Introgression and Parental Conflict Shape Repeated Occurrences of Postzygotic Isolation

Megan E. Frayer[1§*], Hagar K. Soliman[1,2*], Pia F. Schwarz[1], and Jenn M. Coughlan[1§]

1. Department of Ecology & Evolutionary Biology, Yale University, New Haven, CT, 06511
2. Department of Biotechnology, Faculty of Science, Cairo University, Giza, Egypt, 12613

* These authors contributed equally to this manuscript

§ Co-corresponding authors: megan.frayer@yale.edu, jennifer.coughlan@yale.edu

Abstract

Postzygotic reproductive isolation is often thought to accumulate as a byproduct of neutral divergence. Yet, it frequently evolves rapidly, in line with non-neutral evolution. A major driver of intrinsic postzygotic reproductive barriers are intragenomic conflicts, such as conflict between maternal and paternal interests in resource allocation to offspring (i.e. parental conflict). Parental conflict may underlie hybrid seed inviability, a common and rapidly evolving reproductive barrier in angiosperms. Yet, in closely related, hybridizing species, it remains unclear how intragenomic conflicts and introgression interact to determine the fate of incompatibility alleles in nature. Here, we explore repeated incidences of hybrid seed inviability in a rising model: the *Mimulus guttatus* species complex. Using an extensive, range-wide crossing survey, we discover patterns of hybrid seed inviability within the widespread *M. guttatus* that are better described by geography than phylogeny. These patterns of reproductive isolation transgress species boundaries, as geographically-proximate but phylogenetically-distant species also exhibit similar patterns of hybrid seed inviability with allopatric populations of *M. guttatus*. We find strong support that patterns of reproductive isolation are consistent with parental conflict. Lastly, we provide evidence that introgression may underlie shared patterns of hybrid seed inviability between two species within this complex. Such introgression could have led to cascading reproductive isolation with other closely related species, creating a complex landscape of incompatibility. Overall, this work suggests that parental conflict and introgression can interact to shape the rapid and repeated evolution of strong reproductive isolation in the wild.

Keywords

Hybrid seed inviability, *Mimulus,* cascading reproductive isolation, repeated evolution, hybridization, endosperm, Dobzhansky-Muller Incompatibility

Introduction

Understanding the origin and maintenance of species is a fundamental question in biology. Central to both of these processes is the emergence of reproductive isolation—the mechanisms that reduce interspecific fertilization and/or hybrid fitness. The evolution of intrinsic postzygotic isolation is often thought of as a slow accumulation of neutral or nearly neutral differences between two lineages (Coyne and Orr 1989, 1997, 2004). However, intrinsic postzygotic incompatibilities can also evolve rapidly and dynamically at various stages of divergence, suggesting a role of non-neutral processes in their evolution (Barbash et al. 2003; Johnson 2010; Corbett-Detig et al. 2013; Crespi and Nosil 2013; Coughlan and Matute 2020). Such rapid evolution of hybrid inviability and sterility has been observed in diverse species (Barbash et al. 2003; Charistianson et al. 2005; Briscoe Runquist et al. 2014; Schumer et al. 2014; İltaş et al. 2021; Gustafsson et al. 2022; Fusca et al. 2025). Thus, although not every species will rapidly accumulate intrinsic postzygotic isolation, this phenomenon can be found universally, providing important insights into how closely related taxa diverge in fundamental developmental processes, such as gametogenesis or offspring development (Charistianson et al. 2005; Wiley et al. 2009; Bracewell et al. 2011; Moyle et al. 2012; Brekke and Good 2014; Briscoe Runquist et al. 2014; Barnard-Kubow et al. 2016; Gustafsson et al. 2022).

One source of rapid evolution of incompatibilities are intragenomic conflicts, such as parental conflict in angiosperms and placental mammals (Haig and Westoby 1989, 1991; Moore and Haig 1991; Zeh and Zeh 2000; Burt and Trivers 2009; Coughlan 2023b; Soliman and Coughlan 2024; Frayer et al. 2025). Parental conflict occurs when unequal relatedness among offspring in a brood leads to opposing parental optima for resource allocation (Trivers 1974; Haig and Westoby 1989, 1991; Haig 1997). Under this theory, selection should favor paternal alleles that draw more resources from the maternal parent to their offspring, while maternal alleles arise to combat that excess via resource repression (Trivers 1974; Haig 1997). This parental "tug of war" can lead to an evolutionary arms race wherein sets of maternal and paternal alleles become coadapted to each other within a given population or species (Brandvain and Haig 2018). Parental conflict is thought to manifest most significantly in taxa that have evolved specialized tissues that facilitate nutrient exchange from maternal parents to the

62 offspring, namely the endosperm in angiosperms and the placenta in eutherians (Soliman and Coughlan
63 2024). Mechanistically, such tug-of-war battles over resources are proposed to occur through genomic
64 imprinting, a specialized form of gene regulation that has independently evolved in angiosperms and
65 eutherians, wherein genes exhibit parent-of-origin biased expression (Haig and Westoby 1991).

66 Parental conflict theory posits that in crosses within a lineage, maternally- and paternally-acting
67 alleles should be evenly matched, resulting in typical offspring development. However, crosses among
68 lineages with different histories of conflict can reveal selfish alleles with such parent-of-origin effects,
69 leading to dysfunctional offspring development and potentially death (Zeh and Zeh 2000; Gutierrez-
70 Marcos et al. 2003; Coughlan 2023b,c). Several testable predictions arise from parental conflict theory:
71 First, incompatibility should involve tissues that facilitate nutrient exchange among maternal parents
72 and offspring (Soliman and Coughlan 2024). Second, reciprocal F1 offspring should exhibit parent-of-
73 origin specific growth abnormalities. Namely, F1s sired by a lineage that has experienced a longer or
74 more intense history of conflict should exhibit an overgrowth phenotype known as "paternal excess",
75 while the reciprocal cross should exhibit an undergrowth phenotype (i.e. "maternal excess"; Haig and
76 Westoby 1991; Brandvain and Haig 2018). We refer to these as high- and low-conflict lineages, and
77 note that these definitions align with high- and low-endosperm balance number, which is a similar
78 conceptual framework more commonly used in the crop breeding literature (Johnston et al. 1980;
79 Johnston and Hanneman 1982). Third, parental conflict theory suggests that any cross that restores the
80 balance of maternally- and paternally-acting alleles should be compatible. As such, crosses between
81 two lineages with similar histories of conflict should be compatible, even if their histories of conflict
82 are evolutionarily independent (Coughlan et al. 2020). This creates a final testable prediction that the
83 outcome of crosses should be predictable based on inferred histories of conflict.

84 In angiosperms, overgrowth phenotypes often involve delayed cell division or cellularization
85 in the endosperm, both of which tend to be lethal (Rebernig et al. 2015; Oneal et al. 2016; Lafon-Placette
86 et al. 2017, 2018; Roth et al. 2018; Coughlan et al. 2020; Sandstedt and Sweigart 2022). Conversely,
87 undergrowth phenotypes often involve smaller seeds caused by accelerated endosperm development
88 (Rebernig et al. 2015; Oneal et al. 2016; Lafon-Placette et al. 2018; Coughlan et al. 2020; İltaş et al.
89 2021; Sandstedt and Sweigart 2022). Hybrid seed inviability can evolve rapidly (Coughlan 2023b)

including in taxa as recently diverged as 10,000 years ago (İltaş et al. 2021). Importantly, hybrid seed inviability is common—it has been reported in diverse angiosperm species (Grant 1966; Briscoe Runquist et al. 2014; Rebernig et al. 2015; Wolff et al. 2015; Florez-Rueda et al. 2016; Oneal et al. 2016; Lafon-Placette et al. 2017, 2018; Roth et al. 2019; Coughlan et al. 2020; Sandstedt et al. 2020; Ostevik et al. 2021; Sandstedt and Sweigart 2022; Butel et al. 2024). The observation that hybrid seed inviability has evolved rapidly and independently across angiosperms, with consistently similar patterns of dysfunctional seed development, indicates that it is highly repeatable, at least at the phenotypic level. However, it remains unclear how repeatable this barrier is at a genetic level or, if incompatibility alleles are shared, what the source of this shared genetic variation is. These questions have fundamental implications for evolution, as they can reveal how much of the genome is susceptible to genetic conflict and how quickly these alleles can arise and spread.

Introgression is a common way for alleles to spread among populations (Taylor and Larson 2019; Dagilis et al. 2022). While typically thought to hinder speciation, introgression can also contribute to reproductive isolation by introducing novel ecologically-mediated reproductive barriers (Long and Rieseberg 2024; Rosser et al. 2024). Introgression from one species into another can also enable reproductive isolation with a third species (Moran et al. 2024; Aguillon et al. 2025). In the case of incompatibility alleles evolving under intragenomic conflicts, theory predicts that once a selfish allele invades a new population, it should spread (Meiklejohn et al. 2018; Wang et al. 2024). Evidence of introgressed drivers has been found in several species (Meiklejohn et al. 2018; Svedberg et al. 2021; Wang et al. 2024), including those involved in incompatibility (Widen et al. 2023). However, it is unclear under what conditions an intrinsic incompatibility that has evolved via intragenomic conflicts could persist as a reproductive barrier. Furthermore, if and how theory developed for elements such as meiotic drivers translates to an allele evolving via parental conflict remains unknown.

Here, we leverage a rising model system for ecology, evolution, and genetics—the *Mimulus guttatus* species complex—to understand the repeated occurrence of hybrid seed inviability. We identify several instances of hybrid seed inviability across geographically and/or phylogenetically distinct taxa. We test whether these patterns of crossability conform to the prediction of parental conflict. We then assess whether the genetic basis of hybrid seed inviability between two incidences is

shared, and whether they have a history of introgression. Our results support the idea that introgression of selfish elements can lead to the repeated evolution of reproductive isolation between lineages.

Results

Repeated occurrence of hybrid seed inviability in the Mimulus guttatus *species complex*

The *Mimulus guttatus* species complex is a relatively young clade of monkeyflowers endemic to the western half of North America (Vickery 1974, 1978; Fig.1A,B; crown age estimate: 674kya, Sandstedt et al. 2020). Using a range-wide crossing survey of 23 populations of *M. guttatus* crossed in a nearly full diallel design (426/506 unique directions, 1,742 crosses), we identified strong and asymmetric patterns of hybrid seed inviability between geographically distinct populations of *M. guttatus* (Fig.1B,C; Fig.S1; Table S1). Crosses between populations from the Sierra mountains/foothills and coastal or more northern parts of the range were largely inviable, manifesting as flat seeds that did not germinate when the Sierran populations served as the sire. In contrast, the reciprocal crosses produced smaller, viable seeds with high germination rates (Fig.1C; Fig.S2; Sierran x Northern *M. guttatus*: $\chi^2 = 1427$, DF = 3, $p < 0.0001$; Sierran x Coastal *M. guttatus*: $\chi^2 = 2724$, DF = 3, $p < 0.0001$). There was little to no hybrid seed inviability observed in crosses between populations of *M. guttatus* from the north and coast (herein "Northern" and "Coastal") or within the Sierran lines— these crosses largely formed plump, viable seeds with high germination rates (Fig.S2; Northern x Coastal *M. guttatus*: $\chi^2 = 6.88$, DF = 3, $p = 0.07$). Despite broad consistency, we found some population-specificity in the magnitude of hybrid seed inviability. The most variable patterns of hybrid seed inviability arose from crosses between Sierran *M. guttatus* and Northern *M. guttatus*, and were largely caused by two Northern *M. guttatus* accessions that exhibited more viable seeds with Sierran lines and fewer with other Northern lines (i.e. CRES and TOK; Fig.1C). Furthermore, the Sierran *M. guttatus* themselves do not form a monophyletic group (Fig.1A). This finding highlights a novel incidence of hybrid seed inviability segregating within geographically distinct populations of *M. guttatus*, albeit with some population-specificity in the magnitude of incompatibility.

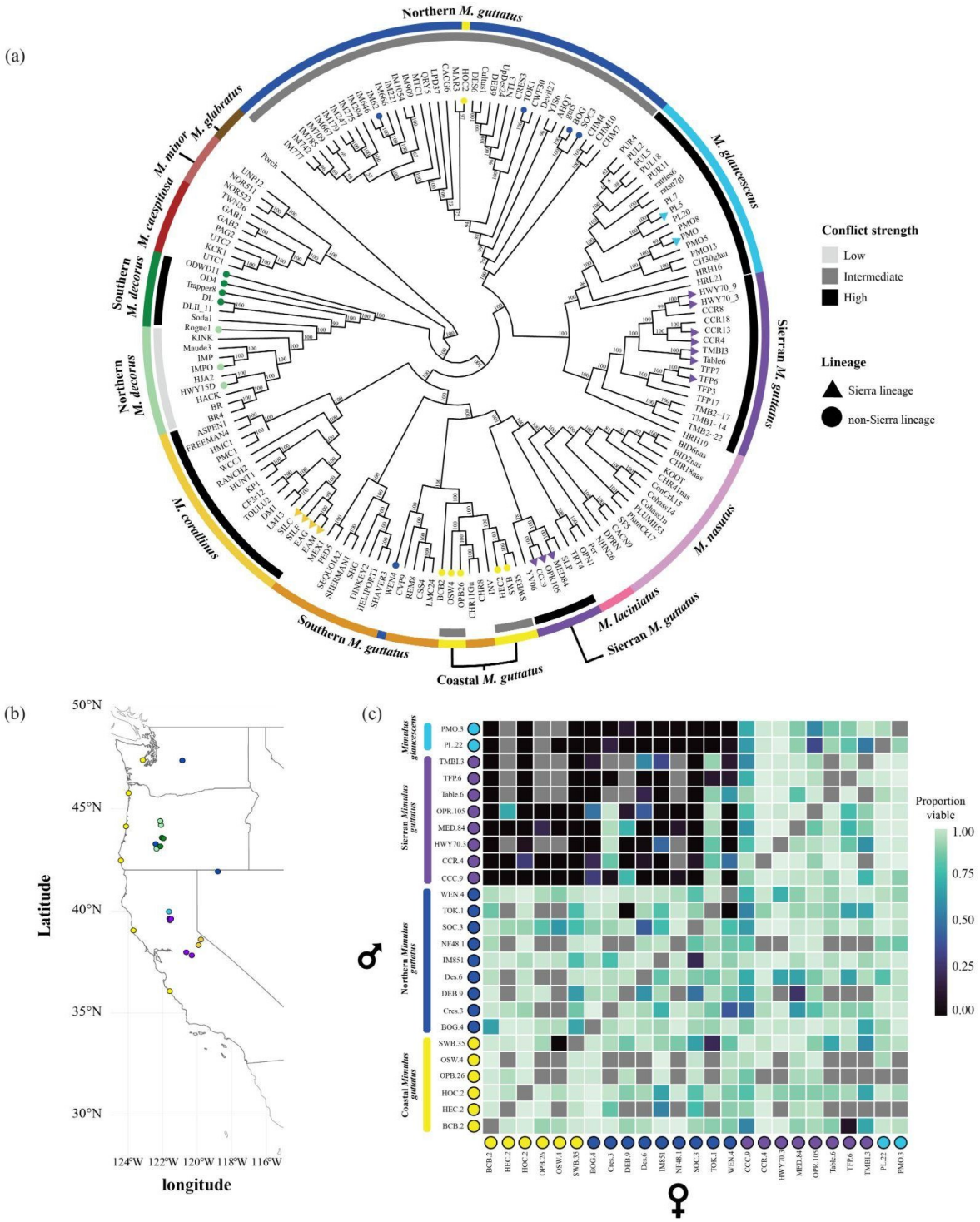

Figure 1. Hybrid seed inviability segregates within the widespread *M. guttatus* species complex. A) A maximum likelihood phylogeny of the *M. guttatus* species complex with lines used in the crossing survey denoted by colored shapes at the tips. Inferences of the history of conflict that a subset of these taxa have experienced are denoted by the inner greyscale ring. These inferences are based on the current work, as well as that of others (Coughlan et al. 2020). The outer colored ring denotes named species or

lineages of *M. guttatus* and *M. decorus* that are inferred to have different histories of conflict. **B)** The geographic distribution of the lines used in the crossing survey. Samples are colored according to species/lineage. **C)** Proportion of viable seeds for pairwise crosses survey between each of the 25 populations. Grey boxes denote incomplete crosses.

Given the geographic pattern of incompatibility, we next tested whether two additional Sierran endemic species (*M. glaucescens* and *M. corallinus*) also exhibited this incompatibility. We crossed 2 populations of *M. glaucescens* to 21 populations of *M. guttatus* from our original crossing survey. Both Sierran *M. guttatus* and *M. glaucescens* behaved similarly in crosses with either Northern or Coastal *M. guttatus*, although seed inviability tended to be more severe in crosses sired by *M. glaucescens* (Fig.1). Although crosses between Sierran *M. guttatus* and *M. glaucescens* produced overall plump, viable seeds, we did observe a small but significant decline in viable seed production when *M. glaucescens* sired the cross (~ 6-7%; $\chi^2 = 36.4$, DF = 3, $p < 0.001$; Fig.S2), consistent with previous work on sympatric populations of *M. glaucescens* and Sierran *M. guttatus* (Ivey et al. 2023). We then crossed another, more distantly related Sierran endemic, *M. corallinus* to a single population of Northern *M. guttatus.* We again found strong and asymmetric hybrid seed inviability, wherein seeds were inviable when *M. corallinus* sired these crosses but were viable when *M. corallinus* served as the dam (Fig.2B; $\chi^2 = 68$, DF = 3, $p < 0.0001$).

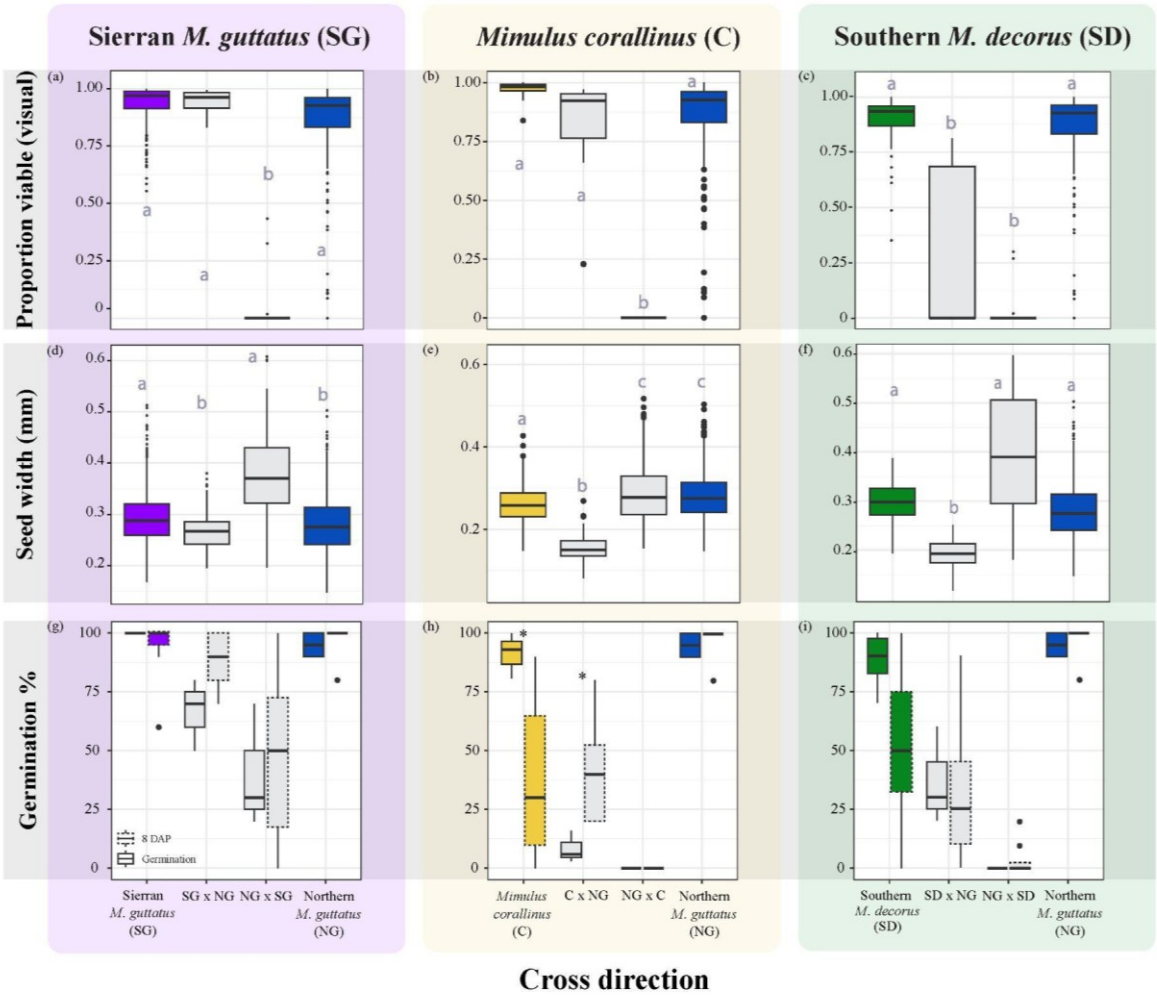

Figure 2. Evidence for parental conflict. Crosses between Sierran *M. guttatus* (SG), *M. corallinus* (C), and Southern *M. decorus* (SD) to Northern *M. guttatus* (SG; columns). **A-C)** The proportion of viable seeds for reciprocal crosses. **D-F)** The seed width for all reciprocal crosses. **G-I)** The result of germination assays (solid outline) and embryo rescued 8 days after pollination (8 DAP; dotted outline) for reciprocal crosses. Groups with significant differences are denoted by letters in plots A-F and by asterisks in plots G-I.

These patterns of hybrid seed inviability mirror those previously described in the *M. guttatus* species complex involving a phylogenetically distinct and allopatric species: Southern *M. decorus* (Fig.1A; Coughlan et al. 2020). Crosses between Southern *M. decorus* and a subset of 9 Northern and Coastal *M. guttatus* lines used in the crossing survey above reveal a broadly consistent pattern, although the pattern here is more symmetric than we have previously reported (Fig.2A-C; Coughlan et al. 2020). In all cases, hybrid seeds are more likely to be inviable when *M. corallinus*, *M. glaucescens,* Sierran *M. guttatus*, or Southern *M. decorus* serve as the sire in crosses with Coastal or Northern *M. guttatus*.

190 Although lines of Coastal and Northern *M. guttatus* did vary from one another in the severity of
191 incompatibility (Fig.1C, Fig.S2), we note that patterns of crossability between any given accession of
192 *M. guttatus* with Sierran *M. guttatus* or Southern *M. decorus* were highly correlated (i.e. lines that
193 exhibit more symmetric patterns of incompatibility with Sierran *M. guttatus* also show more symmetric
194 patterns of incompatibility with Southern *M. decorus*; $r^2 = 0.79$, DF = 13, $p = 0.0005$; Fig.S3). We next
195 sought to test whether patterns of repeated hybrid seed inviability supported a role of parental conflict
196 in its evolution.
197
198 *Parent-of-origin effects on growth partially support a role of parental conflict*
199 Parental conflict predicts rapid and repeated evolution of hybrid seed inviability through
200 continuous antagonistic selection between maternal- and paternal-interests in resource allocation to
201 offspring (Haig and Westoby 1991). Under parental conflict, hybridization should reveal paternally-
202 acting, resource-acquiring alleles and maternally-acting, resource-repressive alleles. We therefore
203 predict that reciprocal crosses should exhibit parent-of-origin-specific growth phenotypes (Haig and
204 Westoby 1989, 1991; Haig 2000). In line with this prediction, we identified distinct parent-of-origin
205 growth effects in reciprocal F1 seeds in all crosses, wherein seeds sired by *M. corallinus, M.*
206 *glaucescens,* Sierran *M. guttatus,* or Southern *M. decorus* were larger than the reciprocal cross (Fig.
207 2D-F; Fig. S4). Given these signatures of paternal excess when *M. corallinus, M. glaucescens,* Sierran
208 *M. guttatus*, or Southern *M. decorus* are the sire, we infer that they have experienced a higher history
209 of conflict than Coastal or Northern *M. guttatus*, in accordance with previous work (Coughlan et al.
210 2020).
211 Under parental conflict, disrupted seed development should manifest in the endosperm (Haig
212 and Westoby 1989). We, therefore, predicted that F1 viability may be rescued if embryos are grown on
213 a nutrient-rich media. We performed embryo rescues for a subset of crosses between Northern *M.*
214 *guttatus* and each of Sierran *M. guttatus, M. corallinus*, and Southern *M. decorus*. While we
215 qualitatively found that germination increased in rescued seeds in 4 out of 6 crosses (Fig.2G-I), only
216 the *M. corallinus* x Northern *M. guttatus* cross was statistically significant (Fig.2H). Despite a lack of

217 statistical significance, we were able to rescue crosses that otherwise never germinate. Moreover,
218 several within-species embryo rescues showed reductions in germination (Fig.2G-I), suggesting that
219 the rescue process itself can be damaging. Therefore, while embryo rescues do not perfectly restore
220 germination, the endosperm likely does play an important role in hybrid seed inviability.

221 Lastly, parental conflict theory dictates that the outcome of crosses should be predictable based
222 on the histories of conflict, even if phylogenetically distant. In line with this hypothesis, crosses among
223 all high-conflict lineages (i.e. Southern *M. decorus, M. corallinus, M. glaucescens,* and Sierran *M.*
224 *guttatus*) yielded plump, viable seeds with no parent-of-origin size differences (Fig.3A,B; Fig.S5).
225 Conversely, crosses between high-conflict lineages (i.e. *M. corallinus* and Sierran *M. guttatus*) and a
226 species that has previously been shown to exhibit the lowest history of conflict of all taxa described
227 herein (i.e. Northern *M. decorus;* Coughlan et al. 2020), exhibit reciprocal and nearly complete hybrid
228 seed inviability (Fig.3C,D). Overall, this suggests that high-conflict lineages behave in predictable
229 ways: inviability is most severe between lineages with the most disparate histories of conflict, and
230 viability is recovered between lineages with more similar histories of conflict, despite varying
231 divergence times, in line with parental conflict. However, this prediction may also be true if
232 incompatibility loci have introgressed, a possibility we explore below.

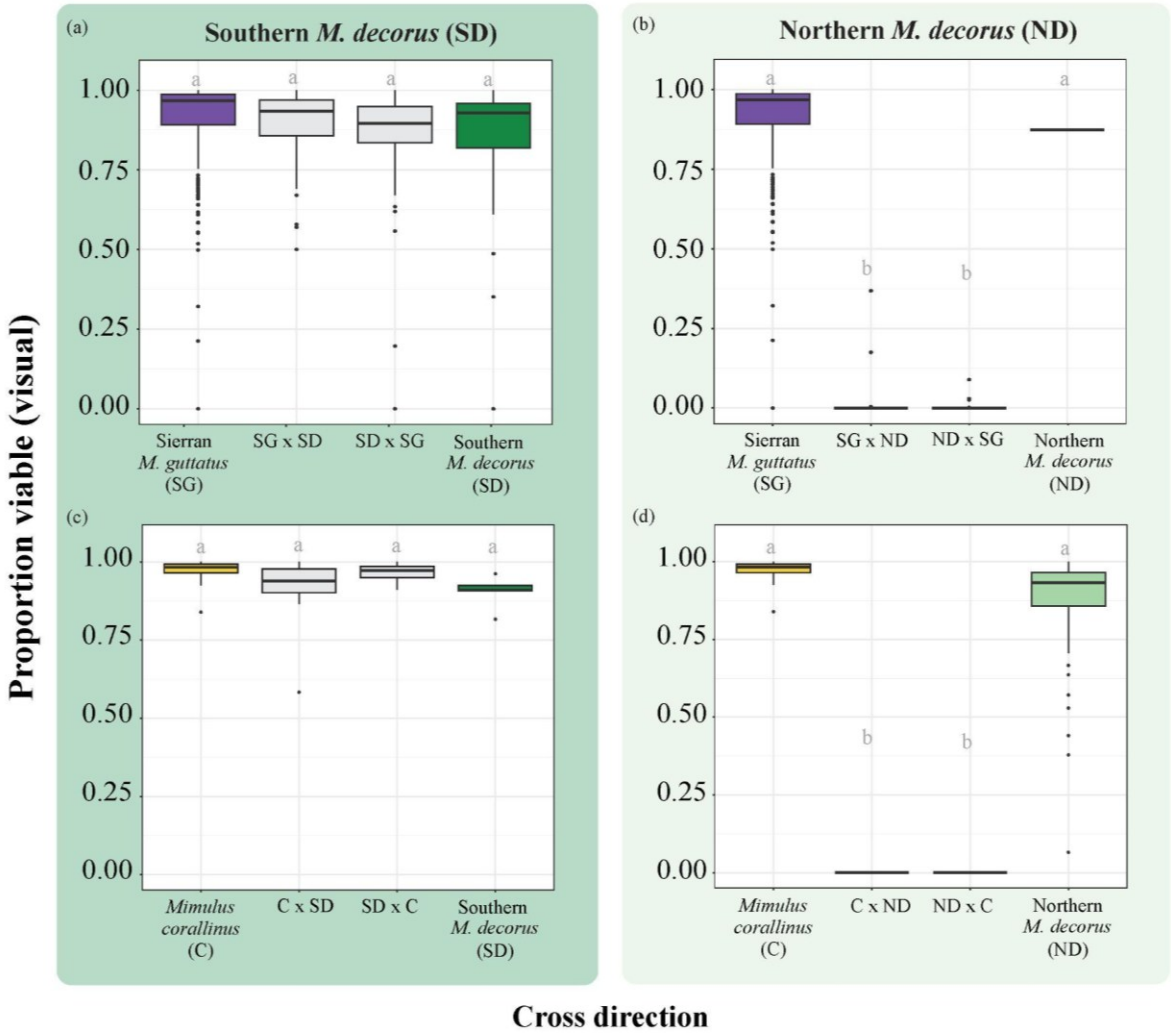

Figure 3. Cross compatibility is predictable based on inferred histories of conflict. High-conflict lineage Southern *M. decorus* is reciprocally crossed to **A)** Sierran *M. guttatus* and **C)** *M. corallinus*, and no hybrid seed inviability is observed. The low-conflict lineage, Northern *M. decorus*, is reciprocally crossed to **B)** Sierran *M. guttatus* and **D)** *M. corallinus*, and nearly complete hybrid seed inviability is observed. Notably, it has been previously shown that the closely related Southern and Northern *M. decorus* have complete hybrid seed inviability when crossed to each other (Coughlan et al. 2020).

Repeated incidences of hybrid seed inviability have a largely shared genetic basis

We next sought to understand if the shared patterns of cross incompatibility were caused by a shared genetic basis. To test this hypothesis, we used a complementation test, where two lines exhibiting a similar phenotype are hybridized, and the presence or absence of phenotypic segregation in F2 hybrids can reveal whether the genetic basis of the focal trait is shared. Using this logic, we created an F2

population between two lineages that exhibit identical patterns of hybrid seed inviability with Northern *M. guttatus*—*M. corallinus* and Southern *M. decorus* (Fig. 2). We then crossed a single inbred line of Northern *M. guttatus* (IM62) to *M. corallinus*, Southern *M. decorus*, their reciprocal F1s, and reciprocal F2s, with Northern *M. guttatus* acting as the dam in all crosses. If the genetic basis of hybrid seed inviability was largely independent, we would expect that F2s should segregate for the ability to cross with Northern *M. guttatus*, and thus some fraction of F2s would be at least partially compatible with Northern *M. guttatus*. However, we find that out of 761 crosses (>41,000 seeds, 170 unique F2 individuals), we did not recover a single viable hybrid seed, strongly suggesting that the genetic basis of hybrid seed inviability between Northern *M. guttatus* and each of *M. corallinus* and southern *M. decorus* is largely shared (Fig.4A; Fig.S8).

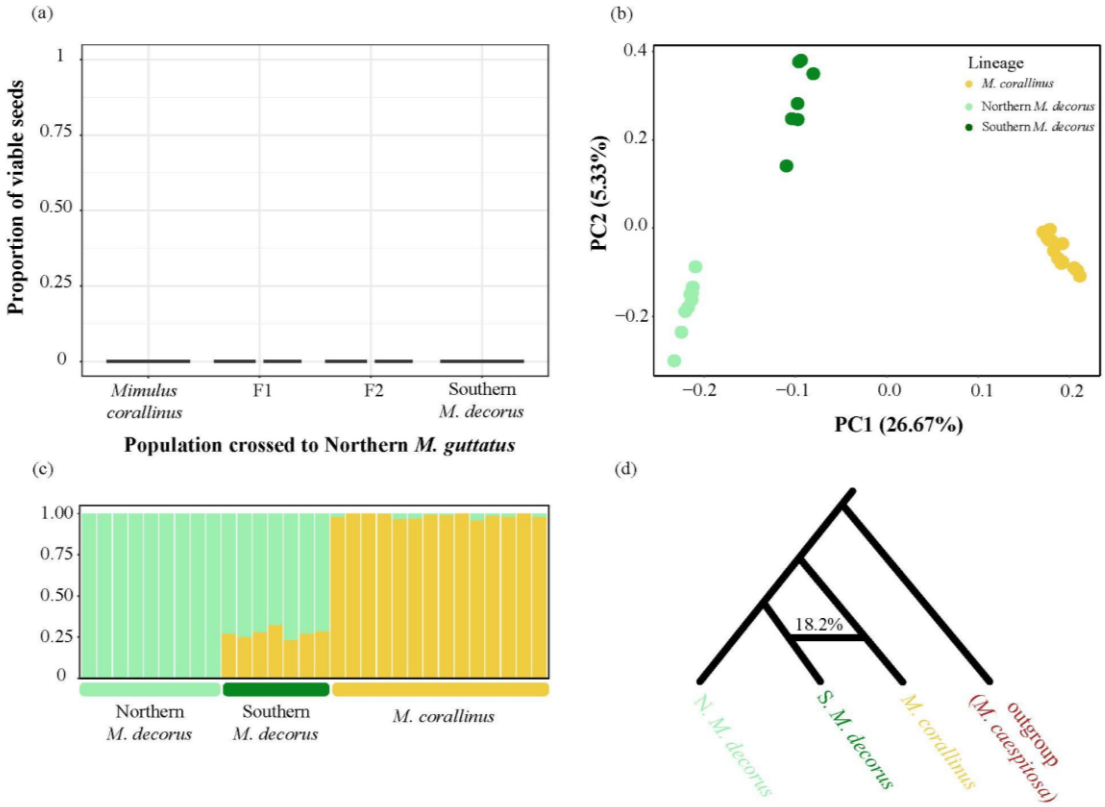

Figure 4. The shared genetic basis of hybrid seed inviability between *M. corallinus* and Southern *M. decorus* may be due to a history of introgression. A) A complementation test supports the shared genetic basis of hybrid seed inviability between Northern *M. guttatus* and each of *M. corallinus* and Southern *M. decorus*. **B)** PCA of Northern *M. decorus,* Southern *M. decorus*, and *M. corallinus*. **C)** PCAdmix results for these three lineages. K=2 was the best supported ancestry grouping. **D)** HyDe estimates that 18.2% of the genome of Southern *M. decorus* comes from *M. corallinus*.

268 *High-conflict lineages exhibit a history of introgression*

269 The shared genetic basis of a trait can be caused by independent mutations in the same or tightly
270 linked gene(s), shared ancestral polymorphisms, or introgression. Given the extensive history of gene
271 flow in this species complex (Brandvain et al. 2014; Twyford and Friedman 2015; Ivey et al. 2023;
272 Mantel and Sweigart 2024; Farnitano et al. 2025), we next tested whether phylogenetically-distinct,
273 high-conflict species may also have a history of introgression. Using four-population tests to examine
274 all combinations of lineages within the *M. guttatus* species complex (with *M. caespitosa* as an
275 outgroup), we find extensive evidence of introgression across the complex, as the minimum value of D
276 is significant in 83% of trios (mean D statistic = 0.059). Moreover, some of these positive tests could
277 be the result of inferred ancient introgression among ancestors of extant lineages (Fig.S6). Given this
278 extensive introgression, disentangling its role in shaping the distribution of hybrid seed inviability is
279 not straightforward, particularly in currently sympatric taxa.

280 However, there is a particularly strong signal of introgression between two high-conflict
281 lineages— *M. corallinus* and Southern *M. decorus*. These lineages exhibit replicated patterns of hybrid
282 seed inviability with other taxa in the complex (i.e. with both Northern *M. guttatus*, Northern *M.*
283 *decorus*; Fig.2,3; Coughlan et al. 2020), our complementation test above supports a shared genetic basis
284 between these two lineages for inviability with Northern *M. guttatus*, and they are currently allopatric,
285 potentially allowing for more straightforward tests of introgression. We therefore next focus on
286 investigating the history of ancient introgression between Southern *M. decorus* and *M. corallinus*.

287 We performed several tests using only the Northern *M. decorus*, Southern *M. decorus,* and *M.*
288 *corallinus* with *M. caespitosa* as an outgroup. While we sequenced 17 Southern *M. decorus* individuals,
289 we found a high degree of relatedness within populations. We therefore limited most genomic analyses
290 to 6 unrelated individuals. In a PCA, Southern *M. decorus* is intermediate to Northern *M. decorus* and
291 *M. corallinus* along PC1, which explains 26.67% of the genomic variance (Fig.4B). Multiple lines of
292 evidence support a high fraction of *M. corallinus* ancestry segregating within Southern *M. decorus* at a
293 genome-wide level. PCAdmix indicates that there are two ancestries among these three taxa, with
294 Southern *M. decorus* being ~29% *M. corallinus* and 71% Northern *M. decorus* (Fig.4C). Additionally,

295 we estimated a global admixture proportion of 18.2% *M. corallinus* with HyDe (Fig.4D; Blischak et al.
296 2018). Using Dsuite, D and f4 also gave broadly consistent estimates of admixture (0.204 and 0.255,
297 respectively; Malinsky et al. 2021). In total, these patterns support a consistent and significant
298 contribution of *M. corallinus* ancestry into Southern *M. decorus*.

299 We next sought to characterize local patterns of introgression in Southern *M. decorus*. We
300 identified windows exhibiting high f_{dM}, indicating putative introgression between Southern *M. decorus*
301 and *M. corallinus*. Furthermore, given that the Northern and Southern lineages of *M. decorus* are quite
302 closely related (Coughlan et al. 2020), we should expect relatively low values of F_{ST} between them,
303 except at regions of the genome that have introgressed between *M. corallinus* and Southern *M. decorus.*
304 Such regions should exhibit high F_{ST} between both Northern *M. decorus* and each of *M. corallinus* and
305 Southern *M. decorus*, but low F_{ST} between *M. corallinus* and Southern *M. decorus.* Using these criteria,
306 we find signals of introgression across the genome, typically in small blocks (Fig.5A). These results
307 were consistent with topology weighting (Martin and Van Belleghem 2017), wherein we found support
308 for a phylogeny that grouped *M. corallinus* and Southern *M. decorus* at low levels across the genome,
309 but little support for the phylogeny that grouped *M. corallinus* and Northern *M. decorus*, in line with a
310 scenario of introgression between *M. corallinus* and Southern *M. decorus* rather than incomplete lineage
311 sorting (Fig.S7A). Finally, we used ancestryHMM to infer local ancestry within Southern *M. decorus*
312 (Corbett-Detig and Nielsen 2017); Fig.S7B,C, Fig.S9). Local ancestry across all Southern *M. decorus*
313 is correlated with f_{dM} ($r^2 = 0.511$; p-value < 2.2e-16; Fig.S7D), but reveals high levels of individual
314 ancestry heterozygosity, suggesting that *M. corallinus* alleles are segregating in Southern *M. decorus*
315 populations. Lastly, we estimated the timing of admixture at 4,703 generations (Fig.S10), suggesting
316 that introgression is not ongoing.

317 Although we find many small blocks of introgression in the genomes of Southern *M. decorus*,
318 one striking signal occurs at the distal end of chromosome 13 (Fig.5B). This region exhibits a high f_{dM},
319 as well as a high difference in F_{ST} when comparing Southern and Northern *M. decorus* to *M. corallinus*.
320 This region harbors a chromosomal inversion known to be distinct between Northern *M. decorus* and
321 *M. guttatus* (Lovell et al. 2025; Joint Genome Institute, pers. comm.). Intriguingly, this inversion has
322 previously been implicated in hybrid seed lethality between *M. guttatus* and *M. tilingii* (Garner et al.

323 2016), and preliminary mapping data supports its role in hybrid seed inviability between Northern *M.*
324 *decorus* and *M. guttatus* (Coughlan unpublished data). Excitingly, this inversion contains multiple
325 genes that are potential targets of parental conflict that are also introgression outliers (i.e. are in the top
326 1% for f_{dM} and F_{ST} difference (Table S2)). Two outlier windows contain genes known to be expressed
327 in the *M. guttatus* endosperm (Tucci et al. 2024), although it is currently unknown whether either of
328 these genes are imprinted in *Mimulus*. One top window contains a homolog of *Napin*, a storage protein
329 found in maturing seeds (Crouch et al. 1983), while the other contains the *Mimulus* homolog of *DOG1*,
330 which plays an important role in seed dormancy (Bentsink et al. 2006). There are also two genes that
331 are known to be imprinted in *Arabidopsis*. The first gene is an *MSI* homolog, located close to *Napin* in
332 one of the top windows. Just downstream of the second top window is *FIE*, a gene that is both a master
333 regulator of imprinting in *Arabidopsis* endosperm and is itself imprinted (Luo et al. 1999, 2000; Köhler
334 et al. 2003, 2021; Batista and Köhler 2020).

335 We hypothesized that this inversion has introgressed from *M. corallinus* into Southern *M.*
336 *decorus*, and may play an important role in the nearly complete hybrid seed inviability between the two
337 sister lineages of *M. decorus*. However, we note that the role of this inversion is not straightforward and
338 cannot explain all patterns of hybrid seed inviability that we describe in the species complex as *M.*
339 *corallinus* and many *M. guttatus* lineages share the same orientation, despite also exhibiting hybrid seed
340 inviability (Lovell et al. 2025). Additionally, we find that the inversion is heterozygous in 15 out of 17
341 Southern *M. decorus* samples (5 out of 6 unrelated samples). The observation that this inversion
342 contributes to hybrid seed inviability in other *Mimulus* crosses but is found largely in heterozygous state
343 may seem perplexing, as segregation of the inversion in the offspring of Southern *M. decorus* should
344 result in segregating hybrid seed inviability– a pattern that we do not see. However, the observed
345 patterns of hybrid seed inviability may be explained if the genetic basis of hybrid seed inviability
346 between the two lineages of *M. decorus* is sufficiently redundant (i.e. composed of several large affect
347 alleles with redundant effects). Although we find strong evidence of introgression of this inversion, it
348 contains potential targets of parental conflict, and is known to contribute to hybrid seed inviability in
349 other crosses, further genetic mapping will be required to explicitly link this chromosomal inversion
350 with hybrid seed inviability between the two lineages of *M. decorus*.

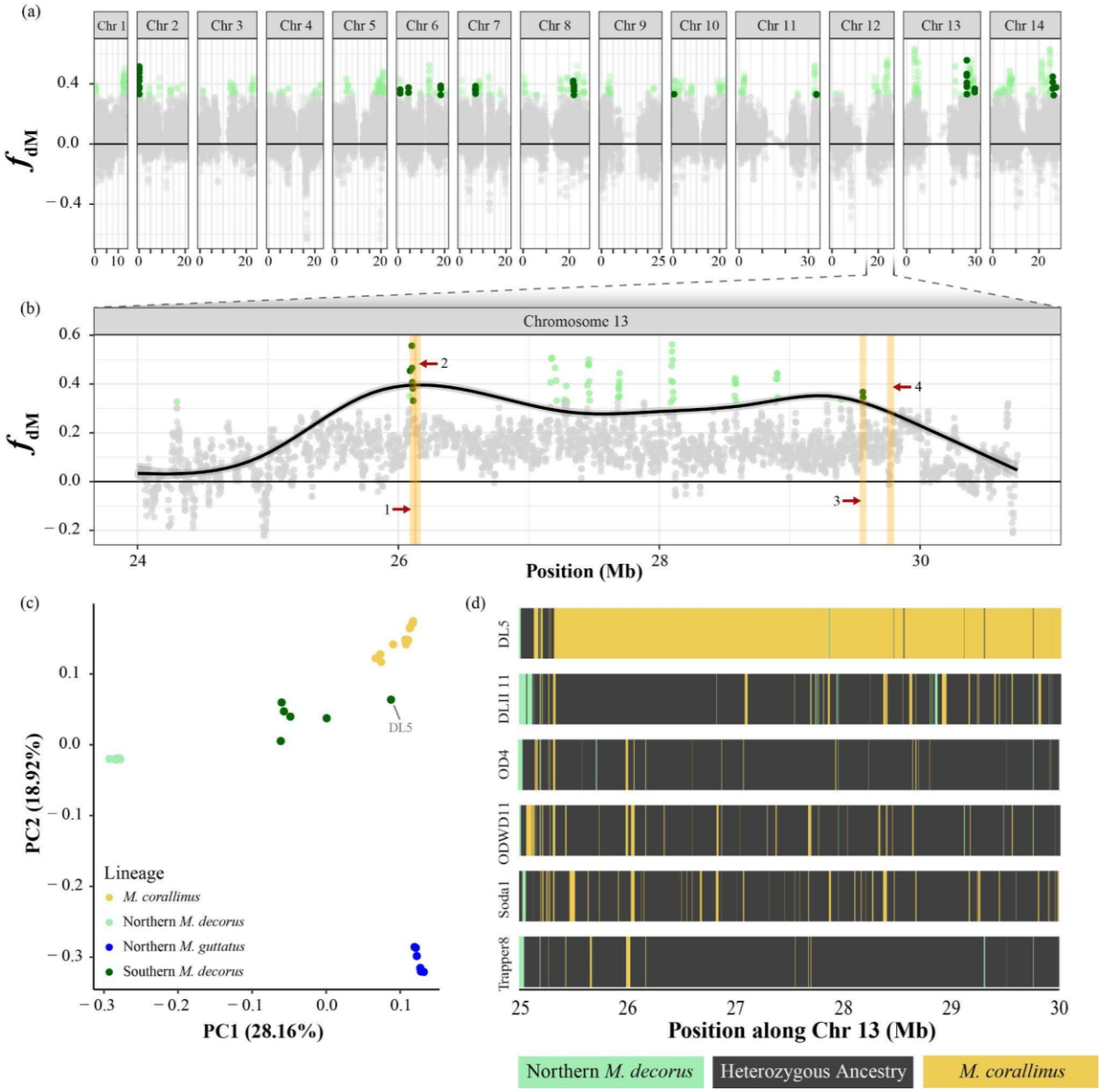

Figure 5. The Southern *M. decorus* genome is a genomic mosaic of Northern *M. decorus* and *M. corallinus*. A) Distribution of f_{dM} values measured in 250 SNP windows. Light green dots are in the top 1% of f_{dM} values. Dark green dots represent the windows that also overlap with the top 1% of windows with low F_{ST} between *M. corallinus* and Southern *M. decorus* and high F_{ST} between northern and southern *M. decorus*. **B)** The putative inversion on chromosome 13. The black line represents the smoothed average of F_{ST} between *M. corallinus* and Southern *M. decorus* minus F_{ST} between Northern and Southern *M. decorus*. Orange blocks indicate the location of genes with known roles in imprinting or endosperm expression: (1) *Napin*, (2) *MSI* homolog, (3) *DOG1*, and (4) *FIE* homolog. **C)** A PCA of SNPs within the inversion region places most Southern *M. decorus* samples intermediate to Northern *M. decorus* and *M. corallinus*, except for DL5, which falls closer to *M. corallinus*. **D)** Each unrelated Southern *M. decorus* sample is colored by the most likely ancestry at each position, determined by AncestryHMM. DL5 is largely homozygous for *M. corallinus* ancestry, while the remaining unrelated samples are largely heterozygous.

368 **Discussion**

369 We show that hybrid seed inviability is widespread and has evolved rapidly and repeatedly
370 within the *Mimulus guttatus* species complex. Hybrid seed inviability segregates among the widespread
371 *M. guttatus*, as well as several other species in the group, yet patterns of hybrid seed inviability are more
372 strongly associated with geography, rather than phylogeny. Crossing patterns within this group also
373 conform to predictions of parental conflict theory: crosses exhibit canonical parent-of-origin effects on
374 growth, incompatibility is at least partially linked with the nutritive endosperm, and patterns of crossing
375 are more predictive based on the inferred history of conflict that lineages have experienced, rather than
376 phylogenetic relatedness. Lastly, two instances of hybrid seed inviability appear to have a shared genetic
377 basis, and these two taxa also exhibit a substantial history of introgression, including at several
378 candidate genes. Although more research is needed to explicitly identify genes involved in hybrid seed
379 inviability and assess their histories of introgression, our results highlight the potential for intragenomic
380 conflicts to drive the rapid evolution of incompatibility alleles, and for introgression to underlie the
381 invasion of incompatibility alleles into new species. We posit that introgression of selfish alleles could
382 then cause cascading reproductive isolation between populations of what was once the same species.
383

384 *Parental conflict as a driver of hybrid seed inviability*

385 The data we have presented here is consistent with the parental conflict theory. This theory
386 posits an ongoing arms race between paternally-acting, resource-acquiring alleles and maternally-
387 acting, resource-repressive alleles within populations. When populations with different histories of
388 conflict hybridize, incompatibilities between these alleles are revealed. A hallmark phenotype of
389 parental conflict is parent-of-origin growth defects in F1 hybrids, which is a consistent observation in
390 all crosses. Furthermore, parental conflict theory predicts that incompatibilities should manifest in
391 endosperm. Therefore, it should be possible to "rescue" embryos by providing them with an external
392 source of nutrition. Our embryo rescue results are less conclusive than the patterns in seed size, but they
393 trend in the expected direction, and we were able to rescue hybrid embryos in crosses that typically do
394 not germinate. Moreover, the procedure itself is delicate, as exemplified in our control embryo rescues

395 (which in some cases are significantly worse than the regular germination assay; Fig. S2), suggesting
396 that our partial hybrid rescue may also be influenced by the rescue procedure itself. Lastly, we find that
397 the inferred histories of conflict are highly predictive of the outcome of future crosses. Despite being
398 more distantly related, all Sierran lineages were entirely compatible with one another, as well as with
399 high-conflict Southern *M. decorus*. By contrast, all taxa were completely incompatible with the lower-
400 conflict lineage, Northern *M. decorus*.

401 The introgressed chromosome 13 inversion is also connected to these predictions. Several genes
402 located in or near our top f_{dM} peaks within this region are expressed in the *Mimulus* endosperm (e.g.,
403 *DOG1* and *Napin;* Tucci et al. 2024) or imprinted in *Arabidopsis* and contribute to parent-of-origin
404 effects when disrupted in that species (e.g., *FIE* and *MSI*; Luo et al. 1999; Köhler et al. 2003). In addition
405 to being imprinted themselves, both *FIE* and *MSI* function as part of the polycomb repressive complex,
406 which establishes the histone modifications needed for imprinting and proper endosperm development
407 (Mozgova et al. 2015; Batista and Köhler 2020). Moreover, this region has been implicated in hybrid
408 seed inviability in at least two crosses (Coastal *M. guttatus* x *M. tilingii* and Northern *M. guttatus* x
409 Northern *M. decorus*; Garner et al. 2016; Coughlan unpublished data). Although some of our
410 observations give rise to more questions (i.e. excess levels of heterozygosity within the inversion), we
411 hypothesize that introgression of this inversion from *M. corallinus* into what is now Southern *M.*
412 *decorus* led to cascading reproductive isolation with what is now Northern *M. decorus*. Nonetheless,
413 explicit tests of its role in generating hybrid seed inviability between Southern *M. decorus* and *M.*
414 *guttatus* will require additional genetic mapping.

415 Our work adds to a growing body of evidence for parental conflict across diverse taxa (Long
416 2005; Schrader and Travis 2008; Chuong et al. 2010; Babak et al. 2015; Cailleau et al. 2018; Raunsgard
417 et al. 2018; Coughlan et al. 2020; Sandstedt and Sweigart 2022; Petrén et al. 2023; Butel et al. 2024).
418 Our results of widespread, asymmetric hybrid seed inviability are not only consistent with other studies
419 in diverse angiosperms, but also mirror the parent-of-origin phenotypes observed in mammals, where
420 the placenta performs an analogous function to the endosperm (Gutiérrez-Marcos et al. 2004; Feil and
421 Berger 2007; Vrana 2007; Briscoe Runquist et al. 2014; Rebernig et al. 2015; Oneal et al. 2016; Lafon-
422 Placette et al. 2017, 2018; Roth et al. 2018, 2019; Arévalo and Campbell 2020; Coughlan et al. 2020;

423 Brekke et al. 2021; Florez-Rueda et al. 2021; İltaş et al. 2021; Sandstedt and Sweigart 2022; Soliman
424 and Coughlan 2024). These parallels suggest that parental conflict could be a common driver of early-
425 onset hybrid embryonic death, especially in cases where isolation is rapidly evolving and/or actively
426 segregating, as is the case in the *M. guttatus* species complex.

427 *A polymorphic incompatibility sorted by geography*

428 One striking finding from our work is that hybrid seed inviability segregates within the
429 widespread *M. guttatus*. Polymorphic incompatibilities are common in nature (Corbett-Detig et al.
430 2013; Sicard et al. 2015; Larson et al. 2018; Zuellig and Sweigart 2018; Calvo-Baltanás et al. 2021),
431 and may represent incompatibilities en route to fixation (or loss; Cutter 2012). Yet, it is often unclear
432 how such alleles would avoid removal from the population, especially when rare (Bank et al. 2012;
433 Lindtke and Buerkle 2015; Xiong and Mallet 2022). If the alleles themselves are selfish, such as targets
434 of parental conflict, the selective advantage conferred by these alleles may aid in overcoming their
435 deleterious epistatic effects (Werren 2011; Meiklejohn et al. 2018; Wang et al. 2024). In the case of
436 other selfish elements, such as meiotic drivers or *Wolbachia*, much theory and empirical work has
437 demonstrated the ability of such selfish loci to invade and spread, even in the face of deleterious effects
438 to the host (Hoffmann et al. 1990; Raychoudhury et al. 2009; Werren 2011; Meiklejohn et al. 2018;
439 Turelli et al. 2018), but an equivalent body of work is lacking in the case of alleles evolving via parental
440 conflict.

441 Additionally, these patterns of hybrid seed inviability do not align with inferred phylogenetic
442 relationships presented here or alternative topologies presented elsewhere (Whitener et al. 2024). For
443 example, all Sierran *M. guttatus* have a similar crossing pattern and are compatible with one another,
444 yet they are not a monophyletic group (Fig.1A). Instead, we identify the Sierra mountains and foothills
445 as a unique group that exhibits cross incompatibility with other geographic regions of *M. guttatus*, but
446 broad compatibility with themselves. Although the *M. guttatus* species complex inhabits a broad range
447 from Alaska to Mexico and from Pacific coastline through to the Rocky mountains (Vickery 1974,
448 1978), the Sierras represent a unique geographic location where two deeply diverged clades within this
449 complex meet (i.e. Northern and Southern *M. guttatus;* Twyford and Friedman 2015), and is home to

450 several endemic species (Ferris et al. 2014, 2017; Twyford and Friedman 2015; Coughlan et al. 2021;
451 Ivey et al. 2023). Although more work on the phylogeography of this group will be needed to further
452 assess the history of the Sierras, one compelling hypothesis is that the Sierras may have contained
453 unique refugia during the last glacial maximum, allowing for population divergence and the
454 accumulation of incompatibility alleles. After glacial retreat and range expansion, such incompatibility
455 alleles may have spread to nearby Northern *M. guttatus* or *M. glaucescens* populations in the Sierra
456 foothills. In line with this hypothesis, we find excessive allele sharing between the Northern and
457 Southern clades of *M. guttatus* in the Sierra mountains and foothills (Fig.S6). Cases of complex
458 population structure and high diversity driven by microrefugia within the Sierras are known in other
459 species (Weng et al. 2021). Alternatively, these incompatibility alleles may predate the split of the
460 Northern and Southern clades, and may have independently fixed in the Sierra mountains and foothills.
461 Given that the entire complex exhibits significant allele sharing some of which is caused by
462 introgression (Brandvain et al. 2014; Twyford and Friedman 2015; Ivey et al. 2023; Mantel and
463 Sweigart 2024), distinguishing patterns of incomplete lineage sorting from introgression within *M.*
464 *guttatus* is challenging and will require a much deeper understanding of the relationship among
465 populations in the Sierras and beyond (though see: Twyford and Friedman 2015; Twyford et al. 2020).
466 Despite this, one clear incidence of introgression is that of *M. corallinus* into the now allopatric
467 Southern *M. decorus*, the consequences of which we explore below.
468
469
470 *Introgression as a source of incompatibilities*

471 Our work supports a shared genetic basis of hybrid seed inviability and a history of
472 introgression between *M. corallinus* and Southern *M. decorus*. Given the low divergence between
473 Southern *M. decorus* and its sister species, Northern *M. decorus*, we infer that this introgression may
474 have led to cascading reproductive isolation between the two *M. decorus* lineages.

475 Yet, many questions remain about this case of introgression. First, the current geographic
476 distance between *M. decorus* and *M. corallinus* suggests that introgression is not ongoing, but it is

477 unclear how and where this introgression originally occurred. The distribution of the *M. guttatus* species
478 complex is likely a result of post-glaciation recolonization, particularly in the Sierras (Twyford and
479 Friedman 2015), which may have facilitated hybridization. In line with this idea, our estimate of the
480 timing of admixture corresponds to a period of geological dynamism in the southern Cascades, wherein
481 many of the current Cascade Lakes were formed (4,703 generations; 4-14kya, assuming a 1-3 year
482 generation time; Johnson 1985). It is, of course, also possible that the source of introgression was a
483 close relative of *M. corallinus* that was not sampled or is no longer extant. Indeed, regions that we
484 identified as fixed for *M. corallinus* ancestry within southern *M. decorus* are not identical (D_{xy} = 0.017;
485 F_{ST} = 0.007). Still, even with the high diversity of this species complex (Lovell et al. 2025), our data
486 support *M. corallinus* as the most likely source of the introgression (Fig. S6).

487 Introgression may be an underappreciated contributor to reproductive isolation. Although
488 introgression is often viewed as a homogenizing force (Owens and Samuk 2020), it can also generate
489 novel biodiversity (Otto and Whitton 2000; Lamichhaney et al. 2018; Long and Rieseberg 2024; Rosser
490 et al. 2024). However, in between these extremes, there are opportunities for lower levels of
491 introgression to move incompatibility alleles between populations (Widen et al. 2023; Aguillon et al.
492 2025). In the case of Northern and Southern *M. decorus* presented in this study, it is possible that
493 introgressed alleles from *M. corallinus* provided an advantage in parental competition to isolated
494 populations of *M. decorus*, leading to the origin of the Southern lineage. We have previously shown
495 that seeds developing alongside hybrids from low-conflict fathers are larger than seeds growing beside
496 full siblings (Coughlan 2023a), suggesting that at the level of individual alleles, selfish solicitation of
497 maternal resources confers a direct benefit to the individual seed (and a direct cost to sibling seeds).
498 Yet, how these small increases in seed size translate into survival and fitness in the wild remains
499 unknown. Few incidences of introgressed incompatibilities have been reported, but given the ubiquity
500 of introgression across eukaryotes (Taylor and Larson 2019; Dagilis et al. 2022), they may be more
501 prevalent than previously thought, as recently exemplified in Swordtail fishes (Aguillon et al. 2025), as
502 well as a potential example of chloroplast capture in a more distantly related group of monkeyflowers
503 (Nelson et al. 2021).
504

Conclusion

Overall, we find that reproductive isolation is segregating in the *Mimulus guttatus* species complex, following a geographic rather than phylogenetic pattern. In at least one instance, we have provided evidence that introgression may have moved incompatibility alleles between lineages, generating reproductive isolation between sister lineages. Our study supports the idea that parental conflict and introgression of selfish elements can contribute to the rapid, repeated, and strong evolution of intrinsic postzygotic reproductive isolation.

Methods

Crossing surveys

To assess hybrid seed inviability within the widespread *M. guttatus*, we grew 3-5 individuals from 1 inbred line/maternal family for each of 23 populations of *M. guttatus,* which span a large part of the species' geographic range (Fig. 1B; Table S1). Seeds were cold stratified at 4°C for 5 days before being transferred to long days in the University of North Carolina greenhouses (16hrs light, 22°C). Upon flowering, we crossed all accessions to each other in a nearly full diallel design, totalling 426 unique cross directions (out of a possible 506), with a mean of 4 replicate crosses per combination (range: 1-12, totalling 1,742 crosses). For all crosses, the buds were emasculated 1-2 days before opening to prevent autogamous self-fertilization, and pollen from a freshly opened flower of the sire was applied to a receptive stigma of the dam.

Based on the results of these crosses, we performed follow-up crossing surveys that incorporated other species within the complex: *Mimulus decorus*, *M. corallinus*, and *M. glaucescens*. Previously, we found that *M. decorus* was two genetically unique lineages, Northern and Southern, that vary in their ability to cross with *M. guttatus*, putatively via differences in the strength of parental conflict within each lineage (Coughlan et al. 2020). Therefore, we crossed 12 accessions of *M. guttatus* (which spanned the observed variation in crossing pattern in the initial survey) to 4 populations of

532 Southern *M. decorus* (high-conflict lineage; mean of 4 replicate crosses/direction, 730 crosses total),
533 and 3 populations of Northern *M. decorus* (low-conflict lineage; mean of 3 replicate crosses/direction,
534 256 crosses total). For *M. glaucescens,* we crossed individuals from 2 populations to 21 *M. guttatus*
535 populations used in the original survey, as well as 2 populations of Northern *M. decorus* and 4
536 populations of Southern *M. decorus* (mean of 4 replicate crosses, 458 crosses total). For *M. corallinus*,
537 we crossed 3 accessions of *M. corallinus* to one accession of each Northern *M. guttatus,* Sierran *M.*
538 *guttatus*, Northern *M. decorus*, and Southern *M. decorus* (3 replicate crosses/direction, 95 crosses total).
539

540 *Estimation of seed viability, germination, and size*

541 We estimated seed viability in two ways. First, we counted all seeds formed per fruit and
542 characterized viability based on morphology (e.g., shriveled/flat corresponding to inviable, or round
543 corresponding to viable). To confirm germination proclivity, we performed a germination assay where
544 10 seeds from each unique cross combination were plated on 0.6% agar, cold stratified for 1 week, then
545 placed in a growth chamber under long-day conditions (18hrs light, 21/18°C day/night) at Yale
546 University. We seeded 3 replicated plates per unique cross. Plates were monitored weekly for 4 weeks
547 for the emergence of a radicle, which was deemed a germinant. These two measurements of viability
548 are highly correlated ($r^2 = 0.81$, DF = 532, $p < 0.0001$), but offer different insights; while one
549 characterizes abnormal phenotypes, the other directly assesses death. To determine seed size, we
550 photographed seeds on white paper with a Nikon D3500 DSLR digital camera and a size standard. We
551 measured the width of 25 seeds for each fruit manually using ImageJ (Schneider et al. 2012). To assess
552 cross compatibility and parent-of-origin differences in seed size, we ran a series of linear mixed models
553 using the *lme4* library in R (Bates et al. 2015), where either the proportion of viable seeds or seed width
554 was the response variable, cross type was a fixed effect, and the identity of the maternal and paternal
555 populations were each random effects. In both sets of analyses, cross type contained four levels (within-
556 lineage for each parent and each reciprocal F1). For each model, we then assessed differences among
557 cross types with a type III Anova using the *car* package and identified significant comparisons using
558 contrasts from the *emmeans* package in R (Fox and Weisberg 2019; Lenth 2025).

559

560 *Embryo Rescue*

561 We further wanted to determine if hybrid seed inviability was caused by defects in the
562 endosperm rather than the embryo itself. To test this, we performed an embryo rescue experiment where
563 early developing seeds were grown on Murashige and Skoog media supplemented with 4% sucrose to
564 functionally compensate for the endosperm by providing external nutrition needed for growth. Under
565 long days at the Yale University greenhouses, we grew four individuals from a single inbred line
566 representing each of the four species used in this assay: Northern *M. guttatus* (IM62), Sierran *M.*
567 *guttatus* (CCC9), *M. corallinus* (SILF), and Southern *M. decorus* (OD11). Given that all incidences of
568 hybrid seed inviability reported here involve Northern *M. guttatus*, we crossed Northern *M. guttatus*
569 reciprocally with the other three species and included intraspecific crosses as controls. For each cross
570 direction, we performed three replicate crosses and dissected the ovaries eight days after pollination
571 (DAP). Ten seeds per cross were placed on Petri dishes with media, sealed with 3M micropore tape,
572 then incubated in growth chambers under long-day conditions (18 hrs light, 22/20°C day/night). Seeds
573 were scored for germination weekly for four weeks. For each cross direction, we tested for significant
574 difference between the germination assay and 8 DAP embryo rescue using unpaired non-parametric
575 Wilcoxon rank-sum test in R.

576

577 *Complementation test*

578 In this study, we report multiple incidences of hybrid seed inviability and shared patterns of
579 incompatibility across phylogenetically and geographically distinct taxa. Given that we find the
580 repeated occurrence of high-conflict lineages within the *M. guttatus* complex, we next sought to
581 understand if the genetic basis of the incompatibility was shared among incidences. To do so, we
582 performed a complementation test between two geographically distinct taxa that showed similar
583 patterns of hybrid seed inviability when crossed with Northern *M. guttatus*- Southern *M. decorus* and
584 *M. corallinus*. We first crossed these two species reciprocally, then self-fertilized one reciprocal F1
585 from each cross direction to create reciprocal F2 populations. We predict that if the genetic basis of

586 hybrid seed inviability with Northern *M. guttatus* was largely shared between *M. corallinus* and
587 Southern *M. decorus*, all F2s should produce inviable seeds when acting as the sire in crosses with
588 Northern *M. guttatus*, just as we see for both *M. corallinus* and Southern *M. decorus.* By contrast, if the
589 genetic basis was independent, then F2s should segregate for their crossability with Northern *M.*
590 *guttatus*, resulting in some fraction of F2s that produced viable hybrid seeds. To test these predictions,
591 we planted 5 replicates of each parent, along with 15 F1s from each cross direction, and roughly 100
592 F2s from each cross direction. After omitting individuals that did not flower, we were left with 3
593 Southern *M. decorus*, 4 *M. corallinus*, 15 Southern *M. decorus* x *M. corallinus* F1s, 12 *M. corallinus* x
594 Southern *M. decorus* F1s, and 170 F2s (83 with Southern *M. decorus* as the grand-dam and 87 with *M.*
595 *corallinus* as the grand-dam). We crossed each experimental plant with an inbred line of Northern *M.*
596 *guttatus* (IM62), with IM62 serving as the dam and each experimental plant as the sire for all crosses.
597 We performed an average of 3 replicate crosses per plant (range: 1-10), totalling 761 crosses. Based on
598 our previous results, hybrid seed inviability should manifest as large, flat, disc-like seeds. We assessed
599 the percentage of viable seed per fruit.

600 Additionally, we performed a simplified power analysis to assess under what genetic conditions
601 we would be able to detect segregating phenotypes among our F2s. For all models, we assumed x
602 unlinked loci with equivalent and additive effect sizes, and assessed the expected fraction of F2
603 individuals that would exhibit complete inviability with *M. guttatus*. Here, x varied from 1 to 10 loci,
604 and we simulated four levels of effect size: each locus having a 100% lethal effect, 50% lethality, 25%
605 lethality, or 10% lethality. We then performed a Fisher's Exact test between our observed counts of
606 individuals that produced no viable seeds (versus those that produced at least some viable seeds) with
607 what would be expected under each set of genetic conditions. We find that, except under the most
608 extreme parameters (i.e. 6 or more loci, each of which exhibits 100% lethality or 9 or more loci each of
609 which exhibits 50% lethality; Fig. S8), we have the power to identify a lack of segregation among F2s.
610 We note that these are quite conservative estimates, as other studies have found that hybrid seed
611 inviability typically involves a smaller number of paternally acting alleles (i.e. 3-6), each of which
612 reduces viability by 20-50% (Rebernig et al. 2015; Garner et al. 2016; Lafon-Placette et al. 2017;
613 Couglan unpublished data).

614

615 *Genomic Analyses*

616 In order to contextualize the patterns observed above with the underlying genetic relationships
617 between lineages, we used whole genome sequences from the lines used in our experiments, combined
618 with numerous new and publicly available sequences. We sequenced 99 new accessions and combined
619 this with 73 publicly available accessions (Table S1). For new sequences, we extracted DNA from bud
620 and leaf tissue using the ThermoFisher GeneJET Plant Genomic DNA kit. Illumina libraries were
621 prepped and sequenced using NovaSeq6000 by the Yale Center for Genome Analysis. For all
622 accessions, we cleaned reads using fastp v0.23.2 (using option "-q 20"; Chen et al. 2018) and aligned
623 to the IM62v3 reference genome (Lovell et al. 2025) using the BWA mem algorithm v0.7.17 (Li and
624 Durbin 2009). We removed duplicates using Picard v2.25.6 (options
625 "VALIDATION_STRINGENCY=LENIENT"; http://broadinstitute.github.io/picard). We called
626 variants using bcftools mpileup and call (v1.16; Li 2011; Danecek et al. 2021), and filtered for quality
627 and depth (-e "FMT/DP<4 | FMT/GQ<20 | QUAL<40") using bcftools v1.16.

628 We estimated relatedness among samples using king (v 2.3.2; Manichaikul et al. 2010). Next,
629 we constructed a phylogeny for all lineages using IQTree2 (Minh et al. 2020). We removed individuals
630 with less than 3X coverage, as well as those suspected to be early generation hybrids, leaving 158
631 sequences. We used only biallelic sites and sites with a minimum minor allele frequency of 0.05. We
632 selected the best substitution model, TVM+F+ASC+R6, using ModelFinder (Kalyaanamoorthy et al.
633 2017), and then we used that model to infer a consensus tree using 1000 bootstraps (Fig. 1A).

634 In order to broadly assess diversity, divergence, and introgression among these lineages, we
635 calculated D and F4 statistics using Dsuite (Malinsky et al. 2021). We split samples into their respective
636 lineages by combining field identification with the inferred relationships from the phylogeny, as well
637 as PCAdmix run using all samples. We ran all combinations of *M. guttatus* species complex lineages
638 with Dtrios using a more distantly related and allopatric species- *M. caespitosa*- as an outgroup. We
639 compared the arrangement of the trios that minimized the value of D to the arrangement predicted by

640 the phylogeny generated above. We also ran Dtrios constrained by the phylogeny, and used these results
641 to obtain f-branch values (Malinsky et al. 2021).

642 We further compared the genomes of the two high-conflict lineages for which we had
643 performed the complementation test (*M. corallinus* and Southern *M. decorus)*, as well as the low-
644 conflict sister species of Southern *M. decorus*: Northern *M. decorus*. We used PCANGSD (Meisner and
645 Albrechtsen 2018) to perform PCA and PCAdmix for global ancestry grouping. Based on these initial
646 findings, we found a striking pattern of ancestry sharing between the two high-conflict lineages. To
647 investigate this shared ancestry further, we constructed explicit tests of introgression using Dsuite and
648 calculated admixture proportion for southern *M. decorus* using HyDe (Blischak et al. 2018), using
649 standard settings with *M. caespitosa* as an outgroup.

650 In order to investigate patterns of introgression along the genome, we used Dinvestigate to
651 calculate f_{dM} in sliding windows of 250 SNPs with a 50 SNP step. We selected fourfold degenerate sites
652 using degenotate (Mirchandani et al. 2024), and used these to calculate F_{ST} with *Pixy* (Korunes and
653 Samuk 2021) using the same sliding windows in which f_{dM} was calculated. We further investigated
654 regions of the genome that appear introgressed by estimating topology weighting along the genome
655 using TWISST (Martin and Van Belleghem 2017). TWISST input files were made using the
656 genomics_general scripts available from Simon Martin
657 (https://github.com/simonhmartin/genomics_general), as recommended by the TWISST manual, again
658 using *M. caespitosa* as an outgroup.

659 To understand the individual-level patterns of introgression, we inferred local ancestry within
660 our Southern *M. decorus* genomes using ancestryHMM (Corbett-Detig and Nielsen 2017). We used all
661 *M. corallinus* and Northern *M. decorus* individuals to calculate population allele frequencies for the
662 parental populations using custom scripts. To assign genetic distances between loci, we used a linkage
663 map generated from crosses between Northern *M. decorus* and *M. guttatus* (Schwarz, unpublished); for
664 sites in between SNPs on the linkage map, we assumed a linear increase in genetic position with physical
665 position. We used a starting ancestry proportion of 20% *M. corallinus* ancestry and allowed the timing
666 of that ancestry to be inferred by the program. We compared three different sets of SNPs: 1) all sites
667 that had variation in the parental populations, 2) fixed differences between the parents, and 3) sites with

668 an allele frequency difference between the parents greater than 50%. We only included SNPs that had
669 no missing data in the parental samples (Fig.S9). We focus on the results from using all variable sites
670 as these analyses yielded the most consistent results with other genomic analyses.

671 Finally, our *M. corallinus* samples displayed significant population structure, with four
672 individuals forming a distinct group in the phylogeny and PCA. Therefore, we repeated all of our
673 analyses with and without those individuals. There was not a substantial difference in the results, so we
674 present only the results using all *M. corallinus* individuals here.

675

676

677 **Author Contributions**

678 All authors contributed to the design and conceptual framing of this work, as well as data collection and
679 data analysis. M.E.F. led the writing of the manuscript with significant contributions from all authors.
680 All authors approved the final version of this manuscript before submission.

681

682

683 **Acknowledgements**

684 We are grateful to Christopher Bolick and Nathan Guzzo for plant care and assistance in the Yale
685 Science Building greenhouses. The Coughlan lab provided helpful feedback on earlier drafts of this
686 work. We also thank Patricia Vallejo Joseph and Quinn Evans for helping with seed counting and
687 viability assessment. This work was supported by an NIH grant to J.M.C. (NIH R35GM150907), an
688 NSF Postdoctoral Research Fellowship in Biology to M.E.F. (2305853), and an NIH grant to Daniel R.
689 Matute that supported J.M.C. (NIH R35GM148244). P.F.S. and H.K.S. were supported by the Yale
690 Graduate School. The Yale Center for Genome Analysis, which provided sequencing, is supported by
691 the NIH NIGMS 1S10OD030363-01A1.

692

693

694

References

Aguillon, S. M., S. K. Haase Cox, Q. K. Langdon, T. R. Gunn, J. J. Baczenas, S. M. Banerjee, A. E. Donny, B. M. Moran, P. Fascinetto-Zago, C. Gutiérrez-Rodríguez, O. Ríos-Cárdenas, M. R. Morris, D. L. Powell, and M. Schumer. 2025. Pervasive gene flow despite strong and varied reproductive barriers in swordtails. Nat. Ecol. Evol. 9:867–878. Nature Publishing Group.

Arévalo, L., and P. Campbell. 2020. Placental effects on the maternal brain revealed by disrupted placental gene expression in mouse hybrids. Proc. R. Soc. B Biol. Sci. 287:20192563. Royal Society.

Babak, T., B. DeVeale, E. K. Tsang, Y. Zhou, X. Li, K. S. Smith, K. R. Kukurba, R. Zhang, J. B. Li, D. van der Kooy, S. B. Montgomery, and H. B. Fraser. 2015. Genetic conflict reflected in tissue-specific maps of genomic imprinting in human and mouse. Nat. Genet. 47:544–549.

Bank, C., R. Bürger, and J. Hermisson. 2012. The Limits to Parapatric Speciation: Dobzhansky–Muller Incompatibilities in a Continent–Island Model. Genetics 191:845–863.

Barbash, D. A., D. F. Siino, A. M. Tarone, and J. Roote. 2003. A rapidly evolving MYB-related protein causes species isolation in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 100:5302–5307. National Academy of Sciences.

Barnard-Kubow, K. B., N. So, and L. F. Galloway. 2016. Cytonuclear incompatibility contributes to the early stages of speciation. Evolution 70:2752–2766.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67:1–48.

Batista, R. A., and C. Köhler. 2020. Genomic imprinting in plants—revisiting existing models. Genes Dev. 34:24–36.

Bentsink, L., J. Jowett, C. J. Hanhart, and M. Koornneef. 2006. Cloning of DOG1, a quantitative trait locus controlling seed dormancy in Arabidopsis. Proc. Natl. Acad. Sci. 103:17042–17047. Proceedings of the National Academy of Sciences.

Blischak, P. D., J. Chifman, A. D. Wolfe, and L. S. Kubatko. 2018. HyDe: A Python Package for Genome-Scale Hybridization Detection. Syst. Biol. 67:821–829.

Bracewell, R. R., M. E. Pfrender, K. E. Mock, and B. J. Bentz. 2011. Cryptic postzygotic isolation in an eruptive species of bark beetle (*Dendroctonus ponderosae*). Evolution 65:961–975.

Brandvain, Y., and D. Haig. 2018. Outbreeders pull harder in a parental tug-of-war. Proc. Natl. Acad. Sci. 115:11354–11356. Proceedings of the National Academy of Sciences.

Brandvain, Y., A. M. Kenney, L. Flagel, G. Coop, and A. L. Sweigart. 2014. Speciation and Introgression between Mimulus nasutus and Mimulus guttatus. PLoS Genet. 10:e1004410.

Brekke, T. D., and J. M. Good. 2014. Parent-of-origin growth effects and the evolution of hybrid inviability in dwarf hamsters. Evolution 68:3134–3148.

Brekke, T. D., E. C. Moore, S. C. Campbell-Staton, C. M. Callahan, Z. A. Cheviron, and J. M. Good. 2021. X chromosome-dependent disruption of placental regulatory networks in hybrid dwarf hamsters. Genetics 218.

Briscoe Runquist, R. D., E. Chu, J. L. Iverson, J. C. Kopp, and D. A. Moeller. 2014. Rapid evolution of reproductive isolation between incipient outcrossing and selfing Clarkia species. Evol. Int. J. Org. Evol. 68:2885–2900.

Burt, A., and R. Trivers. 2009. Genes in Conflict: The Biology of Selfish Genetic Elements. Harvard University Press.

Butel, N., Y. Qiu, W. Xu, J. Santos-González, and C. Köhler. 2024. Parental conflict driven regulation of endosperm cellularization by a family of Auxin Response Factors. Nat. Plants 10:1018–1026. Nature Publishing Group.

Cailleau, A., D. Grimanelli, E. Blanchet, P.-O. Cheptou, and T. Lenormand. 2018. Dividing a Maternal Pie among Half-Sibs: Genetic Conflicts and the Control of Resource Allocation to Seeds in Maize. Am. Nat. 192:577–592. The University of Chicago Press.

Calvo-Baltanás, V., J. Wang, and E. Chae. 2021. Hybrid Incompatibility of the Plant Immune System: An Opposite Force to Heterosis Equilibrating Hybrid Performances. Front. Plant Sci. 11. Frontiers.

Charistianson, S. J., J. G. Swallow, and G. S. Wilkinson. 2005. Rapid evolution of postzygotic reproducive isolation in stalk-eyed flies. Evolution 59:849–857.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890.

Chuong, E. B., W. Tong, and H. E. Hoekstra. 2010. Maternal–Fetal Conflict: Rapidly Evolving Proteins in the Rodent Placenta. Mol. Biol. Evol. 27:1221–1225.

Corbett-Detig, R. B., J. Zhou, A. G. Clark, D. L. Hartl, and J. F. Ayroles. 2013. Genetic incompatibilities are widespread within species. Nature 504:135–137. Nature Publishing Group.

Corbett-Detig, R., and R. Nielsen. 2017. A Hidden Markov Model Approach for Simultaneously Estimating Local Ancestry and Admixture Time Using Next Generation Sequence Data in Samples of Arbitrary Ploidy. PLOS Genet. 13:e1006529. Public Library of Science.

Coughlan, J. M. 2023a. Indirect Effects of Parental Conflict on Conspecific Offspring Development. Am. Nat. 201:154–162. The University of Chicago Press.

Coughlan, J. M. 2023b. The role of conflict in shaping plant biodiversity. New Phytol., doi: 10.1111/nph.19233.

Coughlan, J. M. 2023c. The role of hybrid seed inviability in angiosperm speciation. Am. J. Bot. 110:e16135.

Coughlan, J. M., M. W. Brown, and J. H. Willis. 2021. The genetic architecture and evolution of life-history divergence among perennials in the Mimulus guttatus species complex. Proc. R. Soc. B Biol. Sci. 288:20210077. Royal Society.

Coughlan, J. M., and D. R. Matute. 2020. The importance of intrinsic postzygotic barriers throughout the speciation process. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 375:20190533.

Coughlan, J. M., M. Wilson Brown, and J. H. Willis. 2020. Patterns of Hybrid Seed Inviability in the Mimulus guttatus sp. Complex Reveal a Potential Role of Parental Conflict in Reproductive Isolation. Curr. Biol. 30:83-93.e5. Elsevier Ltd.

Coyne, J. A., and A. H. Orr. 2004. Speciation. Sinauer Associates.

Coyne, J. A., and H. A. Orr. 1989. Patterns of Speciation in Drosophila. Evolution 43:362–381.

Coyne, J. A., and H. A. Orr. 1997. “Patterns of Speciation in Drosophila” Revisited. Evolution 51:295–303.

Crespi, B., and P. Nosil. 2013. Conflictual speciation: species formation via genomic conflict. Trends Ecol. Evol. 28:48–57.

Crouch, M. L., K. M. Tenbarge, A. E. Simon, and R. Ferl. 1983. cDNA clones for Brassica napus seed storage proteins: evidence from nucleotide sequence analysis that both subunits of napin are cleaved from a precursor polypeptide. J. Mol. Appl. Genet. 2:273–283.

Cutter, A. D. 2012. The polymorphic prelude to Bateson–Dobzhansky–Muller incompatibilities. Trends Ecol. Evol. 27:209–218. Elsevier.

Dagilis, A. J., D. Peede, J. M. Coughlan, G. I. Jofre, E. R. R. D’Agostino, H. Mavengere, A. D. Tate, and D. R. Matute. 2022. A need for standardized reporting of introgression: Insights from studies across eukaryotes. Evol. Lett. 6:344–357.

Danecek, P., J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan, M. O. Pollard, A. Whitwham, T. Keane, S. A. McCarthy, R. M. Davies, and H. Li. 2021. Twelve years of SAMtools and BCFtools. GigaScience 10:giab008.

Farnitano, M. C., K. Karoly, and A. L. Sweigart. 2025. Fluctuating reproductive isolation and stable ancestry structure in a fine-scaled mosaic of hybridizing Mimulus monkeyflowers. PLOS Genet. 21:e1011624.

Feil, R., and F. Berger. 2007. Convergent evolution of genomic imprinting in plants and mammals. Trends Genet. 23:192–199.

Ferris, K. G., L. L. Barnett, B. K. Blackman, and J. H. Willis. 2017. The genetic architecture of local adaptation and reproductive isolation in sympatry within the Mimulus guttatus species complex. Mol. Ecol. 26:208–224.

Ferris, K. G., J. P. Sexton, and J. H. Willis. 2014. Speciation on a local geographic scale: the evolution of a rare rock outcrop specialist in Mimulus. Philos. Trans. R. Soc. B Biol. Sci. 369:20140001. Royal Society.

Florez-Rueda, A. M., F. Fiscalini, M. Roth, U. Grossniklaus, and T. Städler. 2021. Endosperm and Seed Transcriptomes Reveal Possible Roles for Small RNA Pathways in Wild Tomato Hybrid Seed Failure. Genome Biol. Evol. 13:evab107.

Florez-Rueda, A. M., M. Paris, A. Schmidt, A. Widmer, U. Grossniklaus, and T. Städler. 2016. Genomic Imprinting in the Endosperm Is Systematically Perturbed in Abortive Hybrid Tomato Seeds. Mol. Biol. Evol. 33:2935–2946. Oxford Academic.

Fox, J., and S. Weisberg. 2019. An R Companion to Applied Regression. Third. Sage, Thousand Oaks, CA.

Frayer, M. E., N. V. Robles, M. J. Rodríguez-Barrera, J. M. Coughlan, and M. Schumer. 2025. The molecular evolutionary basis of species formation revisited. Trends Genet. 0. Elsevier.

Fusca, D. D., M. N. Dall'Acqua, S. Sánchez-Ramírez, and A. D. Cutter. 2025. Phylogenomic timetree-calibrated speciation clocks for Caenorhabditis nematodes reveal slow but disproportionate accumulation of post-zygotic reproductive isolation. bioRxiv.

Garner, A. G., A. M. Kenney, L. Fishman, and A. L. Sweigart. 2016. Genetic loci with parent-of-origin effects cause hybrid seed lethality in crosses between Mimulus species. New Phytol. 211:319–331.

Grant, V. 1966. The Selective Origin of Incompatibility Barriers in the Plant Genus Gilia. Am. Nat. 100:99–118. The University of Chicago Press.

Gustafsson, A. L. S., G. Gussarova, L. Borgen, H. Ikeda, A. Antonelli, L. Marie-Orleach, L. H. Rieseberg, and C. Brochmann. 2022. Rapid evolution of post-zygotic reproductive isolation is widespread in Arctic plant lineages. Ann. Bot. 129:171–184.

Gutiérrez-Marcos, J. F., L. M. Costa, C. Biderre-Petit, B. Khbaya, D. M. O'Sullivan, M. Wormald, P. Perez, and H. G. Dickinson. 2004. maternally expressed gene1 Is a novel maize endosperm transfer cell-specific gene with a maternal parent-of-origin pattern of expression. Plant Cell 16:1288–1301.

Gutierrez-Marcos, J. F., P. D. Pennington, L. M. Costa, and H. G. Dickinson. 2003. Imprinting in the endosperm: a possible role in preventing wide hybridization. Philos. Trans. R. Soc. B Biol. Sci. 358:1105–1111.

Haig, D. 1997. Parental antagonism, relatedness asymmetries, and genomic imprinting. Proc. R. Soc. B Biol. Sci. 264:1657–1662.

Haig, D. 2000. The Kinship Theory of Genomic Imprinting. Annu. Rev. Ecol. Syst. 31:9–32.

Haig, D., and M. Westoby. 1991. Genomic imprinting in endosperm: its effect on seed development in crosses between species, and its implications for the evolution of apomixis. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 333:1–13. The Royal Society London.

Haig, D., and M. Westoby. 1989. Parent-Specific Gene Expression and the Triploid Endosperm. Am. Nat. 134:147–155. [University of Chicago Press, American Society of Naturalists].

Hoffmann, A. A., M. Turelli, and L. G. Harshman. 1990. Factors affecting the distribution of cytoplasmic incompatibility in Drosophila simulans. Genetics 126:933–948.

İltaş, Ö., M. Svitok, A. Cornille, R. Schmickl, and C. Lafon Placette. 2021. Early evolution of reproductive isolation: A case of weak inbreeder/strong outbreeder leads to an intraspecific hybridization barrier in Arabidopsis lyrata. Evolution 75:1466–1476.

Ivey, C. T., N. M. Habecker, J. P. Bergmann, J. Ewald, M. E. Frayer, and J. M. Coughlan. 2023. Weak reproductive isolation and extensive gene flow between Mimulus glaucescens and M. guttatus in northern California. Evolution 77:1245–1261.

Johnson, D. M. 1985. Atlas of Oregon Lakes. Oregon State University Press.

Johnson, N. A. 2010. Hybrid incompatibility genes: Remnants of a genomic battlefield? Trends Genet. 26:317–325. Elsevier Ltd.

Johnston, S. A., T. P. M. den Nijs, S. J. Peloquin, and R. E. Hanneman. 1980. The significance of genic balance to endosperm development in interspecific crosses. Theor. Appl Genet. 57:5–9.

Johnston, S. A., and R. E. Hanneman. 1982. Manipulations of Endosperm Balance Number Overcome Crossing Barriers Between Diploid Solanum Species. Science 217:446–448. American Association for the Advancement of Science.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14:587–589. Nature Publishing Group.

Köhler, C., K. Dziasek, and G. Del Toro-De León. 2021. Postzygotic reproductive isolation established in the endosperm: mechanisms, drivers and relevance. Philos. Trans. R. Soc. B Biol. Sci. 376:20200118. Royal Society.

Köhler, C., L. Hennig, R. Bouveret, J. Gheyselinck, U. Grossniklaus, and W. Gruissem. 2003. Arabidopsis MSI1 is a component of the MEA/FIE Polycomb group complex and required for seed development. EMBO J. 22:4804–4814. John Wiley & Sons, Ltd.

Korunes, K. L., and K. Samuk. 2021. pixy: Unbiased estimation of nucleotide diversity and divergence in the presence of missing data. Mol. Ecol. Resour. 21:1359–1368.

Lafon-Placette, C., M. R. Hatorangan, K. A. Steige, A. Cornille, M. Lascoux, T. Slotte, and C. Köhler. 2018. Paternally expressed imprinted genes associate with hybridization barriers in Capsella. Nat. Plants 4:352–357. Nature Publishing Group.

Lafon-Placette, C., I. M. Johannessen, K. S. Hornslien, M. F. Ali, K. N. Bjerkan, J. Bramsiepe, B. M. Glöckle, C. A. Rebernig, A. K. Brysting, P. E. Grini, and C. Köhler. 2017. Endosperm-based hybridization barriers explain the pattern of gene flow between Arabidopsis lyrata and Arabidopsis arenosa in Central Europe. Proc. Natl. Acad. Sci. U. S. A. 114:E1027–E1035.

Lamichhaney, S., F. Han, M. T. Webster, L. Andersson, B. R. Grant, and P. R. Grant. 2018. Rapid hybrid speciation in Darwin's finches. Science 359:224–228. American Association for the Advancement of Science.

Larson, E. L., D. Vanderpool, B. A. J. Sarver, C. Callahan, S. Keeble, L. L. Provencio, M. D. Kessler, V. Stewart, E. Nordquist, M. D. Dean, and J. M. Good. 2018. The Evolution of Polymorphic Hybrid Incompatibilities in House Mice. Genetics 209:845–859.

Lenth, R. V. 2025. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.11.2-00002.

Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27:2987–2993.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinforma. Oxf. Engl. 25:1754–1760.

Lindtke, D., and C. A. Buerkle. 2015. The genetic architecture of hybrid incompatibilities and their effect on barriers to introgression in secondary contact. Evolution 69:1987–2004.

Long, T. a. F. 2005. The influence of mating system on the intensity of parent–offspring conflict in primates. J. Evol. Biol. 18:509–515.

Long, Z., and L. H. Rieseberg. 2024. Documenting homoploid hybrid speciation. Mol. Ecol. 00:e17412.

Lovell, J. T., R. Walstead, A. Lawrence, E. Stark-Dykema, M. C. Farnitano, A. Harder, T. Brůna, K. Barry, D. Goodstein, J. Jenkins, A. Lipzen, L. Boston, J. Webber, M. Chovatia, J. Eichenberger, J.

Talag, J. Grimwood, J. Schmutz, J. K. Kelly, A. L. Sweigart, L. Fishman, and J. H. Willis. 2025. Comparative Analyses of Four Reference Genomes Reveal Exceptional Diversity and Weak Linked Selection in the Yellow Monkeyflower (Mimulus guttatus) Complex. Mol. Ecol. Resour. e70012.

Luo, M., P. Bilodeau, E. S. Dennis, W. J. Peacock, and A. Chaudhury. 2000. Expression and parent-of-origin effects for FIS2, MEA, and FIE in the endosperm and embryo of developing Arabidopsis seeds. Proc. Natl. Acad. Sci. U. S. A. 97:10637–10642.

Luo, M., P. Bilodeau, A. Koltunow, E. S. Dennis, W. J. Peacock, and A. M. Chaudhury. 1999. Genes controlling fertilization-independent seed development in Arabidopsis thaliana. Proc. Natl. Acad. Sci. 96:296–301. Proceedings of the National Academy of Sciences.

Malinsky, M., M. Matschiner, and H. Svardal. 2021. Dsuite - Fast D-statistics and related admixture evidence from VCF files. Mol. Ecol. Resour. 21:584–595.

Manichaikul, A., J. C. Mychaleckyj, S. S. Rich, K. Daly, M. Sale, and W.-M. Chen. 2010. Robust relationship inference in genome-wide association studies. Bioinformatics 26:2867–2873.

Mantel, S. J., and A. L. Sweigart. 2024. Postzygotic barriers persist despite ongoing introgression in hybridizing Mimulus species. Mol. Ecol. 33:e17261.

Martin, S. H., and S. M. Van Belleghem. 2017. Exploring Evolutionary Relationships Across the Genome Using Topology Weighting. Genetics 206:429–438.

Meiklejohn, C. D., E. L. Landeen, K. E. Gordon, T. Rzatkiewicz, S. B. Kingan, A. J. Geneva, J. P. Vedanayagam, C. A. Muirhead, D. Garrigan, D. L. Stern, and D. C. Presgraves. 2018. Gene flow mediates the role of sex chromosome meiotic drive during complex speciation. eLife 7:e35468. eLife Sciences Publications, Ltd.

Meisner, J., and A. Albrechtsen. 2018. Inferring Population Structure and Admixture Proportions in Low-Depth NGS Data. Genetics 210:719–731.

Minh, B. Q., H. A. Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. von Haeseler, and R. Lanfear. 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol. Biol. Evol. 37:1530–1534.

Mirchandani, C. D., A. J. Shultz, G. W. C. Thomas, S. J. Smith, M. Baylis, B. Arnold, R. Corbett-Detig, E. Enbody, and T. B. Sackton. 2024. A Fast, Reproducible, High-throughput Variant Calling Workflow for Population Genomics. Mol. Biol. Evol. 41:msad270.

Moore, T., and D. Haig. 1991. Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet. 7:45–49.

Moran, B. M., C. Y. Payne, D. L. Powell, E. N. K. Iverson, A. E. Donny, S. M. Banerjee, Q. K. Langdon, T. R. Gunn, R. A. Rodriguez-Soto, A. Madero, J. J. Baczenas, K. M. Kleczko, F. Liu, R. Matney, K. Singhal, R. D. Leib, O. Hernandez-Perez, R. Corbett-Detig, J. Frydman, C. Gifford, M. Schartl, J. C. Havird, and M. Schumer. 2024. A lethal mitonuclear incompatibility in complex I of natural hybrids. Nature 1–9. Nature Publishing Group.

Moyle, L. C., M. Levine, M. L. Stanton, and J. W. Wright. 2012. Hybrid Sterility over Tens of Meters Between Ecotypes Adapted to Serpentine and Non-Serpentine Soils. Evol. Biol. 39:207–218.

Mozgova, I., C. Köhler, and L. Hennig. 2015. Keeping the gate closed: functions of the polycomb repressive complex PRC2 in development. Plant J. 83:121–132.

Nelson, T. C., A. M. Stathos, D. D. Vanderpool, F. R. Finseth, Y. Yuan, and L. Fishman. 2021. Ancient and recent introgression shape the evolutionary history of pollinator adaptation and speciation in a model monkeyflower radiation (Mimulus section Erythranthe). PLOS Genet. 17:e1009095. Public Library of Science.

Oneal, E., J. H. Willis, and R. G. Franks. 2016. Disruption of endosperm development is a major cause of hybrid seed inviability between Mimulus guttatus and Mimulus nudatus. New Phytol. 210:1107–1120. Blackwell Publishing Ltd.

Ostevik, K. L., J. L. Rifkin, H. Xia, and M. D. Rausher. 2021. Morning glory species co-occurrence is associated with asymmetrically decreased and cascading reproductive isolation. Evol. Lett. 5:75–85.

Otto, S. P., and J. Whitton. 2000. Polyploid incidence and evolution. Annu. Rev. Genet. 34:401–437.

Owens, G. L., and K. Samuk. 2020. Adaptive introgression during environmental change can weaken reproductive isolation. Nat. Clim. Change 10:58–62. Nature Publishing Group.

Petrén, H., H. Thosteman, M. Stift, P. Toräng, J. Ågren, and M. Friberg. 2023. Differences in mating system and predicted parental conflict affect post-pollination reproductive isolation in a flowering plant. Evolution 77:1019–1030.

Raunsgard, A., Ø. H. Opedal, R. K. Ekrem, J. Wright, G. H. Bolstad, W. S. Armbruster, and C. Pélabon. 2018. Intersexual conflict over seed size is stronger in more outcrossed populations of a mixed-mating plant. Proc. Natl. Acad. Sci. 115:11561–11566. Proceedings of the National Academy of Sciences.

Raychoudhury, R., L. Baldo, D. C. S. G. Oliveira, and J. H. Werren. 2009. Modes of Acquisition of Wolbachia: Horizontal Transfer, Hybrid Introgression, and Codivergence in the Nasonia Species Complex. Evolution 63:165–183.

Rebernig, C. A., C. Lafon-Placette, M. R. Hatorangan, T. Slotte, and C. Köhler. 2015. Non-reciprocal Interspecies Hybridization Barriers in the Capsella Genus Are Established in the Endosperm. PLOS Genet. 11:e1005295. Public Library of Science.

Rosser, N., F. Seixas, L. M. Queste, B. Cama, R. Mori-Pezo, D. Kryvokhyzha, M. Nelson, R. Waite-Hudson, M. Goringe, M. Costa, M. Elias, C. Mendes Eleres de Figueiredo, A. V. L. Freitas, M. Joron, K. Kozak, G. Lamas, A. R. P. Martins, W. O. McMillan, J. Ready, N. Rueda-Muñoz, C. Salazar, P. Salazar, S. Schulz, L. T. Shirai, K. L. Silva-Brandão, J. Mallet, and K. K. Dasmahapatra. 2024. Hybrid speciation driven by multilocus introgression of ecological traits. Nature 628:811–817. Nature Publishing Group.

Roth, M., A. M. Florez-Rueda, S. Griesser, M. Paris, and T. Städler. 2018. Incidence and developmental timing of endosperm failure in post-zygotic isolation between wild tomato lineages. Ann. Bot. 121:107–118.

Roth, M., A. M. Florez-Rueda, and T. Städler. 2019. Differences in Effective Ploidy Drive Genome-Wide Endosperm Expression Polarization and Seed Failure in Wild Tomato Hybrids. Genetics 212:141–152.

Sandstedt, G. D., and A. L. Sweigart. 2022. Developmental evidence for parental conflict in driving Mimulus species barriers. New Phytol. 236:1545–1557.

Sandstedt, G. D., C. A. Wu, and A. L. Sweigart. 2020. Evolution of multiple postzygotic barriers between species of the Mimulus tilingii complex. Evolution 1–14.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9:671–675. Nature Publishing Group.

Schrader, M., and J. Travis. 2008. Testing the Viviparity-Driven-Conflict Hypothesis: Parent-Offspring Conflict and the Evolution of Reproductive Isolation in a Poeciliid Fish. Am. Nat. 172:806–817. The University of Chicago Press.

Schumer, M., R. Cui, D. L. Powell, R. Dresner, G. G. Rosenthal, and P. Andolfatto. 2014. High-resolution mapping reveals hundreds of genetic incompatibilities in hybridizing fish species. eLife 3:e02535. eLife Sciences Publications, Ltd.

Sicard, A., C. Kappel, E. B. Josephs, Y. W. Lee, C. Marona, J. R. Stinchcombe, S. I. Wright, and M. Lenhard. 2015. Divergent sorting of a balanced ancestral polymorphism underlies the establishment of gene-flow barriers in Capsella. Nat. Commun. 6:7960. Nature Publishing Group.

Soliman, H. K., and J. M. Coughlan. 2024. United by conflict: Convergent signatures of parental conflict in angiosperms and placental mammals. J. Hered. esae009.

Svedberg, J., A. A. Vogan, N. A. Rhoades, D. Sarmarajeewa, D. J. Jacobson, M. Lascoux, T. M. Hammond, and H. Johannesson. 2021. An introgressed gene causes meiotic drive in Neurospora sitophila. Proc. Natl. Acad. Sci. 118:e2026605118. Proceedings of the National Academy of Sciences.

Taylor, S. A., and E. L. Larson. 2019. Insights from genomes into the evolutionary importance and prevalence of hybridization in nature. Nat. Ecol. Evol. 3:170–177. Nature Publishing Group.

Trivers, R. L. 1974. Parent-Offspring Conflict. Am. Zool. 14:249–264.

Tucci, A., M. A. Flores-Vergara, and R. G. Franks. 2024. Machine Learning Inference of Gene Regulatory Networks in Developing Mimulus Seeds. Plants 13:3297.

Turelli, M., B. S. Cooper, K. M. Richardson, P. S. Ginsberg, B. Peckenpaugh, C. X. Antelope, K. J. Kim, M. R. May, A. Abrieux, D. A. Wilson, M. J. Bronski, B. R. Moore, J.-J. Gao, M. B. Eisen, J. C. Chiu, W. R. Conner, and A. A. Hoffmann. 2018. Rapid Global Spread of wRi-like Wolbachia across Multiple Drosophila. Curr. Biol. CB 28:963-971.e8.

Twyford, A. D., and J. Friedman. 2015. Adaptive divergence in the monkey flower Mimulus guttatus is maintained by a chromosomal inversion. Evol. Int. J. Org. Evol. 69:1476–1486.

Twyford, A. D., E. L. Y. Wong, and J. Friedman. 2020. Multi-level patterns of genetic structure and isolation by distance in the widespread plant Mimulus guttatus. Heredity 125:227–239.

Vickery, R. K. 1978. Case Studies in the Evolution of Species Complexes in Mimulus. Evol. Biol. 11.

Vickery, R. K. J. 1974. Crossing barriers in the yellow monkey flowers of the genus Mimulus (Scrophulariceae). Genet Lect.

Vrana, P. B. 2007. Genomic Imprinting as a Mechanism of Reproductive Isolation in Mammals. J. Mammal. 88:5–23.

Wang, H., L. Planche, V. Shchur, and R. Nielsen. 2024. Selfing Promotes Spread and Introgression of Segregation Distorters in Hermaphroditic Plants. Mol. Biol. Evol. 41:msae132.

Weng, Y.-M., D. H. Kavanaugh, and S. D. Schoville. 2021. Drainage basins serve as multiple glacial refugia for alpine habitats in the Sierra Nevada Mountains, California. Mol. Ecol. 30:826–843.

Werren, J. H. 2011. Selfish genetic elements, genetic conflict, and evolutionary innovation. Proc. Natl. Acad. Sci. U. S. A. 108:10863–10870.

Whitener, M. R., H. Mangelson, and A. L. Sweigart. 2024. Patterns of genomic variation reveal a single evolutionary origin of the wild allotetraploid Mimulus sookensis. Evolution 78:1464–1477.

Widen, S. A., I. C. Bes, A. Koreshova, P. Pliota, D. Krogull, and A. Burga. 2023. Virus-like transposons cross the species barrier and drive the evolution of genetic incompatibilities. Science 380:eade0705. American Association for the Advancement of Science.

Wiley, C., A. Qvarnström, G. Andersson, T. Borge, and G.-P. Sætre. 2009. Postzygotic isolation over multiple generations of hybrid descendents in a natural hybrid zone: how well do single-generation estimates reflect reproductive isolation? Evolution 63:1731–1739.

Wolff, P., H. Jiang, G. Wang, J. Santos-González, and C. Köhler. 2015. Paternally expressed imprinted genes establish postzygotic hybridization barriers in Arabidopsis thaliana. eLife 4:e10074. eLife Sciences Publications, Ltd.

Xiong, T., and J. Mallet. 2022. On the impermanence of species: The collapse of genetic incompatibilities in hybridizing populations. Evolution 76:2498–2512.

Zeh, D. W., and J. A. Zeh. 2000. Reproductive mode and speciation: the viviparity-driven conflict hypothesis. BioEssays 22:938–946.

Zuellig, M. P., and A. L. Sweigart. 2018. A two-locus hybrid incompatibility is widespread, polymorphic, and active in natural populations of *Mimulus* *. Evolution 72:2394–2405. Society for the Study of Evolution.