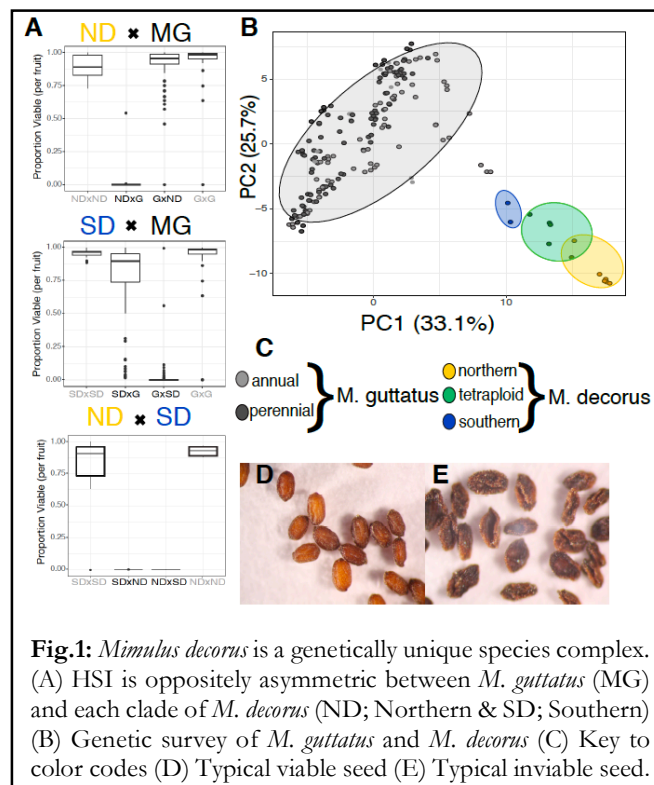


A major goal of evolutionary biology is to determine how species first form then persist through time. Examining the reproductive barriers that minimize gene flow and allow divergence to accumulate is essential to understand speciation. Yet, we know little of the genetic basis and evolutionary drivers of most barrier traits. In addition, there is growing evidence that even good species can experience gene flow in nature, and this introgression can have significant evolutionary consequences. Determining the types of barriers that are effective against introgression and the consequences of introgression are important, and relatively unexplored questions in speciation biology. **My research aims to understand the evolutionary drivers and genetic basis of reproductive barriers, test the importance of different barrier traits in halting gene flow, and assess the consequences of introgression in natural systems** by integrating quantitative and population genomics, development, ecology, field work, and experimental approaches.

### Current & Previous Work

#### What are the evolutionary causes of reproductive isolation?

Reproductive isolation mostly evolves as a byproduct of population divergence. This divergence can be caused by shifts in ecology, intra-genomic conflicts, or by genetic drift. While much theory has explored the evolutionary drivers of reproductive isolation, far fewer empirical tests exist. I have previously explored the genomic basis of reproductive isolation conferred by ecological divergence<sup>1,2</sup>, and provided one of the first empirical tests of the role of conflict in driving hybrid inviability<sup>4</sup>. This work leverages the exceptional diversity of a model system for ecology, evolution, and genetics; the *Mimulus guttatus* species complex.



**Fig.1:** *Mimulus decorus* is a genetically unique species complex. (A) HSI is oppositely asymmetric between *M. guttatus* (MG) and each clade of *M. decorus* (ND; Northern & SD; Southern) (B) Genetic survey of *M. guttatus* and *M. decorus* (C) Key to color codes (D) Typical viable seed (E) Typical inviable seed.

A major focus of my research is to understand how intra-genomic conflicts can result in hybrid sterility and inviability. A fundamental source of conflict in viviparous organisms stems from differences between maternal and paternal interests in resource allocation to offspring (i.e. parental conflict)<sup>3</sup>. Under parental conflict, variance in paternity drives the evolution of paternally derived, resource-acquiring alleles, and maternally derived alleles that distribute resources equally among offspring<sup>3</sup>. In hybrids, mismatches between these parent of origin effect alleles cause inappropriate development of essential nutritive tissues (e.g. placenta or endosperm), and subsequently embryo death. I previously uncovered a phenotypically cryptic species-*M. decorus*- that is recently diverged and reproductively isolated from *M. guttatus* primarily via Hybrid Seed Inviability (HSI)<sup>4</sup>. HSI has evolved repeatedly in this group, as distinct diploid lineages of *M. decorus* exhibit oppositely asymmetric HSI with *M. guttatus*<sup>4</sup> (Fig.1). I leveraged this diversity of reproductive isolation to show that patterns

of HSI conform to the predictions of parental conflict and that independent incidences of HSI exhibit parallel developmental defects<sup>4</sup>. Additionally, genetic mapping suggests that HSI is conferred by nuclear, parent-of-origin effect loci (i.e. loci that affect the probability of death if maternally *or* paternally derived), some of which have been implicated in other incidences of HSI. Together, these results suggest that HSI has rapidly and repeatedly evolved in this group, and may be driven by parental conflict. Additionally, it highlights the potential for developmental and genetic parallelism in putatively conflict-driven processes.

#### What are the dynamics of introgression in nature?

The diversity of life on earth is a product of not only the accumulation of reproductive isolation, but also the ability of species to persist. A major potential threat to species persistence is the exchange of genetic materials among close relatives (e.g. introgression)<sup>4</sup>. In my postdoc, I am using an experimental evolution approach to quantify the effects of reproductive isolation, divergence time between species, and

environment conditions on the outcomes of introgression in replicated synthetic hybrid swarms of four species pairs *Drosophila* grown in four environmental conditions. This project will result in sequencing of tens of thousands of hybrid genomes. Additionally, I am quantifying rates, patterns, and consequences of introgression in both natural *Drosophila* populations and natural contact zones of *Mimulus*, and assessing how different reproductive barriers (e.g. mate choice, hybrid inviability) influence the outcomes of introgression in nature. Together, these projects will provide a holistic examination of the factors that influence the dynamics of introgression and the rates and consequences of introgression in natural systems.

### Proposed Future Work

#### 1. What are the genes that cause reproductive isolation?

HSI is a common reproductive barrier, yet **the genes underlying HSI have yet to be discovered in any diploid system**. I have uncovered a group where HSI has rapidly and repeatedly evolved and incidences of HSI exhibit both developmental and genetic parallelism. I will: (a) combine comparative QTL mapping, traditional fine mapping, and admixture mapping (outlined below) to determine the genetic basis of multiple incidences of HSI between *M. guttatus* and *M. decorus*. This work can shed light on the degree of parallelism in HSI and thus the mutational target size for parental conflict. (b) By combining the results from (1a) in concert with expression work (outlined below), I will create a list of candidate genes, whose effects on HSI can be functionally validated; the tools for which have already been developed in *Mimulus*<sup>5</sup>. Genes that exhibit parent of origin specific expression in endosperm and co-localize with regions identified in (1a) are good candidate for functional work. This work will be the first to genetically characterize HSI in naturally co-occurring diploid populations.

#### 2. What are the molecular mechanisms underlying reproductive isolation?

One mechanism by which nuclear loci can have parent of origin effects is genomic imprinting; an epigenomic phenomenon wherein alleles are expressed based on their parent of origin<sup>1,6</sup>. Imprinted expression is a canonical feature of normal endosperm (and placenta) development<sup>6</sup>. Paired with the observation that many incidences of HSI exhibit parent of origin effects on growth and development, this has lead to the hypothesis that misregulation of imprinted genes underlies HSI. Yet, there is little known about the extent to which closely related species are divergent in imprinted expression, if/how natural selection has shaped this divergence, and the consequences of divergence for hybrids. a) I will use a combination of developmental genetics approaches (e.g. RNAseq, bisulfite sequencing) to assess the extent of divergence in imprinted expression in *M. guttatus* and each diploid clade of *M. decorus* and quantify the epigenomic mechanisms associated with divergence in imprinting. I can then leverage phylogenetically informed models of gene expression evolution to assess if imprinted expression evolves faster than neutral divergence, as would be expected under parental conflict. Additionally, because *M. guttatus* and *M. decorus* differ in their inferred histories of parental conflict<sup>4</sup>, I can assess if the number of imprinted genes or severity of imprinted expression is associated with inferred histories of parental conflict. This experiment will allow me to quantify the extent to which natural selection via parental conflict is responsible for the rapid divergence in genomic imprinting. b) To determine the role of imprinting in HSI, I will quantify misexpression of normally imprinted genes in all reciprocal F1s within and among *M. guttatus* and *M. decorus*. These data will allow me to categorize genes that are normally imprinted during seed development, show divergent expression between closely related species, and exhibit abnormal gene expression in hybrids. I can then leverage variation in the severity of HSI within and between species pairs (e.g. between species pairs that exhibit asymmetric versus symmetric HSI, or between viable and inviable hybrid seeds produced from a single species pair) to determine if the extent of misexpression is associated with the severity of HSI. Together, these experiments can provide a quantitative assessment of if/how natural selection shapes divergence in imprinted expression, and the consequences of this divergence for reproductive isolation.

#### 3. How effective are barrier traits in nature? What is their evolutionary significance?

Intrinsic barriers can play a crucial role in the speciation process both by impeding gene flow upon secondary contact, but also as an engine of diversity through processes such as reinforcement<sup>7</sup>. I will test the evolutionary significance of different barrier traits by assessing these processes using both broad scale comparative approaches, and in-depth examinations of specific barriers in natural contact zones. a) I will survey sister species pairs from across *Mimulus* for key barriers (e.g. ecogeographic isolation, floral and phenological divergence, pollen competition, hybrid seed inviability, hybrid sterility, hybrid necrosis), and use population genomics to detect and quantify rates of introgression. This project will not only test what

barriers are, on average, more effective at minimizing overall introgression, but can also be used to determine the rate of evolution of different types of barriers. I can then assess whether the rate of evolution of each barrier differs in sympatric vs allopatric sister species to estimate the frequency of reinforcement. b) I have previously discovered ~5 contact zones between *M. decorus* and *M. guttatus*. Contact zones are an exceptional resource for evolutionary biology, because they represent repeated evolutionary experiments which can be used to (i) assess the fate of reproductive barriers upon secondary contact (ii) leverage phenotype-blind approaches to elucidate incompatibilities via long-range patterns of linkage disequilibrium, and (iii) accelerate mapping of reproductive barriers (or other traits of interest) using admixture mapping. By pairing long-term temporal genomic transects of contact zones with greenhouse experiments and manipulative crosses, I can not only track the fate of HSI alleles, but also identify new reproductive barriers and track their fate in nature. These projects can help us to understand what types of barriers generally evolve first during speciation and what barriers are ultimately important in halting gene flow.

#### 4. What are the consequences of introgression in natural systems?

Even good species can experience some gene flow in nature. In my postdoc, I am using population genomic tools to understand the consequences of introgression in both natural and synthetic hybrid zones in *Drosophila* and *Mimulus*. In my future research group, I am keen to apply these methods to natural contact zones in *Mimulus* to understand the factors that influence the dynamics of introgression at a genome-wide scale and the effects of introgression at specific genomic regions. In particular, I aim to determine how life history impacts the dynamics of introgression between members of the *M. guttatus* species complex. This system exhibits tremendous variation in life history; from rapidly lived annuals to largely clonal perennials<sup>1,2</sup>. The rate of clonality differs both within and between species. While there are many benefits to clonal reproduction, clonality should also reduce effective population size, decrease efficacy of selection, and result in a potentially higher genetic load (e.g. more mildly deleterious alleles). Thus, loci that are relatively neutral in more clonal individuals may be deleterious when introgressed into less clonal individuals, while, introgression from less clonal individuals into more clonal individuals may actually eradicate mildly deleterious alleles and reduce genetic load. In this case, introgression from annuals into perennials, and from less clonal perennials into more clonal perennials might actually be favored. However, the importance of life history in the rates and consequences of introgression are relatively unexplored. Introgression may also play a more direct role in adaptive evolution. Perennial and annual species within the complex often co-occur and introgress<sup>8</sup>. Annual populations of *M. guttatus* exhibit exceptional genetic variation for flower size, which is maintained by spatially and temporally varying selection<sup>9</sup>, but the source of this variation is unknown. Many populations of annual *M. guttatus* co-occur with perennial *M. decorus* throughout their shared range. Introgression from large-flowered *M. decorus* into annual *M. guttatus* may provide a source of large flower alleles that are then maintained in annual *M. guttatus*. By combining quantitative and population genetic approaches, I will determine if flower-size alleles are more likely to be introgressed than expected by chance. This work will shed light on the dynamics of introgression in nature, with particular focus on how life history affects introgression across genomic-scales.

#### Long Term Vision

**My work strives to understand speciation and adaptation through an evolutionary genetics lens by exploring the evolutionary drivers and genetic basis of reproductive isolation, and the dynamics and consequences of introgression in nature.** The projects I have outlined above will provide substantial phenotypic and genomic resources for the *Mimulus* community. My research program lends well to collaboration with researchers from diverse backgrounds, including ecology, population genomics, development, and molecular biology. These integrative and collaborate projects have implications for evolution, food security, and medicine, strengthening potential funding from the USDA, DOE, NSF, and NIH. My goal in developing a research group is to foster a highly collaborative, interdisciplinary community of researchers, which I think will be highly fruitful given the outstanding group of geneticists, evolutionary biologists, and ecologists at Yale.

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