based on a K-means clustering analysis (see text). Asterisks indicate *M. guttatus* samples

collected from within an area of sympatry with *M. glaucescens*.

857 Fig. 4. Violin plots of jack-knifed estimates of fraction of admixture (f_G) in *M. glaucescens*

858 and Northern *M. guttatus* accessions with allopatric or sympatric distributions. Estimates of

859 f_G were significantly higher from sympatric accessions (χ^2=91.2, *df*=1, *p*<0.0001).

Mimulus glaucescens

A

Mimulus guttatus

B