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Research

Genetic loci with parent-of-origin effects cause hybrid seed lethality in crosses between *Mimulus* species

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Summary

- In flowering plants, F_1 hybrid seed lethality is a common outcome of crosses between closely related diploid species, but the genetic basis of this early-acting and potentially widespread form of postzygotic reproductive isolation is largely unknown.
- We intercrossed two closely related species of monkeyflower, *Mimulus guttatus* and *Mimulus tilingii*, to characterize the mechanisms and strength of postzygotic reproductive isolation. Then, using a reciprocal backcross design, we performed high-resolution genetic mapping to determine the genetic architecture of hybrid seed lethality and directly test for loci with parent-of-origin effects.
- We found that F_1 hybrid seed lethality is an exceptionally strong isolating barrier between *Mimulus* species, with reciprocal crosses producing < 1% viable seeds. This form of postzygotic reproductive isolation appears to be highly polygenic, indicating that multiple incompatibility loci have accumulated rapidly between these closely related *Mimulus* species. It is also primarily caused by genetic loci with parent-of-origin effects, suggesting a possible role for imprinted genes in the evolution of *Mimulus* hybrid seed lethality.
- Our findings suggest that divergence in loci with parent-of-origin effects, which is probably driven by genomic coevolution within lineages, might be an important source of hybrid incompatibilities between flowering plant species.

Introduction

Understanding the genetics of interspecific incompatibility provides insight into both the origins of species barriers and the processes driving genomic divergence within species. In plants, postmating incompatibilities are often significant, and, in particular, many early plant geneticists noted difficulties in generating F_1 hybrids from experimental crosses between closely related species (Thompson, 1930; Stebbins, 1957; Valentine & Woodell, 1963; Vickery, 1978). Some of these crossing problems are probably caused by pollen–pistil interactions that prevent fertilization altogether, but for others, successful fertilization occurs only to end in a high rate of F_1 hybrid seed lethality. This failure of F_1 seeds is also a common outcome of interploidy crosses (known as triploid block; Ramsey & Schemske, 1998), and represents a form of postzygotic reproductive isolation that acts early in the life cycle. Relative to other forms of hybrid incompatibility, which may act only in one sex (e.g. hybrid sterility) or after plants have already reproduced (e.g. hybrid necrosis), F_1 hybrid seed lethality might be a particularly strong barrier to interspecific gene flow. Moreover, its prevalence in plant crosses from diverse taxa suggests that F_1 hybrid seed failure might represent a major class of reproductive isolation in plants – albeit one that has gone largely unmentioned in modern accounts of speciation (e.g. Coyne & Orr, 2004; but see Tiffin *et al.*, 2001; Turelli & Moyle, 2007).

Hybrid seed inviability has long been thought to result from developmental defects in the endosperm, an important nutritive tissue for the developing embryo (Brink & Cooper, 1947; Woodell & Valentine, 1961). In the seeds of flowering plants, a triploid endosperm is one of the two products of double fertilization. The male gametophyte (pollen tube) releases two sperm into the ovule: one fuses with the egg nucleus to produce the zygote, and the other fuses with the two nuclei of the central cell to form the endosperm. Classic work showed that deviations from the ratio of two maternal to one paternal genome (2m : 1p) disrupt endosperm development (Johnston *et al.*, 1980), explaining the failure of many interploidy crosses, which double the maternal or paternal contribution. A similar disturbance to endosperm 'balance' also seems to cause hybrid seed lethality in many diploid crosses between species (Valentine & Woodell, 1963; Johnston & Hanneman, 1982; Dilkes & Comai, 2004; Josefsson *et al.*, 2006; Ishikawa *et al.*, 2011).

The sensitivity of the endosperm to mismatches in parental genome dosage led to the hypothesis that genomic imprinting is the molecular cause of both interploidy and interspecific seed lethality (Haig & Westoby, 1991; Birchler, 1993; Gutierrez-Marcos *et al.*, 2003). The expression of an imprinted gene is

dependent on its parent-of-origin as a consequence of differential epigenetic modifications established during male and female gametogenesis (Köhler *et al.*, 2012). These epigenetic 'imprints' result in different expression levels of alleles inherited from the paternal vs maternal parent in post-fertilization tissues (in angiosperms, genomic imprinting occurs primarily in the endosperm and early embryo). If imprinted genes encode dosage-sensitive regulators, quantitative changes in parental contributions could cause a stoichiometric imbalance with downstream targets (Birchler *et al.*, 2001; Birchler & Veitia, 2012). More generally, Dilkes & Comai (2004) have argued that mismatches between *any* differentially expressed dosage-sensitive genes (including but not limited to imprinted genes) are expected to affect seed development in a parent-of-origin-dependent manner. For example, genes involved in female gametophyte development might be misregulated in hybrids, resulting in parent-of-origin seed phenotypes (Dilkes & Comai, 2004). As with genomic imprinting, dosage imbalance involving gametophyte-specific genes provides an explanation for the observation that reciprocal interploidy crosses often differ in seed phenotypes (e.g. Thompson, 1930; Brink & Cooper, 1947; Scott *et al.*, 1998; Sekine *et al.*, 2013); dosage changes in maternally vs paternally derived alleles have different consequences for endosperm and/or embryo development. In line with these expectations, several recent studies in *Arabidopsis thaliana* have shown that imprinted genes are misexpressed in seeds derived from interploidy crosses (Erilova *et al.*, 2009; Jullien & Berger, 2010; Wolff *et al.*, 2011; Kradolfer *et al.*, 2013; Wolff *et al.*, 2015).

In principle, hybrid seed lethality between diploid plant species might also be caused by genetic loci with parent-of-origin effects (Dilkes & Comai, 2004; Köhler *et al.*, 2010), but, apart from a few studies (Josefsson *et al.*, 2006; Burkart-Waco *et al.*, 2012; Rebernig *et al.*, 2015), this idea remains largely untested. Because F_1 hybrid seeds combine divergent gene sequences, in addition to potentially divergent patterns of gene expression, interactions among heterospecific alleles (i.e. Dobzhansky–Muller incompatibilities; Dobzhansky, 1937; Muller, 1942) at loci without parent-of-origin effects might also contribute to hybrid lethality. In *Drosophila* and fish, there are many cases of hybrid lethality as a result of incompatibilities between genes with biparental expression (i.e. genes for which alleles from the two parents are equally expressed; e.g. Wittbrodt *et al.*, 1989; Presgraves *et al.*, 2003; Brideau *et al.*, 2006; Tang & Presgraves, 2009). In contrast, parent-of-origin effects on hybrid growth are common in crosses between mammalian species (Wolf *et al.*, 2014). This fact is intriguing because mammals, like flowering plants, have evolved a nutritive structure (the placenta) that regulates the growth of the developing embryo in part through genomic imprinting (Piedrahita, 2011). Moreover, classic theory suggests that parental conflict over maternal investment might have driven the evolution of genomic imprinting in the endosperm of angiosperms and the placenta of mammals (Haig & Westoby, 1989; but see Wolf & Hager, 2006; Köhler *et al.*, 2012; Spencer & Clark, 2014 for alternative evolutionary scenarios). As a byproduct of genomic coevolution within species (i.e. to resolve the conflict), it is possible that both taxonomic groups are predisposed to evolve hybrid lethality between species. This idea is compelling, but, apart from one case in deer mice (Vrana *et al.*, 2000) and three in flowering plants (*Arabidopsis*: Josefsson *et al.*, 2006; Burkart-Waco *et al.*, 2012; *Capsella*: Rebernig *et al.*, 2015), evidence for a direct role of deregulated imprinted genes in hybrid inviability has been lacking. As a first step, additional studies are needed to determine if loci with parent-of-origin effects contribute disproportionately to hybrid seed lethality between species of flowering plants.

Here, we explore the mechanisms and genetics of reproductive isolation between two diploid species of *Mimulus*, *Mimulus guttatus* and *Mimulus tilingii*. In nature, these species are mostly allopatric, but they occasionally co-occur in high alpine areas. Because both species are primarily outcrossing with large, bee-pollinated flowers, sympatric populations might be expected to experience interspecific gene flow. However, early crossing studies reported that F_1 hybrids between *M. guttatus* and *M. tilingii* are often difficult to generate (Vickery, 1978), suggesting that there might be some degree of postmating, prezygotic isolation that prevents fertilization, postzygotic isolation, or both in the form of hybrid seed lethality. To investigate these possibilities, we began our study by intercrossing the two *Mimulus* species, characterizing both the mechanisms and strength of reproductive isolation. In reciprocal crosses, we found that there was a substantial reduction in hybrid seed number, suggesting that pollen–pistil incompatibilities might contribute to reproductive isolation. Even more striking, among the F_1 hybrid seeds that were produced, almost all of them (> 99%) were misshapen and inviable.

Despite the potential importance of hybrid seed lethality for plant speciation, a mechanistic understanding of this isolating barrier has been lacking in systems other than *A. thaliana* and its close relatives. This *Mimulus* species pair thus presents a rare opportunity to test the extent to which hybrid seed lethality between diploid species involves parent-of-origin effects. The finding that hybrid seed lethality is severe in *both* cross directions does not necessarily rule out reciprocal differences in its underlying genetic basis. That is, loci for *Mimulus* hybrid seed lethality might differ depending on their parent of origin. In this study, we used a powerful breeding design – backcrossing F_2 hybrids reciprocally to each parent – to assess both the maternal and paternal contributions to *Mimulus* hybrid seed inviability. At the outset, our genetic analyses revealed two correlates of genomic divergence, transmission ratio distortion (TRD) in F_2 hybrids and chromosomal differentiation, which both provide insights into *Mimulus* speciation. Additionally, by performing high-resolution genetic mapping of *Mimulus* hybrid seed lethality, we addressed two key evolutionary questions: is hybrid seed lethality between *M. guttatus* and *M. tilingii* attributable primarily to loci with parent-of-origin effects; and what is the genetic architecture of hybrid seed lethality? The first question is of fundamental importance for *Mimulus* speciation because genes with parent-of-origin effects (e.g. imprinted genes) might represent a special class of loci subject to genomic coevolution within species, rather than the independent fixation of alleles that only interact in hybrids. Answering the second question, too, is critical for determining whether reproductive isolation evolves by the fixation of alleles at

relatively few major loci or instead by the accumulation of multiple small incompatibilities over time.

Materials and Methods

Study system and plant material

The yellow monkeyflower *Mimulus guttatus* DC. is highly polymorphic with natural populations distributed across much of western North America. The species occupies diverse environments, ranging from sand dunes along the Pacific coast to high alpine habitats. *Mimulus tilingii* Regel, a mat-forming perennial, occurs throughout much of the same geographic area, but is largely restricted to high elevations (>2000 m). Both species are self-compatible, but predominantly outcrossing with large, bee-pollinated flowers. *Mimulus guttatus* and *M. tilingii* are closely related (Beardsley *et al.*, 2003) and belong to the same *Simiolus* section (Phrymaceae) of primarily yellow-flowered taxa. Still, the two species have been classified as members of different species complexes (Vickery, 1978) and variation at 16 nuclear loci suggests that they form distinct genetic groups (Oneal *et al.*, 2014). In areas of sympatry, there have been a few reports of putative hybridization (Lindsay & Vickery, 1967; C. Wu pers. comm.), but classic crossing experiments have shown that F_1 hybrids are difficult to generate (Vickery, 1978), suggesting that reproductive isolation between *M. guttatus* and *M. tilingii* is strong.

In this study, we used one inbred line for each of the two focal species. The *M. guttatus* parental line (DUN10) is derived from a population located in the Oregon Dunes National Recreation Area along the Pacific coast. The *M. tilingii* parental line (LVR) originated from a high-alpine population in California's Yosemite Valley (at 2751 m). Both of these inbred lines were formed by more than six generations of self-fertilization with single-seed descent.

To determine the extent of genetic differentiation between *M. tilingii* and the well-studied *M. guttatus* complex (Brandvain *et al.*, 2014), we measured genome-wide divergence (i.e. average pairwise nucleotide differences) among *M. tilingii*, *M. guttatus*, and *Mimulus nasutus* Greene (Supporting Information Methods S1; Notes S1). For *M. tilingii*, we generated new sequence data for the parental line LVR (see Materials and Methods below). For *M. guttatus* and *M. nasutus*, we used six previously published lines (Table S1), including the parental *M. guttatus* line DUN10.

Measurement of reproductive isolation and genetic crosses

To characterize postmating reproductive isolation between *M. guttatus* and *M. tilingii*, we performed crosses within and between the two parental lines ($n = 20$ individuals each for DUN10 × DUN10, LVR × LVR, DUN10 × LVR, and LVR × DUN10). For each of these crosses, we dissected one ripened fruit and measured total seed set. For each fruit, we also assessed seed viability by determining the proportion of viable seeds (number of viable seeds/total number of seeds). Seed viability was straightforward to score by eye: viable seeds were plump and tan in color, whereas inviable seeds were collapsed, darker in color, and often adhering to each other (Fig. S1). The majority of what we called inviable seeds had enlarged to > 50% the size of viable ones, suggesting that, in most cases, successful fertilization had occurred, but that seed development did not proceed properly (Searcy & Macnair, 1990). This phenotype of mostly enlarged but collapsed seeds is consistent with failure of the endosperm (Cooper & Brink, 1945) and might be caused by its precocious or delayed cellularization (e.g. Scott *et al.*, 1998). Adding support to this idea, a similar hybrid inviability phenotype (i.e. shriveled, collapsed hybrid seeds) in a different *Mimulus* cross (*M. guttatus* × *M. nudatus*) was recently shown to be the result of disrupted endosperm development (Oneal *et al.*, 2015). To determine if our measure of seed viability is correlated with germination rate, we planted the seeds from a single fruit for crosses within parental lines ($n = 2$ each) and for a subset of our F_2 × parental crosses ($n = 20$; see Fig. 1). Because we found that our visual assessment of seed viability correlates strongly with germination rate (Spearman's correlation, $rho = 0.92$; $P < 0.0001$), all further measurements of seed viability were performed by eye.

To study the genetics of reproductive isolation between *M. guttatus* and *M. tilingii*, we intercrossed DUN10 (maternal parent) and LVR (paternal parent) to form F_1 hybrids. We then backcrossed F_1 hybrids ($n = 20$) reciprocally to each parental line and assessed seed viability. To generate a recombinant population for genetic mapping, we self-fertilized a single F_1 to form an F_2 generation ($n = 240$). For each of these F_2 hybrids, we performed reciprocal backcrosses to each parental line; seed viability was measured from a single fruit for each of the four cross treatments (Fig. 1). Note that, because the DUN10 line was used as the original maternal parent, all F_1 and F_2 hybrids carried an *M. guttatus* cytoplasm. Our aim with this crossing design was to determine to what extent loci with parent-of-origin effects contribute to *Mimulus* hybrid seed lethality; if seed phenotypes or the underlying genetic loci differ between reciprocal backcrosses to *M. guttatus* (which carry the same cytoplasm), it would suggest an influence of loci with parent-of-origin effects. If instead progeny from the reciprocal backcrosses do not differ in seed lethality, 'regular' hybrid incompatibilities might be involved.

All plants were grown using similar conditions at the University of Georgia. Seeds were planted into 2.5-inch pots containing Fafard 3b potting mix (Sun Gro Horticulture, Agawam, MA, USA), chilled for 7 d at 4°C to promote germination, and then placed in a Conviron (Winnipeg, Canada) growth chamber with lights set to 16-h days. Plants were bottom-watered daily and temperatures were maintained at 22°C during the day and 16°C at night.

DNA extraction, library preparation, and sequencing

For each of the 240 F_2 individuals, we collected bud tissue into 96-well plates, immediately placed samples on dry ice, and stored them at −80°C. We isolated genomic DNA using a standard CTAB/chloroform extraction protocol as described in Holeski *et al.* (2014). Following extraction, we quantified DNA using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen) and diluted each sample to 5 ng μl^{-1}.

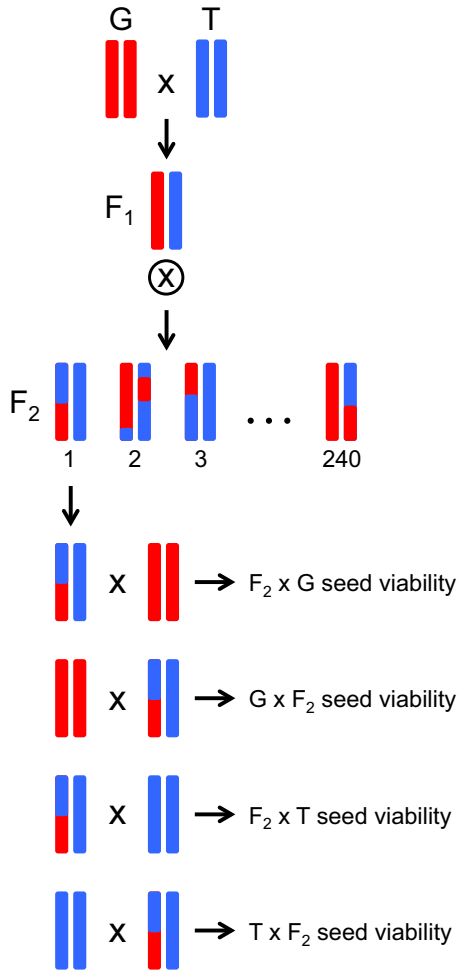

Fig. 1 Crossing design to determine the genetic basis of seed lethality in crosses between *Mimulus guttatus* and *Mimulus tilingii*. We formed F_1 hybrids by intercrossing *M. guttatus* (G; red) and *M. tilingii* (T; blue), and self-fertilized a single F_1 to generate the F_2 generation. We then reciprocally crossed F_2 hybrids to each parental line and assessed seed viability. The diagram shows a single pair of chromosomes for each individual with the maternal parent listed first.

We generated a genomic library for genotyping the 240 F_2 hybrids by using the multiplexed shotgun genotyping (MSG) method of Andolfatto *et al.* (2011). Briefly, we digested 50 ng of genomic DNA with *Mse*I, ligated 48 unique barcoded adapters to each sample, and pooled samples to give five pools of 48 samples each. For each of these five pools, we removed ligated linker dimers with AMPure beads (Beckman Coulter, Indianapolis, IN, USA) and size-selected fragments of 375–425 bp from cleaned products on an agarose gel. Flow-cell sequences were attached to ligation products using PCR. Additional details on MSG library preparation are provided in Methods S2.

We sent our library of 240 F_2 individuals to the Genome Sequencing Facility at Duke University for sequencing. We also included a sample of genomic DNA from the LVR *M. tilingii* line (the library was prepared by the Duke Genome Sequencing Facility). Because LVR is a highly inbred line, this genomic DNA should be nearly identical to that of the maternal line used to generate the F_2 mapping population. Sequencing was performed across two lanes on an Illumina Hiseq 2500 (Illumina, San Diego, CA, USA) for paired-end, 150-bp reads. The raw short read data generated from this study have been submitted to the NCBI Sequence Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra) under accession number SRP068507.

Determining F_2 genotypes

We used Multiplexed Shotgun Genotyping v.0.4.3 (Andolfatto *et al.*, 2011) to genotype 240 F_2 hybrids between *M. guttatus* (DUN10) and *M. tilingii* (LVR). This method uses a hidden Markov model (HMM) to estimate ancestry probabilities for all markers in each individual of a mapping population. We generated 'pseudo-reference' genomes for both parental lines (required by the MSG HMM algorithm) to which we aligned sequence reads from the 240 F_2 hybrids. Using the MSG pipeline, we obtained ancestry probabilities for a total of 151 669 single nucleotide polymorphisms (SNPs), which we then converted to hard genotype calls. We used a cut-off ancestry probability of 95% for genotype calling. The number of genotypes removed per marker by this processing step was rather small: 98% of the 151 669 SNP markers retained > 90% of their genotype calls. We then filtered markers with identical genotypes across all F_2 hybrids, resulting in a final set of 2960 markers to be used for linkage map construction. See Methods S3 for additional details on genotyping.

Linkage mapping, transmission ratio distortion, and quantitative trait loci mapping

We performed linkage mapping in JoinMap 4.0 (Van Ooijen, 2011) using a logarithm of odds (LOD) threshold of 10.0, the Haldane mapping function, and the default maximum likelihood settings. In some cases, marker positions disagreed with their predicted locations from the *M. guttatus* v2.0 reference genome (often in regions known to be misassembled). For each linkage group, we assessed map quality using the nearest neighbor stress parameter in JoinMap, rlod score output from the MSG pipeline, and visual inspection of genotypes to minimize double recombinants. Note that many of the regions that conflict between our genetic map and the genome assembly were also found in Holeski *et al.* (2014). Among the 240 F_2 hybrids, six individuals were discovered to be pure *M. guttatus* (all markers were homozygous for DUN10 alleles); these six individuals were removed from all subsequent analyses. We also used JoinMap to test markers for significant non-Mendelian genotype frequencies. To identify putative inversions between *M. guttatus* and *M. tilingii*, we compared genetic distances between the markers in our linkage map to physical distances from the *M. guttatus* v2.0 assembly (Methods S4).

We performed quantitative trait locus (QTL) mapping using MapQTL®6 (Van Ooijen & Kyazma, 2009). As a first step, we performed interval mapping (IM) using a 1-cM step size. The significance of QTLs detected by IM was evaluated at the 5% significance level by permutation tests ($n = 1000$ permutations) at both

the chromosome level and a genome-wide level. Markers surrounding the QTL (i.e. with LOD scores exceeding the empirical threshold) were selected as cofactors for restricted multiple QTL mapping (MQM; analogous to composite interval mapping). To narrow the set of cofactors, we used the automatic cofactor selection tool in MapQTL with a threshold of $P = 0.005$. Automatic cofactor and restricted MQM analyses were repeated until a stable set of significant cofactors remained (note that restricted MQM excludes linked cofactors). At the end of this process, selected cofactors were used for a final round of restricted MQM to estimate for each QTL the maximum LOD and additive genotypic effect (a). As before, QTLs were considered significant if LOD peaks reached the genome-wide and/or chromosome significance threshold of 5% ($n = 1000$ permutations). Finally, we calculated 1.5-LOD intervals for QTLs found through MQM.

Results

Nucleotide divergence among *Mimulus* species

Several recent studies have characterized population genomic variation within and between closely related species of the *M. guttatus* complex (Brandvain *et al.*, 2014; Puzey & Vallejo-Marín, 2014; Twyford & Friedman, 2015), but samples from *M. tilingii*, which classic crossing studies suggest is more distantly related (Vickery, 1978), were not included in these analyses. As a first step toward understanding the extent of genetic differentiation between these species, we examined genome-wide nucleotide variation within and between *M. guttatus*, *M. nasutus*, and *M. tilingii* (Table S2). Consistent with classic taxonomic groupings (Vickery, 1978) and previous phylogenetic work (Beardsley *et al.*, 2003), genome-wide divergence between *M. guttatus* and *M. tilingii* (d (divergence) = 6.94%; SE = 0.28%) was substantially higher than divergence between *M. guttatus* and *M. nasutus* ($d = 4.38\%$, SE = 0.09%). The latter pair is largely interfertile with ongoing introgression (Brandvain *et al.*, 2014), and species divergence was comparable to levels of diversity within *M. guttatus* (π (nucleotide diversity) = 4.02%; SE = 0.19%). Higher genomic divergence between *M. guttatus* and *M. tilingii* suggests an earlier split for this pair and, potentially, stronger interspecific isolating barriers.

Pattern of hybrid seed production and viability in crosses between *M. guttatus* and *M. tilingii*

To examine the strength of postmating reproductive isolation between *M. guttatus* and *M. tilingii*, we compared seed sets from reciprocal interspecific crosses to those from crosses within each parental line (Fig. S2). The DUN10 line of *M. guttatus* is a large-flowered ecotype and produces significantly more seeds per fruit than the smaller flowered LVR line of *M. tilingii* (*M. guttatus* × *M. guttatus* (G × G): mean = 313; SE = 16.6; $n = 20$; *M. tilingii* × *M. tilingii* (T × T): mean = 188; SE = 16.6; $n = 20$; Tukey–Kramer honest significant difference test (HSD): $P < 0.0001$). For both of the interspecifc crosses, seed sets were significantly lower than parental seed sets: *M. guttatus* × *M. tilingii* crosses produced 53% as many seeds as crosses within *M. guttatus* (G × T: mean = 167; SE = 16.6; $n = 20$; Tukey–Kramer HSD: $P < 0.0001$), and *M. tilingii* × *M. guttatus* crosses produced 60% as many seeds as crosses within *M. tilingii* (T × G: mean = 113; SE = 16.2; $n = 20$; Tukey–Kramer HSD: $P = 0.029$). Overall, there was a considerable reduction in interspecific seed set, suggesting that reproductive isolating barriers (e.g. pollen–pistil incompatibilities or very early hybrid seed abortion) might partially interfere with the production or development of F_1 hybrid seeds.

An even more dramatic isolating barrier was observed when we compared the proportion of viable seeds produced within and between species (Fig. 2). Although seed viability differed significantly among parental lines (*M. guttatus*: mean = 0.95; SE = 0.02; $n = 20$; *M. tilingii*: mean = 0.56; SE = 0.02; $n = 20$), both had much higher proportions than either interspecific cross. Indeed, F_1 hybrid seed viability – in both directions of the cross – was < 2% of the mid-parent value and not significantly different from zero (G × T: mean = 0.004; SE = 0.02; $n = 20$; T × G: mean = 0.01; SE = 0.02; $n = 20$). This F_1 seed lethality represents an extremely strong barrier to interspecific reproduction, allowing < 1% of F_1 hybrid seeds to survive.

Moreover, this strong F_1 hybrid lethality appears to be driven, at least in part, by loci with parent-of-origin effects: in backcrosses to either of the recurrent parents, hybrid lethality was much more severe when the F_1 acted as the paternal parent. For the backcross to *M. guttatus*, this effect cannot be explained by cytonuclear interactions because the reciprocal backcross progeny inherit the same DUN10 cytoplasm. By contrast, the progeny of reciprocal backcrosses to *M. tilingii* do carry different cytoplasms; this fact, along with the possible maternal effects from *M. tilingii*, might explain the particularly low levels of seed viability observed when *M. tilingii* acted as the seed parent (in Fig. 2, compare seed viability for T × F_1 vs F_1 × T). For the other backcross classes, seed viability was generally additive, with levels of seed failure among the progeny intermediate to those from parental and interspecific crosses (e.g. in Fig. 2, compare seed viability for F_1 × G to that for G × G and T × G).

Transmission ratio distortion and evidence for chromosomal inversions in the F_2 hybrid mapping population

Our genetic map is based on 2960 markers and has a total length of 1318 cM with an average spacing of 0.4 cM. Two-thirds (1987) of the markers genotyped in our F_2 mapping population deviated from the expected 1 : 2 : 1 genotype ratios at $\alpha = 0.05$, and nearly one-third (940) showed significant TRD at a higher threshold ($\alpha = 0.001$). The bias we observed was highly directional. Of the 940 markers distorted at $\alpha = 0.001$, 692 had an excess of *M. guttatus* (GG) homozygous genotypes and a deficit of *M. tilingii* (TT) genotypes, whereas only 70 markers showed the opposite pattern (an excess of TT and a deficit of GG). The vast majority of these markers (749 of 762) also showed a significant bias in allele frequency from the expected 1 : 1 ratio ($\alpha = 0.05$). The remaining markers with significant genotypic distortion (178) showed an excess of heterozygotes, whereas no markers showed a deficit of heterozygous genotypes.

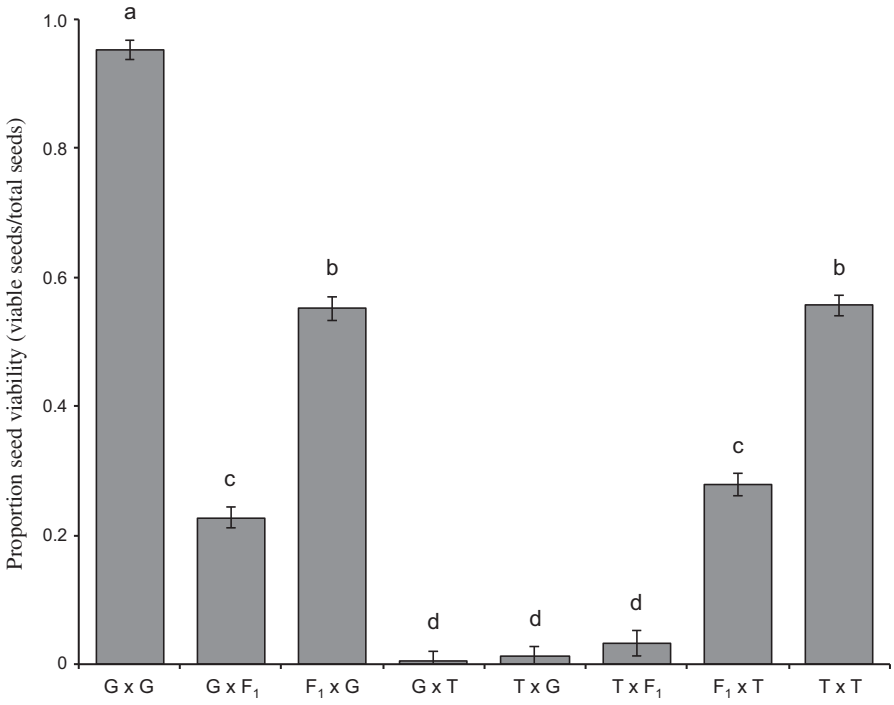

Fig. 2 Mean seed viability varies among experimental crosses. Seed viability is highest from crosses within parental lines (*Mimulus guttatus*: G × G; *Mimulus tilingii*: T × T) and lowest in reciprocal interspecific crosses (G × T and T × G). Seed viability is generally intermediate in the four classes of backcross progeny (G × F_1, F_1 × G, T × F_1, and F_1 × T). For all crosses, the maternal parent is listed first. Bars indicate ± SE and lower case letters above show significant differences by Tukey's honest significant difference (HSD) test.

TRD was highly variable across the genome and affected 12 of the 14 linkage groups (Figs 3, S3). By examining the genome-wide distribution of transmission bias, we identified 16 regions that contained genetically linked clusters of distorted markers. Ten of these regions showed an excess of *M. guttatus* genotypes, three showed an excess of *M. tilingii* genotypes, and three had an excess of heterozygotes (red, blue, and purple horizontal bars, respectively, in Figs 3, S3). Two linkage groups (LG2 and LG6) had highly distorted markers across their entire lengths – both were overrepresented for *M. guttatus*.

Across our genetic map, we observed three regions where a large number of physically dispersed markers mapped to the same location, suggesting the presence of inversions between *M. guttatus* and *M. tilingii*. One of these regions extends from *c.* 0.9 to 7.6 Mb on chromosome 8 and overlaps with a previously discovered inversion known as *DIVERGENCE1* (*DIV1*) that differentiates *M. guttatus* annual and perennial ecotypes (Lowry & Willis, 2010; Oneal *et al.*, 2014; Twyford & Friedman, 2015). Because the DUN10 *M. guttatus* parent is known to carry the 'perennial' *DIV1* arrangement (Lowry & Willis, 2010), our results strongly suggest that *M. tilingii* is collinear with annual forms of *M. guttatus*. The two other putative inversions are from *c.* 13.4 to 18.8 Mb on chromosome 5 and *c.* 15.9 to 20.5 Mb on chromosome 13. This region on chromosome 5 overlaps with a putative inversion segregating within *M. guttatus* (Holeski *et al.*, 2014).

QTL mapping of hybrid seed lethality and characterization of parent-of-origin effects

The distribution of hybrid seed viability varied substantially among the four different F_2 cross types (Fig. 4). Intriguingly, the distinct patterns of phenotypic variation in reciprocal crosses (F_2 × G vs G × F_2; F_2 × T vs T × F_2) suggested parent-of-origin effects on hybrid seed viability (but note that in cross types involving *M. tilingii* we cannot rule out an effect of cytoplasm). In three of the four cross types, seed viability appeared generally consistent with additivity, with the means for F_1 and F_2 hybrids similar to the midparent values (see the first three panels of Fig. 4). By contrast, seed viability from the *M. tilingii* × F_2 crosses was skewed strongly to the left, potentially suggesting a role for cytonuclear interactions and/or maternal effects.

Our QTL analyses showed a polygenic basis for hybrid seed lethality in crosses between *M. guttatus* and *M. tilingii*. For three of the four cross types, we detected multiple QTLs (a total of five were significant at the genome level and another 13 at the chromosomal level; Fig. 5; Table 1). In the *M. tilingii* × F_2 cross, we found only a single QTL, but our power of detection was probably limited by a smaller sample size (only 158 F_2 hybrids were phenotyped for this cross type) and nonnormal distribution of seed viability (Fig. 4; Beavis, 1998). For 17 of the 18 QTLs, additive effects were in the expected directions based on parental values. Individual QTLs often had strong effects on seed lethality: reductions in backcross seed viability for the 'lethal' vs alternative homozygote ranged from 20% to 61% (Table 1). On average, the seed viability of F_2 hybrids carrying a lethal genotype at one of the QTLs was reduced by 39%.

In crosses to both species, we discovered distinct sets of QTLs for hybrid seed lethality in reciprocal F_2 crosses, implying that the allelic effects of these loci differ depending on whether they are inherited from the maternal or paternal parent. Indeed, there was little overlap between QTLs mapped in F_2 × *M. guttatus* crosses and those mapped in *M. guttatus* × F_2 crosses (orange vs red bars in Fig. 5). The same is true for QTLs mapped in the two cross types involving *M. tilingii* (blue vs green bars in Fig. 5; note

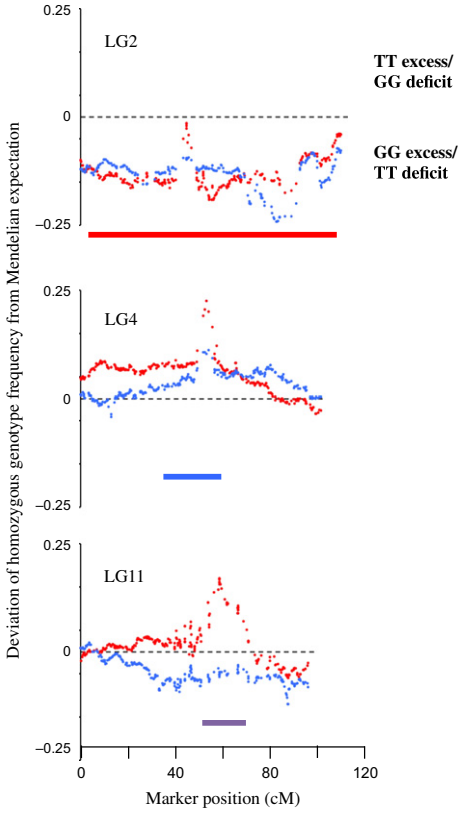

Fig. 3 Transmission ratio distortion across three linkage groups of the *Mimulus guttatus* × *Mimulus tilingii* genetic linkage map. For each of the three linkage groups shown, homozygous parental genotypes at marker loci are shown in red (*M. guttatus*; GG) and blue (*M. tilingii*; TT). For each marker, the vertical positions of the red and blue dots show the deviations of genotype frequencies from the Mendelian expectation of 0.25. Note that the *M. tilingii* homozygote (TT) deviations are plotted directly (deviation = (frequency of TT) – 0.25) and the *M. guttatus* (GG) deviations are plotted as negative (deviation = – ((frequency of GG) – 0.25)). As a result, the values above the zero line show excesses of TT homozygotes or deficits of GG homozygotes and values below the zero line show excesses of GG homozygotes or deficits of TT homozygotes. Horizontal bars beneath linkage groups indicate regions with excess GG (red), TT (blue), or heterozygotes (purple).

that, in this comparison, we cannot rule out an effect of cytoplasm, which differed between reciprocal F_2 backcrosses to *M. tilingii*). Additionally, two genomic regions contain overlapping QTLs that showed opposite allelic effects – in the predicted directions – when crossed to different species. The QTLs in one of these regions (on chromosome 4) affected hybrid seed lethality when they segregated in the paternal parent. The QTLs in the other region (on chromosome 14 at *c.* 72–78 cM) affected seed lethality through the maternal parent. Taken together, these results provide strong evidence that QTLs with parent-of-origin effects play a major role in *M. guttatus*–*M. tilingii* hybrid seed lethality.

One exception to this general pattern is the QTL on chromosome 2. At this locus, the *M. guttatus* allele increased seed fertility in three of the four cross types irrespective of direction or species. This result suggests that the chromosome 2 QTL is part of a hybrid incompatibility or simply reflects the segregation of deleterious alleles from the *M. tilingii* parent (in Fig. 2, note the lower seed viability for this inbred line).

Discussion

In this study, we have shown that hybrid seed lethality is a highly effective isolating barrier between *M. guttatus* and *M. tilingii*, with F_1 seed viability being < 1%. Additionally, we used a high-resolution mapping experiment to characterize the genetic basis of this widespread and exceptionally strong form of postzygotic reproductive isolation. Along with identifying hybrid lethality QTLs, our genetic analyses revealed both TRD in F_2 hybrids and chromosomal differentiation resulting in suppression of hybrid recombination. These phenomena are common in other plant mapping populations, and may provide insights into functional and genomic divergence between species. Furthermore, our reciprocal cross design allowed for direct tests of parent-of-origin effects on hybrid seed lethality at the QTL level. Strikingly, most of the QTLs we identified contributed to hybrid seed lethality when inherited from only one of the parents (maternal or paternal). This finding is consistent with recent work in *A. thaliana* revealing that imprinting plays a central role in triploid block (Kradolfer *et al.*, 2013; Wolff *et al.*, 2015), but our study is the first to identify both maternal- and paternal-effect loci for hybrid seed lethality in crosses between diploid species. Further work will be needed to identify the causal genes, but the results presented here suggest that divergence in genes with parent-of-origin effects can generate strong postzygotic isolation between plant species in the early stages of divergence.

Loci with parent-of-origin effects cause reproductive isolation in *Mimulus*

At first glance, the finding that severe F_1 hybrid seed lethality occurs in both reciprocal crosses between *M. guttatus* and *M. tilingii* seems contrary to the idea that genes with parent-of-origin effects are involved. Unlike many interploidy and interspecies crosses, which often show pronounced reciprocal differences in seed viability (Thompson, 1930; Haig & Westoby, 1991), we detected no parent-of-origin effects on viability in these first-generation *Mimulus* hybrid seeds. So, if imprinted or differentially expressed genes *do* cause F_1 hybrid seed lethality, there must be an independent genetic basis for the phenotype in each cross direction, implying that genetic changes have evolved in both *Mimulus* lineages. Indeed, this is exactly what our mapping of distinct maternally and paternally contributed QTLs reveals: many loci with parent-of-origin effects contribute to *Mimulus* hybrid seed lethality. A more detailed phenotypic characterization of *Mimulus* F_1 hybrid seed lethality might also reveal reciprocal differences at the level of endosperm growth and/or development that are associated with these distinct sets of genetic loci. Consistent with this possibility, seed size and morphology appear somewhat distinct depending on the cross direction (Fig. S1), but these differences have not yet been quantified. Interestingly, a recent study of hybrid seed lethality between

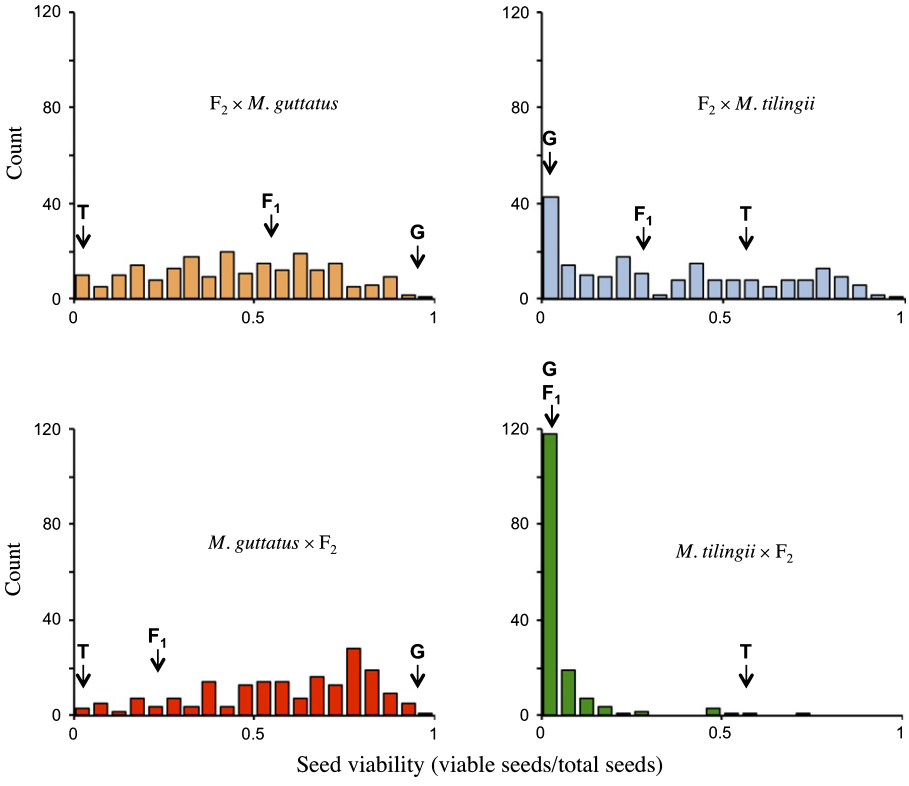

Fig. 4 Frequency distributions of F_2 hybrid seed viability phenotypes for the four experimental crosses: F_2 × *Mimulus guttatus* (average = 0.45; *n* = 215), *M. guttatus* × F_2 (average = 0.58; *n* = 190), F_2 × *Mimulus tilingii* (average = 0.36; *n* = 207), and *M. tilingii* × F_2 (average = 0.05; *n* = 158). Approximate values for parental (*M. guttatus*: G; *M. tilingii*: T) and F_1 means are indicated with arrows in each histogram.

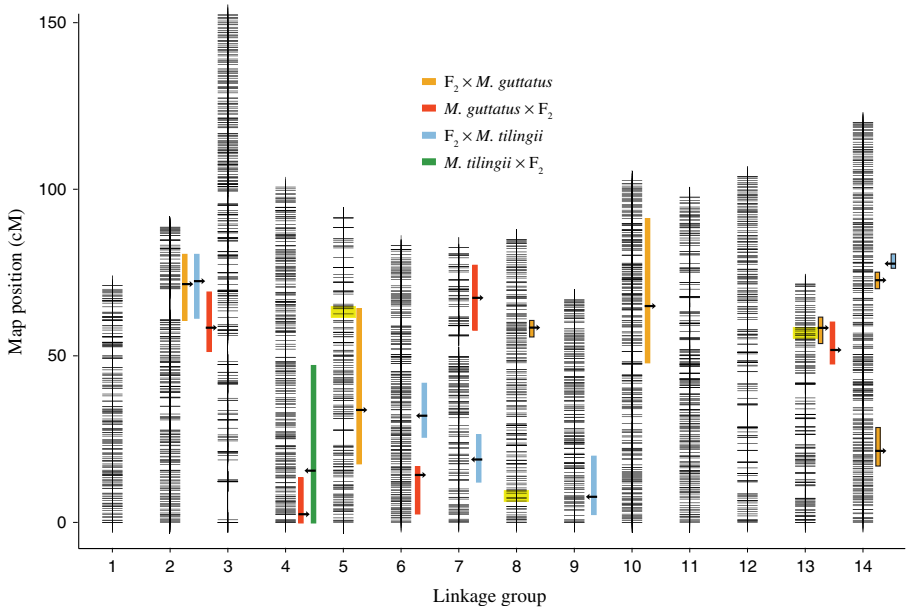

Fig. 5 *Mimulus guttatus* × *Mimulus tilingii* genetic map and seed viability quantitative trait loci (QTLs). Each of the 14 linkage groups corresponds to a chromosome from the *M. guttatus* v2.0 sequence assembly (http://www.phytozome.org), which represents *c.* 294 Mb of the genome (total haploid genome size is *c.* 450 Mb; http://www.mimulusevolution.org). Each marker is shown as a horizontal line and the three hypothesized inversions are indicated by yellow shading. Arrows show the QTL logarithm of odds (LOD) score peak locations and directions of additive effects (right-pointing arrows indicate that *M. guttatus* alleles increase the trait value; left-pointing arrows indicate that *M. tilingii* alleles increase the trait value). QTL bars show the 1.5 LOD drop confidence intervals and those outlined in black are significant at the genome-wide level (all others are significant at the chromosome level).

M. guttatus and another closely related diploid species, *M. nudatus*, found endosperm defects in both directions of the cross (Oneal *et al.*, 2015).

We found a polygenic basis for hybrid seed lethality in crosses between *M. guttatus* and *M. tilingii*. Across the four F_2-backcross treatments, we detected 18 QTLs (five significant at the genome-wide level), although several of these QTLs seem not to be independent. On chromosome 14, for example, two maternally contributed QTLs mapped to overlapping regions and had opposite allelic effects in backcrosses to *M. guttatus* and *M. tilingii*. The simplest explanation for this pattern is that one QTL causes both effects: F_2 hybrids carrying *M. guttatus* alleles at the causal locus promote seed compatibility in crosses to *M. guttatus*, whereas *M. tilingii* alleles improve seed viability in crosses to *M. tilingii*. Similarly, on chromosome 4, overlapping paternal QTLs had opposite phenotypic effects in the two cross treatments

Table 1 Summary of seed lethality quantitative trait locus (QTL) peak locations, statistical significance (logarithm of odds (LOD) score), additive effect estimates (*a*), and viability effects

Cross	Linkage group	Position	LOD	1.5-LOD drop	*a*[a]	Reduction in viability[b]
F_2 × *Mimulus guttatus*	2	70.53	3.62	60.10–79.10	0.078	0.42
	5	33.88	2.74	17.68–64.38	0.066	0.35
	8	58.39	5.00**	55.26–60.08	0.080	0.42
	10	64.05	3.51	47.69–91.19	0.069	0.37
	13	58.35	5.59**	53.55–61.52	0.093	0.50
	14	21.23	5.14**	16.70–28.35	0.131	0.61
	14	72.46	6.74**	70.17–75.10	0.120	0.49
M. guttatus × F_2	2	57.95	3.06	50.93–68.89	0.063	0.20
	4	2.36	2.95	0–13.50	0.067	0.21
	6	13.41	2.63	1.53–15.73	0.082	0.25
	7	66.99	3.14	57.44–76.89	0.086	0.26
	13	51.32	3.31	47.09–59.64	0.089	0.27
F_2 × *M. tilingii*	2	72.73	2.93	60.89–80.00	0.092	0.41
	6	31.94	3.91	25.34–41.34	−0.094	0.41
	7	20.17	3.09	12.91–27.04	−0.073	0.33
	9	7.73	3.17	2.02–19.53	−0.156	0.45
	14	78.10	7.57**	76.81–81.19	−0.140	0.55
M. tilingii × F_2	4	18.59	2.92	0–47.51	−0.037	0.53

**QTL is significant at a genome-wide threshold of 5% ($n = 1000$). All other QTLs are significant at the chromosomal level with a threshold of 5% ($n = 1000$).

[a]Positive values indicate that the *M. guttatus* allele increases seed viability; negative values indicate that the *M. tilingii* allele increases seed viability.

[b]Relative seed viability of the alternative homozygotes. The value is 1 minus the mean seed viability of the 'incompatible' homozygote relative to the 'compatible' homozygote (analogous to a selection coefficient).

(in the predicted directions), suggesting a common genetic basis. (Note that, although cytonuclear interactions cannot contribute to hybrid seed lethality in the backcrosses to *M. guttatus*, they might be involved in the backcrosses to *M. tilingii* (see the Materials and Methods section). However, a common genetic cause for the QTLs on chromosome 4 and on chromosome 14 would rule out an effect of cytonuclear interactions for these two *M. tillingi*-backcross QTLs.) For two additional regions – on chromosomes 2 and 13 – we detected overlapping QTLs from reciprocal crosses, implying that allelic effects at the underlying loci do not depend on parent-of-origin. For all remaining, non-overlapping QTLs, dominance relations and/or genetic background effects might make detection more likely in one backcross than in the other. Fortunately, our four-way crossing design maximizes the chances of mapping such QTLs despite these complicating effects. In total, we found eight QTLs with seed lethality effects only through the maternal parent, three QTLs with effects only through the paternal parent, and two QTLs with effects through both parents. In general, alleles at these loci appear to be additive, which is not surprising given that our crossing scheme is designed to measure the effects of F_2 gametes on backcross seed viability (it is possible that dominance variation plays a role at maternally expressed loci, which contribute two alleles to the triploid endosperm, but it cannot at paternally expressed loci because they are effectively haploid).

Similar to these results in *Mimulus*, postzygotic barriers affecting hybrid seed survival between *Arabidopsis* (Burkart-Waco *et al.*, 2012) and *Capsella* species (Rebernig *et al.*, 2015) are also determined by many genetic loci. In *Arabidopsis*, epistasis among the causal loci indicates that hybrid seed incompatibility is controlled by a complex genetic network (Burkart-Waco *et al.*, 2012). Thus, in three diverse systems, strong F_1 postzygotic isolation seems to have evolved between closely related species as a result of the accumulation of multiple incompatibilities that combine to cause severe defects in hybrid seed development. This scenario contrasts sharply with the relatively simple genetic basis found for hybrid sterility and other forms of hybrid inviability in many studies of plant species (e.g. Fishman & Willis, 2006; Sweigart *et al.*, 2006; Bomblies *et al.*, 2007; Yang *et al.*, 2012; Sicard *et al.*, 2015).

As a first step toward identifying candidate genes for parent-of-origin effects in *Mimulus* hybrid seed lethality, we performed a blast search of the *M. guttatus* reference genome using genes previously identified as imprinted in the *A. thaliana* endosperm (Hsieh *et al.*, 2009; Wolff *et al.*, 2011; Pignatta *et al.*, 2014). We blasted 438 maternally expressed and 150 paternally expressed *A. thaliana* genes and recovered 303 and 92 best hits, respectively, from the *M. guttatus* genome (using an e-value cut-off of 1E-06). Among these 395 *M. guttatus* genes, 26 co-localized with maternal QTLs and six co-localized with paternal QTLs. Based on their annotations, none of these genes has an obvious functional role in endosperm development, and of course, there is no guarantee that any of them are imprinted in *Mimulus*. Because patterns of genomic imprinting are highly variable even among strains of *A. thaliana* (Pignatta *et al.*, 2014), information from other species may have limited predictive value for identifying candidate genes in our *Mimulus* seed lethality QTLs. Thus, further fine-mapping and functional/expression analyses will be

necessary to identify the molecular basis of parent-of-origin effects in *Mimulus* hybrids; such analyses will also enlarge our understanding of this highly dynamic phenomenon beyond a few model systems.

A key question is which evolutionary forces might have led to divergence at hybrid seed lethality loci between *M. guttatus* and *M. tilingii*. One intriguing possibility is that hybrid lethality in both reciprocal crosses is the outcome of unique coevolutionary histories between imprinted genes and their targets in each of the two *Mimulus* lineages. According to the parental conflict model, maternally expressed genes should be selected to restrict endosperm growth, whereas paternally expressed genes might function to promote growth (Haig & Westoby, 1989). If patterns of genomic imprinting have evolved in *Mimulus* because of parental conflict over maternal investment, it is possible that different species have resolved this conflict using distinct genetic routes. In nature, both *M. guttatus* and *M. tilingii* are predominantly outcrossing, so conflict has the potential to be strong within each species (Brandvain & Haig, 2005). Of course, lineage-specific changes may also occur if imprinted genes have evolved by some other selective mechanism (e.g. coadaptation between maternal and offspring traits; Wolf & Hager, 2006) or by incidental proximity to silenced transposable elements (TEs) (Gehring *et al.*, 2009; Hsieh *et al.*, 2009). In the latter case, patterns of genomic imprinting might be particularly dynamic given the high degree of variation in TE position within and between plant species (Tenaillon *et al.*, 2010; Cao *et al.*, 2011). Interestingly, F_1 seed lethality also occurs in some crosses between geographically distant populations of *M. tilingii* (A. L. Sweigart *et al.*, unpublished), suggesting that, as in *A. thaliana* and maize (*Zea mays*) (Waters *et al.*, 2013; Pignatta *et al.*, 2014), there may be variation within *M. tilingii* for patterns of genomic imprinting.

Transmission ratio distortion and chromosomal differentiation

In addition to QTLs for hybrid lethality, our mapping experiment revealed two common correlates of species divergence, TRD and evidence for chromosomal rearrangements. Just within the *M. guttatus* complex, TRD has been instrumental in the discovery of centromere-associated female meiotic drive (Fishman & Willis, 2005; Fishman & Saunders, 2008), loci underlying gamete competition and conspecific pollen precedence (Fishman & Saunders, 2008), and potential cytoplasm-dependent hybrid incompatibilities (Lowry *et al.*, 2009). In addition to these mechanisms, TRD in hybrids may reflect inbreeding depression, barriers to fertilization, and postzygotic hybrid seed lethality. Similarly, suppression of recombination can reveal inversions or other rearrangements distinguishing species, which may be important in the development of both premating (Kirkpatrick & Barton, 2006) and postzygotic barriers (Noor *et al.*, 2001; Navarro & Barton, 2003).

A large number of markers had distorted genotypic frequencies in our F_2 mapping population, similar to other crosses within (Hall & Willis, 2005) and between (Fishman *et al.*, 2001, 2015) *Mimulus* species. The proportion of distorted markers in our F_2 population (67% at $\alpha = 0.05$) is somewhat higher than the proportion found in an interspecific cross between *M. guttatus* and *M. nasutus* (49%; Fishman *et al.*, 2001), consistent with the higher divergence between our focal species. In general, the distorted markers cluster in particular regions, suggesting that patterns of transmission distortion are caused by underlying loci, rather than by chance or error.

F_2 mapping alone does not allow differentiation of the many mechanisms of TRD; however, our seed-set data provide some clues about likely contributors. One possibility in our cross is inbreeding depression. Both parental lines were highly inbred, but they did not have equivalent fertility. Whereas the *M. guttatus* DUN10 line has high fitness, the *M. tilingii* LVR line produces a large fraction of inviable selfed seed (Fig. 2). Segregation of deleterious recessive alleles fixed in the LVR parent could account, in part, for the genome-wide excess of *M. guttatus* genotypes and alleles we observe throughout the genome. Alternatively, TRD could reflect genetic interactions unique to hybrids. These heterospecific interactions can arise in gametes, where they have the potential to influence viability or fertilization success, or in F_2 zygotes, where they might cause differential survival. Consistent with the action of gametic incompatibilities, in both reciprocal crosses of *M. guttatus* and *M. tilingii*, we observed a reduction in seed set (Fig. S2), suggesting that certain loci might interfere with interspecific fertilization. Finally, the presence of strong F_1 seed lethality, as well as the common observation of postzygotic hybrid incompatibilities between less divergent *Mimulus* species (e.g. Christie & Macnair, 1987; Fishman & Willis, 2006; Sweigart & Flagel, 2015), suggests that Dobzhansky–Muller interactions causing F_2 hybrid seed lethality might also contribute to TRD.

One possibility is that some of the same genetic loci might cause both hybrid seed lethality in our F_2 crossing experiments and TRD in the F_2 mapping population. At the LG2 QTL, F_2 hybrids that carry *M. guttatus* alleles produce a higher proportion of viable seeds in three of four cross treatments. Similarly, if F_2 seeds with *M. guttatus* alleles at LG2 are more likely to be viable themselves, it would lead to an overrepresentation of *M. guttatus* alleles in the F_2 adults. The same effect might also occur at the distorted region on LG13; when the LG13 QTL carries *M. guttatus* alleles, it increases seed viability in reciprocal F_2 crosses to *M. guttatus*. Interestingly, both the QTL and TRD on LG13 map to a putative inversion (Fig. 5), suggesting that this region might contain multiple loci contributing to these phenotypes.

Importantly, for understanding the history of divergence between *M. guttatus* and *M. tilingii*, and for further studies of adaptation and speciation in this system, our genetic mapping revealed strong suppression of recombination in the *DIV1* region on chromosome 8 (Fig. 5). The *DIV1* inversion defines widespread annual and perennial ecotypes of *M. guttatus* and contains QTLs for flowering time and growth-related traits that differentiate them (Hall *et al.*, 2006; Lowry & Willis, 2010). Our finding that the LVR strain of *M. tilingii* is not collinear with the perennial DUN10 parent suggests that the perennial

arrangement might be evolutionarily derived, consistent with an observed reduction in genetic diversity in the *DIV1* region in *M. guttatus* perennials (Oneal *et al.*, 2014; Twyford & Friedman, 2015).

Conclusions

In this study, we carried out the first investigation of reproductive isolation and its genetic basis between *M. guttatus* and *M. tilingii*. Both species have abundant natural populations that occasionally co-occur, potentially providing the opportunity for interspecific gene flow. However, we have shown that there is incredibly strong postzygotic reproductive isolation between these species. Our quantitative genetic analysis of hybrid seed lethality – the most comprehensive to date between diploid plant species – confirms a central role for genetic loci with parent-of-origin effects. Until now, a mechanistic understanding of hybrid seed development and failure has been lacking in systems other than *A. thaliana* and its close relatives. Our findings set the stage for future fine-mapping, phenotypic characterizations of *Mimulus* hybrid seed development, and gene expression studies to identify the underlying genes. This possibility, along with the potential for discovering natural variation in genomic imprinting, makes this *M. guttatus–M. tilingii* system particularly rich for investigating the evolutionary mechanisms of this early-acting form of postzygotic isolation.

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Author contributions

A.L.S. and L.F. conceived of the idea for the study. A.L.S., A.G.G. and A.M.K. planned and designed the research. A.G.G., A.M.K. and A.L.S. performed experiments and analyzed data. A.L.S. wrote the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Images of seeds from crosses within and between parental lines of *Mimulus guttatus* and *Mimulus tilingii*.

Fig. S2 Mean seed set varies among experimental crosses.

Fig. S3 Transmission ratio distortion across the *Mimulus guttatus* × *Mimulus tilingii* linkage map.

Table S1 Information about the biology, geography, inbreeding and sequencing of *Mimulus* lines used in this study

Table S2 Pairwise comparisons of genome-wide nucleotide diversity and divergence for *Mimulus guttatus* (G), *Mimulus nasutus* (N), and *Mimulus tilingii* (T)

Methods S1 Measuring genomic divergence among *Mimulus* species.

Methods S2 MSG library preparation.

Methods S3 Determining F_2 genotypes.

Methods S4 Identifying putative inversions.

Notes S1 Python script for calculating genome-wide average pairwise nucleotide diversity.

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