Parallel patterns of development between independent cases of hybrid seed inviability in *Mimulus*

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Author Contributions: *JMC designed this project design, performed data collection and analysis, and wrote the manuscript. JHW contributed substantially to the ideas presented here and the project design.*

Key Words: *Cryptic Species, Endosperm, Parental Conflict, Reproductive Isolation*

Running headline: *Parental conflict and hybrid seed inviability in Mimulus*

Summary

Rationale: The evolutionary drivers of many reproductive barriers are unknown. Hybrid seed inviability (HSI) is a common barrier in angiosperms. We test the idea that conflict between maternal and paternal interests in resource allocation to developing offspring (i.e. parental conflict) drives HSI in two independent incidences of HSI.

Methods: We quantified HSI between *M. guttatus* and two clades of *M. decorus* with oppositely asymmetric HSI. In the northern, weaker-conflict clade, seeds are inviabile when *M. decorus* is the mother, while in the southern, stronger-conflict clade, inviability occurs when *M. decorus* is the father. We quantified whole-seed development for hybrids and parents to assess whether reciprocal F1 seeds show parent-of-origin dependent growth phenotypes, as predicted by conflict.

Key Results: Crosses between *M. guttatus* and both clades of *M. decorus* show parent-of-origin effects on reciprocal F1 seed development. Inviable hybrid seeds exhibit paternal-excess phenotypes, wherein endosperm is large and chaotic. In contrast, viable hybrid seeds show maternal-excess phenotypes, wherein endosperm development is precocious.

Main Conclusions: We find strong parent-of-origin effects on development in reciprocal F1s that are repeated across independent incidences of HSI in *Mimulus.* These patterns suggest that parental conflict may play a strong role in shaping the evolution of HSI.

Introduction

The repeatability of speciation is a contentious topic in evolutionary biology. Much pre-zygotic reproductive isolation can be attributed to adaptations to the abiotic or biotic environment (Coyne & Orr 2004), and the repeatability of extrinsic reproductive isolation may be directly related to the repeatability of adaptation (i.e. Rundle *et al.* 2000; Rolán-Alvarez *et al.* 2004; Stankowski 2013; Soria-Carrasco *et al.* 2014; Stuart *et al.* 2017). Yet, intrinsic post-zygotic reproductive isolation also often manifests as canonical hybrid phenotypes (i.e. abdominal ablation in hybrid *Drosophila* species pairs- Gavin-Smyth & Matute 2013; CMS hybrid sterility phenotypes in plants- reviewed in Hanson & Bentolila 2004; Chase 2007; Rieseberg & Blackman 2010; hybrid necrosis in multiple plant and fungi species pairs- reviewed in Bomblies & Weigel 2007), and much less is known about the evolutionary mechanisms that cause this repeatability at the phenotypic level. Moreover, in many systems it is unclear whether these common and repeated incompatibility phenotypes are developmentally similar, and whether this can inform our understanding of the drivers of hybrid incompatibilities. A classic hybrid incompatibility phenotype in plants is that of hybrid seed inviability (HSI).

Proper seed development is achieved through the effective coordination of three tissues with differing genetic compositions: the maternally derived seed coat, the developing embryo (1m:1p genetic composition), and a nutritive tissue that sustains the embryo until germination- the endosperm. In diploid species, seed development begins with a double fertilization event; one in which the ovule is fertilized and the developing embryo is formed, while the other involves the fertilization of a diploid central cell that gives rise to a triploid endosperm tissue. The endosperm is thus comprised of 2m:1p genetic composition. This ratio is essential for appropriate seed development, because many genes exhibit parent-of-origin specific expression in the endosperm (i.e. imprinting; Köhler & Weinhofer-Molisch 2010). In *Arabidopsis*, this is caused primarily by DNA methylation and histone methylation (Köhler *et al.* 2012), the later of which are controlled by the Fertilization Independent Seed- Polycomb Group Complex (FIS-PcG complex; Köhler *et al.* 2003; Köhler *et al.* 2005; Gehring *et al.* 2006; Makarevich *et al.*

74 2006; Köhler & Makarevic 2006; Gehring *et al.* 2009; Hsieh *et al.* 2009; Wolff *et al.*
75 2011; Waters *et al.* 2011; Derkacheva *et al.* 2016). Indeed, imprinting in plants is thought
76 to have evolved as a mechanism to decrease conflict over paternal and maternal interests
77 in nutritive tissue growth (i.e. parental conflict; Trivers 1974; Haig & Westoby 1989;
78 Köhler *et al.* 2010).

79 Parental conflict is the hypothesis that maternal and paternal interests in resources
80 allocation to offspring differ in non-monogamous systems (Trivers 1974; Haig &
81 Westoby 1989). As maternity is guaranteed, selection should favor equal distribution of
82 resources from mothers to offspring, but when paternity is variable, selection should
83 favor the evolution of paternally derived alleles which increase resource expenditure on
84 developing offspring (Trivers 1974; Haig & Westoby 1989). If populations have evolved
85 differentially in this co-evolutionary arms race, then mismatches between resource-
86 restrictive maternal alleles and resource-acquiring paternal alleles in hybrids can lead to
87 developmental defects, and ultimately, death. One prediction of parental conflict is that
88 reciprocal F1s should show significantly different developmental trajectories (Willi 2013;
89 Raunsgard *et al*. 2018). When strong-conflict populations are crossed to weaker conflict
90 populations as the maternal donor, they should produce growth-restrictive phenotypes, as
91 strong resource-repressing alleles outweigh the relatively weaker resource-acquiring
92 paternal alleles. In contrast, when strong conflict populations are crossed to weaker
93 conflict populations as the paternal contributor, seed should exhibit growth-excessive
94 phenotypes, as strong, resource-acquiring paternal alleles outweigh weaker maternal
95 resource-restrictive alleles (Raunsgard *et al*. 2018).

96 In *Arabidopsis* hybrid seed inviability in interploidy crosses, maternal-restrictive
97 and paternal-excess phenotypes are observed (Scott *et al.* 1998). Inviability in this case
98 arises because of de-repression of normally imprinted genes, particularly that of *ADM,*
99 *SUVH7, PEG2*, and *PEG9*, which are all associated with carbohydrate sequestration and
100 cellularization in normally developing seeds (Kradofler *et al.* 2013; Wolff *et al.* 2015).
101 Overexpression of these alleles causes excessive growth and improper cellularization of
102 the endosperm associated with paternal excess, while maternal excess is often associated
103 with precocious endosperm development (Scott *et al.* 1998; reviewed in Lafon-Placette &

104 Köhler 2016). Defects in endosperm growth are the cause of hybrid seed inviability; both
105 embryo rescues and dosage rescue experiments strongly suggest that in these interploidy
106 crosses, hybrid seed inviability is due specifically to inappropriate development and
107 growth of the endosperm, rather than incompatibilities arising in the embryo itself
108 (Bushell *et al.* 2003; Josefsson *et al.* 2006; Dilkes *et al.* 2008; Erilova *et al.* 2009;
109 Kradolfer *et al.* 2013).

110 While hybrid seed inviability is quite common in diploid species pairs (Tiffin *et*
111 *al.* 2001), far fewer studies have determined the mechanistic underpinnings of hybrid
112 seed inviability among diploids. In plants with nuclear endosperm development, such as
113 *Arabidopsis* and *Capsella,* hybrid seed inviability mimic interploidy crosses with a
114 delayed or premature endosperm cellularization, depending on the maternal and paternal
115 donor in the cross, and endosperm defects are intrinsically linked with hybrid seed
116 inviability (Joseffson *et al.* 2006; Burkart-Waco *et al.* 2012; Rebernig *et al.* 2015; Lafon-
117 Placette *et al.* 2017l Lafon-Placette *et al.* 2018). In plants with cellular endosperm
118 development (i.e. *Solanum* and *Mimulus*), hybrid seed inviability is much less explored,
119 although there is some evidence to suggest that inappropriate replication of endosperm is
120 at least partially causative of hybrid seed inviability in both *M. nudatus* and *M. guttatus*
121 inviable hybrid seeds (Oneal *et al.* 2016), and in wild tomato varieties (Lafon-Placette *et*
122 *al.* 2016; Roth *et al.* 2017).

123 We previously reported a wide diversity of hybrid seed barriers between *M.*
124 *guttatus* and its morphological variant, *M. decorus* which confer relatively strong
125 reproductive isolation (Coughlan *et al.* 2018). We found that *M. decorus* is in fact
126 multiple biological species; a diploid northern clade that exhibits strong asymmetric
127 hybrid seed inviability when it is the maternal donor in crosses to *M. guttatus*; a diploid
128 southern clade that exhibits strong asymmetric hybrid seed inviability when it is the
129 paternal donor in crosses to *M. guttatus;* and at least one tetraploid taxon that exhibits
130 variable levels of hybrid seed inviability (Coughlan *et al.* 2018). Because the direction of
131 inviability differs between *M. guttatus* and each clade of diploid *M. decorus*, there must
132 be independent evolution of maternal and paternal alleles causing inviability between
133 northern and southern *M. decorus*, even though they are each other's closest relative

134 (Coughlan *et al.* 2018). Hybrid seed inviability between *M. guttatus* and each clade of *M.*
135 *decorus* thus represents two independent incidences of hybrid seed inviability. Here, we
136 delve into the developmental basis of hybrid seed inviability using the *Mimulus guttatus*
137 species complex as a model. We test whether hybrid seed inviability between different
138 diploid clades of *M. decorus* and *M. guttatus* are associated with similar developmental
139 defects, whether these defects are endosperm related, and whether they are consistent
140 with paternal excess/maternal restriction, as hypothesized by parental conflict. In
141 addition, we assess the level of developmental parallelism of inviable and viable hybrid
142 seeds between these two independent incidences of hybrid seed inviability.
143

144 **Methods**

145 *Growth conditions and plant crossing*

146 Based on previous results, *Mimulus decorus* exhibits two distinct crossing
147 patterns: the northern clade of *M. decorus* produces inviable seeds when they are the
148 maternal plant in crosses with *M. guttatus*, while for the southern clade produces inviable
149 seeds when they are the paternal plant in crosses with *M. guttatus*. To determine the
150 extent of developmental parallelism, we chose a representative diploid population from
151 each crossing type of *M. decorus* (northern clade: *IMP*; southern clade: *Odell Creek*) to
152 cross with an annual population of *M. guttatus*- Iron Mountain (*IM*). For each population,
153 we planted seed from 3-4 maternal families or inbred lines. For all plants, seeds were
154 sprinkled onto moist Fafard 4-P soil, left to stratify at 4°C for 1 week, then moved to the
155 Duke greenhouses (North Carolina, USA). Plants were transferred within the first 3 days
156 after germination to one plant per 2" pot, then were grown in long days (18h days, 21C
157 day/ 18C nights).

158

159 *Crossing Assays*

160 Plants were emasculated and crossed reciprocally, as well as self pollinated to
161 yield an average of 6 crosses for each pairwise combination of maternal families between
162 IM×Odell Creek, and IM×IMP (IM×Odell range: 2-13 replicate crosses, IM×IMP: 2-10
163 replicate crosses for a total of 298 crosses). Fruits were allowed to develop until ripe but
164 before dehiscence, then seeds for each cross were counted and categorized as inviable or
165 viable based on morphology for each fruit.

166

167 *Microscopy and measuring growth*

168 We sought to determine the extent of developmental parallelism of hybrid seeds
169 in two independent incidences of hybrid seed inviability. We crossed plants, as in above,
170 but collected fruits at 4,6,8,10 and 14 Days After Pollination (DAP). Fruits were
171 collected and immediately placed in FAA fixative (2 formaldehyde: 1 acetic acid: 10
172 ethanol). Plants were processed in a similar manner to White & Turner (2012). In brief,
173 after at least 24 hours in fixative, fruits were gradually dehydrated in a Tetrabutyl-
174 Alcohol dehydration series, then mounted in paraffin wax with ~5% gum elemi resin to
175 increase hardness. Paraffin mounted specimens were then sliced to 8 micron ribbons and
176 mounted onto slides. We performed a staining series using Safranin-O and Fast-Green,
177 which stain for nucleic acids and carbohydrates, respectively. Cover slips were then
178 adhered for permanent storage.
179 We visualized and photographed at least three developing seeds from a single
180 fruit for each cross type and time point combination (40 unique combinations, 120 seeds
181 measured). At least 25 endosperm cell widths were measured per seed using ImageJ
182 (Scheider *et al.* 2012).

183

184 *Embryo Rescues*

185 To assess whether hybrid seed inviability was due largely to inappropriate
186 development of the endosperm tissue, we also performed embryo rescue assays on both
187 IM×Odell seeds and IMP×IM seeds, which would normally be inviable if left to develop
188 fully in the seed pod. For this, we crossed IM reciprocally to both IMP and Odell creek,
189 as well as performed self fertilizations for IMP, Odell Creek, and IM. Fruits were left to
190 mature for 7 or 9 days. Based on our developmental time series, embryos appear to die
191 between 10-14 DAP for both sets of crosses. We plated whole developing seeds for each
192 cross type on a sucrose rich growth media (1/2 MS salts, 0.7% agar, 4% sucrose, adjusted
193 to pH 5.9) at 7 and 9 DAP. Plates were cold stratified for 2 days, then placed in the Duke
194 Greenhouses (18h days, 21C day/ 18C nights).

195

196 *Statistical Analyses*

197 Cross incompatibilities were determined with a linear mixed model using the
198 package *lme4* and a type III ANOVA using *car* (Bates *et al.* 2015; Fox & Weisberg
199 2011) in the statistical interface R. All analyses were completed separately for IM×Odell
200 and IM×IMP crosses. For both total seed number, and proportion viable seed, we treated
201 cross type as a fixed factor and replicate maternal and paternal lines as random effects
202 (i.e. *Proportion viable seed ~ Cross Type + (1|maternal replicate) + (1|paternal*
203 *replicate)* or *total seed count ~ Cross Type + (1|maternal replicate) + (1|paternal*
204 *replicate)*). Pairwise T-tests with Holm correction for multiple testing were performed to
205 determine which cross types varied significantly from one another. Differences in
206 developmental trajectories were also assessed using a linear mixed model, as above. In
207 this case, cross type, DAP, and their interaction were the fixed effects and the seed
208 replicate was treated as a random effect.

Results

Post-Mating Reproductive Isolation

IMP and Odell Creek show consistent patterns of strong, and asymmetric hybrid seed inviability when crossed with individuals from Iron Mountain, but in opposing directions (Figure 1; Odell Creek: X^2= 4598, *df*=3, p<0.0001; IMP: X^2= 2969, *df*=3, p<0.0001). IMP produces entirely inviable seeds when the maternal donor in these crosses, while Odell Creek produces entirely inviable seeds when the paternal donor (and some inviable seeds when the maternal donor; Figure 1; Coughlan *et al.* 2018). Inviable hybrid seeds appear larger, disc-like, and shriveled, while viable hybrid seeds appear smaller overall (as in Coughlan *et al.* 2018). As these crosses differ in what direction of the cross yields a viable vs inviable seed, there must be independent evolution of maternal and paternal alleles causing HIS between these two clades of *M. decorus*, even though they are each other's closest relative (Coughlan *et al.* 2018).

We next wanted to determine if there were any post-mating, pre-zygotic barriers in these crosses. While there is some evidence of pollen-pistil incompatibilities between annual *M. guttatus* and Odell Creek (Odell Creek: X^2= 49.33, *df*=3, p<0.0001; Figure S1), wherein IM lines set less seed when pollinated by Odell Creek than any other cross type. We find no evidence of pollen-pistil interactions between IM and IMP, although we note that IMP has substantially larger ovaries and produces more seeds, regardless of the pollen donor (X^2= 23.7, *df*=3, p<0.0001; Figure S1).

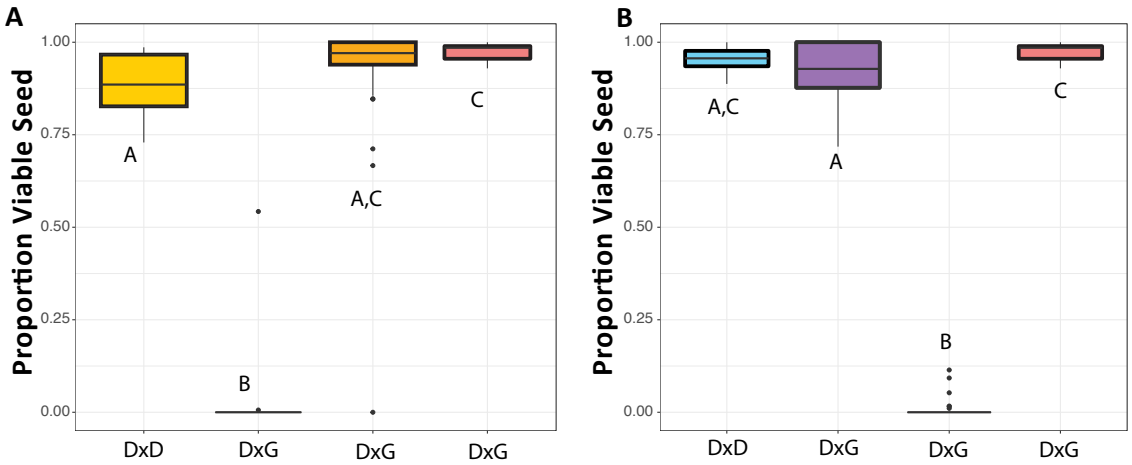


Figure 1: Crosses between *M. guttatus* and each clade of *M. decorus* shows oppositely asymmetric patterns of hybrid seed inviability. Proportion viable seed between three lines of *M. guttatus* (IM) and (A) four maternal families of northern *M. decorus* (IMP) and (B) three maternal families of southern *M. decorus* (Odell Creek). Maternal parent is listed first.

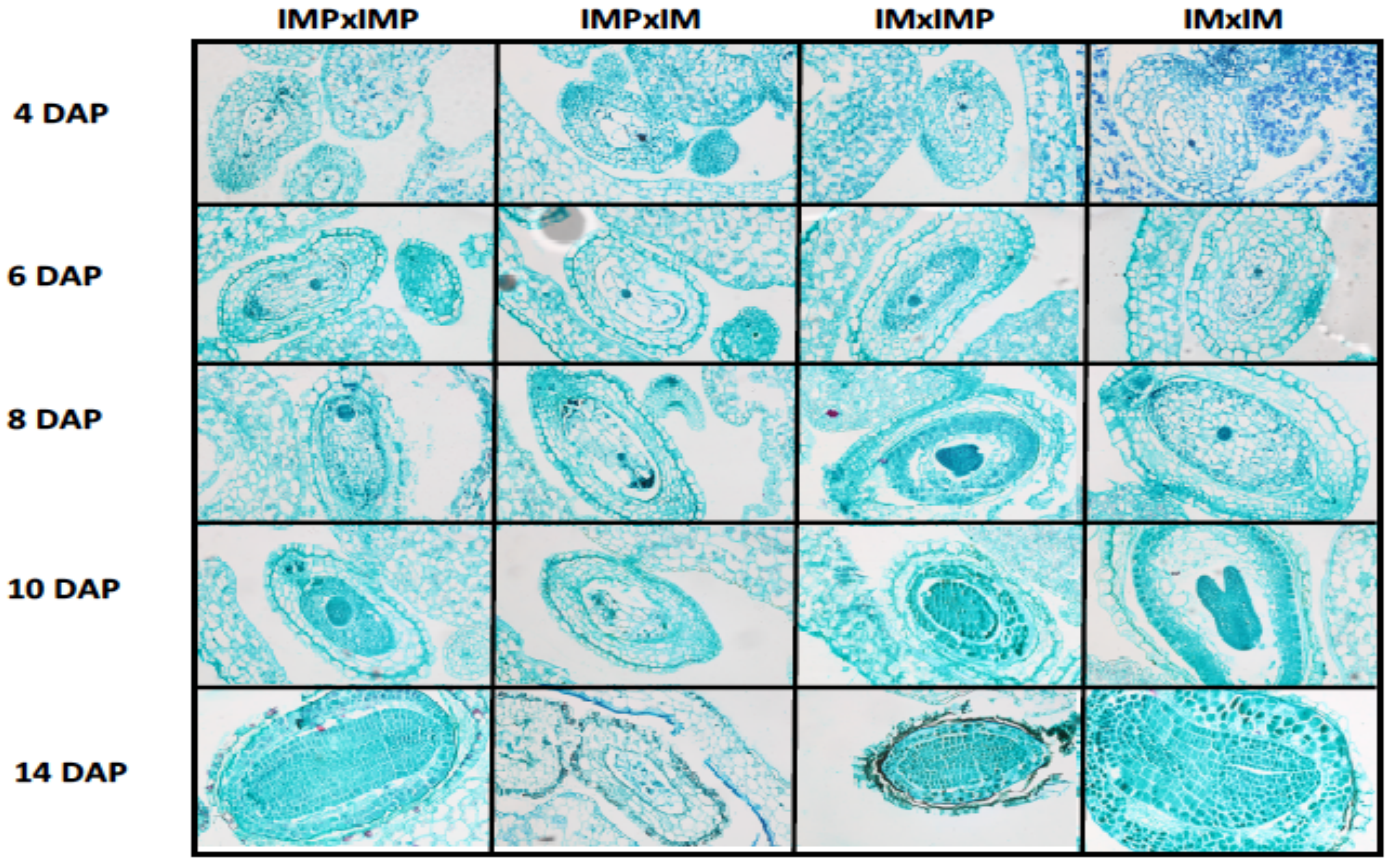
Developmental Trajectories

The three parental species have similar seed development in the context of their morphological attributes. In all three parents, endosperm is tightly packed around the embryo and continues to grow until around 8-10 DAP. At this point, the endosperm is depleted, presumably to feed the growing embryo. Embryo development among the three parents is also quite similar, with IM displaying a slightly accelerated growth, relative to either Odell or IMP (i.e. they reach a heart-shaped embryo around 10 DAP, while this is not achieved in Odell or IMP until around 14 DAP; Figure 2; Figure 3). By 14 DAP the embryo comprises most of the embryo sac, with a thin layer of endosperm remaining.

Hybrid seeds from both the IM×IMP and IM×Odell crosses show distinct developmental trajectories based on the maternal versus paternal donor of the cross, but both hybrid crosses display similar seed trajectories, depending on the fate of the seed (i.e. whether it will remain viable or not). In both sets of crosses, seeds that remain viable

250 (e.g. IM×IMP and Odell×IM) display a precocious development, with several small
251 endosperm cells that are quickly degraded by the embryo (Figure 2; Figure 3) . By 14
252 DAP, the only remaining endosperm is a thin layer which surrounds the embryo (Figure
253 2; Figure 3). The overall seed size is also much smaller than either parent or the
254 reciprocal hybrid (Coughlan *et al.* 2018). In contrast, the hybrid seeds which will
255 eventually become inviable (i.e. IMP×IM and IM×Odell) exhibit a chaotic endosperm
256 growth, producing fewer, but larger and more diffuse endosperm cells (Figure 2; Figure
257 3). Embryos in this direction of the cross remain small, relative to both parents and the
258 reciprocal hybrid (Figure 2; Figure 3). By 14 DAP, most embryos were entirely degraded.
259 The patterns of precocious endosperm development in viable hybrid seeds and chaotic
260 endosperm development in inviable hybrid seeds showcase substantial parent-of-origin
261 effects on endosperm development as a potential cause of embryo inviability. These
262 maternal-excess and paternal-excess phenotypes are also consistent with conflict between
263 maternal and paternal interests in resource allocation to endosperm as an evolutionary
264 explanation for hybrid seed inviability.
265

266

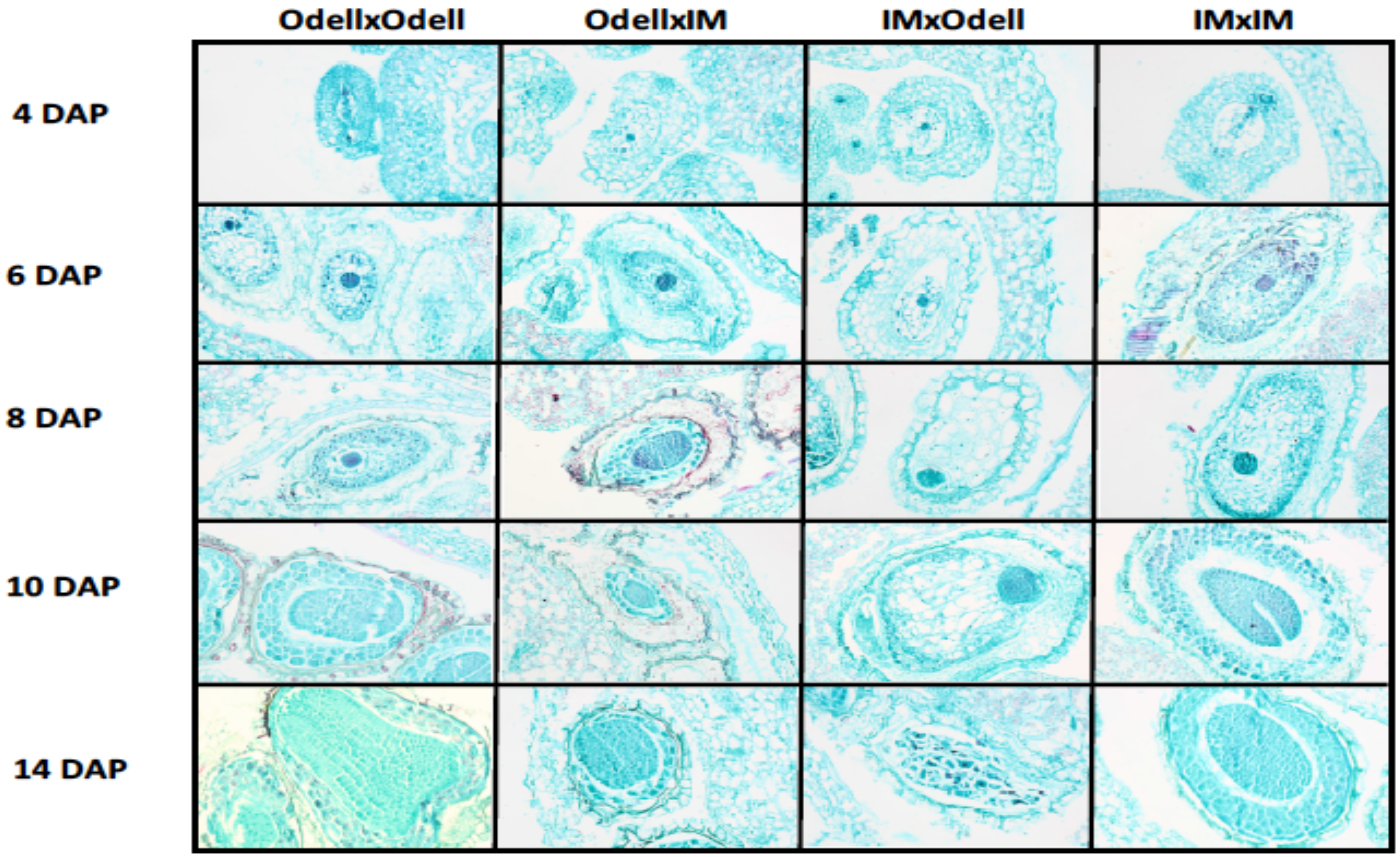


267

268 **Figure 2: Reciprocal hybrids between *M. guttatus* and northern *M. decorus* show significant parent-of-origin effects on**
269 **endosperm growth. Developing seeds between IM62 x IMP at 4, 6, 8, 10, and 14 Days After Pollination (DAP) are shown.**
270 **Maternal parent is listed first.**

Patterns of maternal-restriction and paternal-excess are recapitulated when looking simply at endosperm cell width. In both crosses, the type of cross significantly affected the endosperm cell width (Table 1; Figure 4, Figure S2). Reciprocal F1 hybrids display the most extreme endosperm cell sizes, with inviable seeds exhibiting the largest endosperm cell size, and viable seeds displaying the smallest endosperm cells. For both IM×IMP and IM×Odell Creek crosses, the relationship between cell size and cross type was dependent on DAP (although we note a significant interaction term, suggesting that the rate of growth in endosperm cells through time depends on genotype. Table 1; Figure S2).

282



283

284 **Figure 3: Reciprocal hybrids between *M. guttatus* and southern *M. decorus* show significant parent-of-origin effects on**
285 **endosperm growth. Developing seeds between IM62 x Odell Creek at 4, 6, 8, 10, and 14 Days After Pollination (DAP) are**
286 **shown. Maternal parent is listed first.**

While the two different sets of crosses display quite similar overall patterns of hybrid seed development, there are some notable distinctions between hybrid seeds formed between IM×IMP, and IM×Odell Creek. For example, in IMP×IM crosses, the embryo remains comparatively small, and does not appear to grow past the 6 or 8 DAP. In contrast, seeds formed from a IM×Odell cross often exhibit a developing embryo up until 10 DAP, suggesting that the seed is aborted slightly later in this cross.

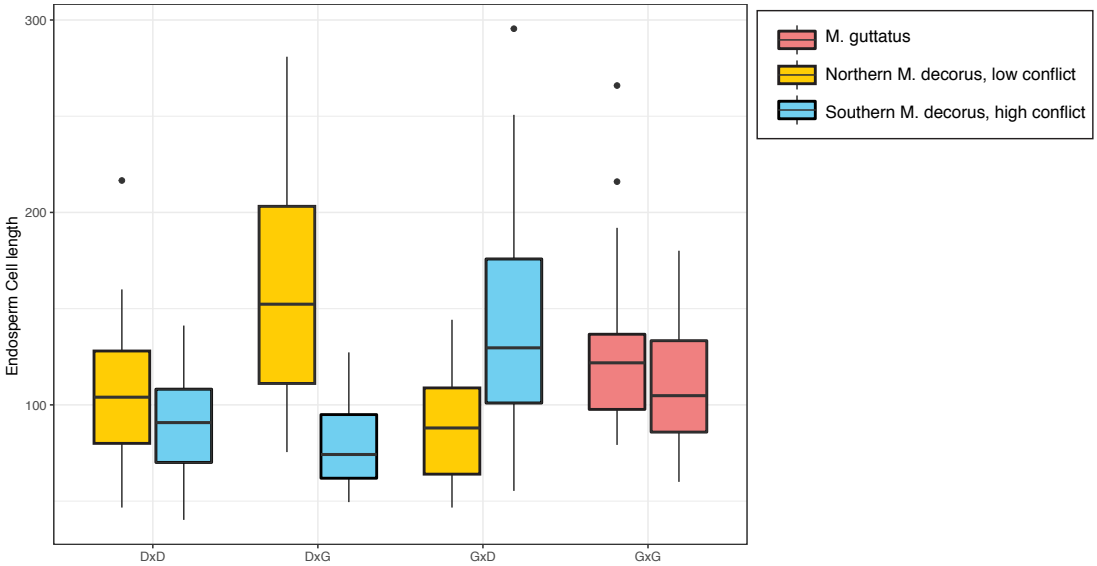


Figure 4: Parent-of-origin effects on endosperm growth are recapitulated at the cellular level. Cell width at 8 Days After Pollination (DAP) for each cross type (maternal parent listed first), for both *M. guttatus* crossed to IMP (yellow) and Odell Creek (blue) are shown. See Supplemental Figure 2 for all sampled time points.

Overall, we see striking parallelism between two distinct seed incompatibilities with reciprocal F1 seeds displaying either excessive and chaotic growth patterns or precocious and stunted patterns, depending on whether the seeds are eventually aborted or not. Our findings suggest that hybrid seed inviability in this group is strongly associated with endosperm overgrowth phenotypes. We next sought to determine if hybrid seed inviability was primarily due to abnormal endosperm growth, or whether incompatibilities manifesting in the embryo also contribute.

307 **Table 1: Mixed model for type III ANOVAs for seed cell size across developmental time for**
308 **IMP and Odell Creek crossed reciprocally to IM62. Cross Type= both reciprocal F1**
309 **crosses and self-fertilizations from each species treated as a separate level; treated as a**
310 **fixed effect), DAP= Days After Pollination (fixed effect). Seed replicate was treated as a**
311 **random effect.**

	IMP			Odell Creek		
	X^2	*DF*	*p*	X^2	*DF*	*p*
Intercept	295.04	1	<0.0001	123.74	1	<0.0001
Cross Type	119.848	3	<0.0001	14.92	3	0.0018
DAP	36.033	1	<0.0001	0.0029	1	0.96
Cross Type*DAP	42.583	3	<0.0001	32.95	3	<0.0001

312

313 *Embryo Rescues*

314 Embryo rescues were generally unsuccessful in these crosses. No seeds germinated when
315 collected 7 DAP. Only a small fraction of IM62 and hybrids which were viable (i.e. OdellxIM
316 and IMxIMP) germinated in the 9 DAP collections (i.e. 5-10%). Overall, this suggests that 7
317 DAP may be too premature for proper embryo development to occur outside of the maternal
318 environment, while only rapidly developing seeds are able survive transplants at 9 DAP.
319 Unfortunately, determining whether hybrid seed inviability is due solely to endosperm
320 malformation is yet to be determined in this group.

321

322 **Discussion**

323 As previously reported, the northern and southern clades of *M. decorus* exhibit strong,
324 and asymmetric reproductive isolation with *M. guttatus*, but in opposite directions of the cross.
325 This observation is true for all maternal family/line combinations tested here (as well as multiple
326 populations tested in Coughlan *et al.* 2018), suggesting that HSI is not geographically restricted,
327 as other intrinsic post-zygotic barriers have been found to be (Sweigart *et al.* 2007; Case &

328 Willis 2008; Martin & Willis 2010; Sicard *et al.* 2015; Barnard-Kubow & Galloway 2017;
329 Zuellig & Sweigart 2018). Total reproductive isolation in these crosses was ~52% for both both
330 species pairs, and thus may represent a significant barrier to reproduction. Hybrid seed
331 inviability has been shown to be a formidable barrier to reproduction in other systems (i.e. Tiffin
332 *et al.* 2001; Lowry *et al.* 2008), but the developmental causes of this incompatibility have really
333 only been quantified for a small number of diploid species pairs. We aimed to determine the
334 developmental trajectories of inviable and viable hybrid seeds in two independent incidences of
335 hybrid seed inviability between close relatives in the *Mimulus guttatus* species complex.
336 We show that hybrid seed inviability is strongly associated with developmental defects in
337 endosperm deposition. In inviable directions of the cross, seeds show a paternal-excess
338 phenotype, where endosperm is chaotically distributed, and the cells are few and large. In the
339 viable directions of the cross, seeds show a maternal-restrictive phenotype, where endosperm
340 develops precociously, as witnessed by the general lack of endosperm remaining when seeds are
341 fully developed, as well as the generally smaller size of both the cells and final seeds (Coughlan
342 *et al.* 2018). Overall, these developmental patterns fit with a parental conflict model of hybrid
343 inviability, wherein paternal excess is more likely to lead to seed abortion. Unfortunately, given
344 the generally low success rate of the embryo rescues, it remains unclear whether hybrid seed
345 inviability is due strictly to malformation of the endosperm, or whether genetic incompatibilities
346 in the embryo also contribute.
347 Maternal-restriction/paternal excess endosperm phenotypes have been shown in hybrids
348 between wild species pairs of both *Arabidopsis* and *Capsella*, which have a nuclear-type
349 endosperm development (Rebernig *et al.* 2015; Lafon-Placette *et al.* 2017). In diploid
350 *Arabidopsis* and *Capsella* crosses, as well as interploidy crosses, endosperm over-proliferation is
351 accompanied by a failure of endosperm cells to cellularize (Scott *et al.* 1998; Bushell *et al.* 2003;
352 Rebernig *et al.* 2015; Lafon-Placette *et al.* 2017), and at least in *Capsella,* paternal excess is
353 associated with inviability, but maternal excess still produces viable seed (Rebernig *et al.* 2015).
354 Initial findings from species with cellular-type endosperm development (e.g. wild tomatoes and
355 other *Mimulus* species pairs) also show endosperm defects as a major cause of hybrid inviability
356 (Roth *et al.* 2017; Oneal *et al.* 2016). However, these defects to not represent defects in rates of
357 cellularization, but rather rates of proliferation, cell size and distribution, and optical density
358 (Oneal *et al.* 2016; Roth *et al.* 2017). Our results are consistent with findings from other *Mimulus*

359 and wild tomato studies, where inviable seeds display large-celled, chaotically distributed
360 endosperm cells, but there are fewer of them, suggesting that issues in the regular timing of
361 mitosis may be at play.

362 The developmental trajectories of viable vs non-viable hybrid seeds are strikingly similar
363 between Odell×IM, and IMP×IM crosses, despite the fact that inviability occurs in the opposite
364 cross direction. Because hybrid seed inviability in this group manifests in opposite directions of
365 the cross between *M. guttatus* and each of the diploid clades of *M. decorus*, there must be
366 independent evolution of alleles contributing to hybrid seed inviability between these clades of
367 *M. decorus.* This degree of developmental parallelism in independent incidences of hybrid seed
368 inviability suggests that similar evolutionary pressures may be causing hybrid seed inviability
369 within this complex. Given the maternal-restriction/paternal-excess phenotypes that we observe,
370 one potential hypothesis is that of parental conflict. Despite considerable developmental
371 parallelism, it remains to be seen how constrained the genetic targets of selection are between
372 these species pairs. In other post-zygotic reproductive barriers, canonical hybrid incompatibilities
373 often share similar genetic bases. For example, hybrid necrosis is often caused by interactions
374 involving R genes (reviewed in Bomblies & Weigel 2007), and CMS almost always involves
375 PPR genes (polcentric peptide repeat genes; reviewed in Rieseberg & Blackman 2010). Yet,
376 whether this remains true for hybrid seed inviability remains to be seen. Indeed, one may predict
377 that the genomic target size for influencing resource provisioning to seeds is presumably larger
378 than the number of genes responsible for auoto-immune function or managing organelle
379 expression, as is the case with hybrid necrosis and CMS, respectively. However, this prediction
380 remains to be tested. Indeed, a thorough examination of the molecular and developmental
381 genetics of hybrid seed inviability in this system is necessary to answer these questions, and can
382 inform our understanding of the proximate and ultimate causes of hybrid seed inviability.
383

384 **Acknowledgements**

385 We thank members of the Willis lab- particularly Elen Oneal, as well as Miguel Florez and Bob
386 Franks for useful discussions of this project. We are deeply grateful to Melvin Turner for
387 teaching JMC histological techniques, and to Dick White for allowing us access to his histology
388 lab. This project was funded from NSF grants EF-0328636 and EF-0723814 to JHW, a DDIG

(DEB- 1501758), ASN Student research award and SSE student research award to JMC. The Duke Graduate School also provided funding to JMC with the Myra and William Waldo Boone fellowship, and financial support was provided from the Duke Biology Department.

Data Availability

Data for the manuscript will be made publically available.

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Supplemental Figures

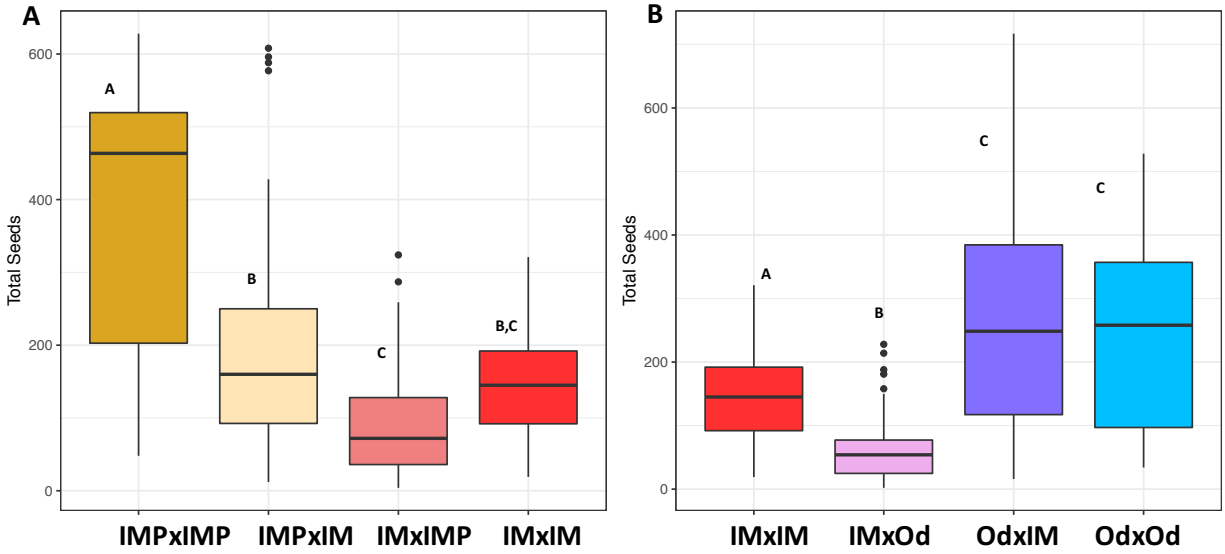
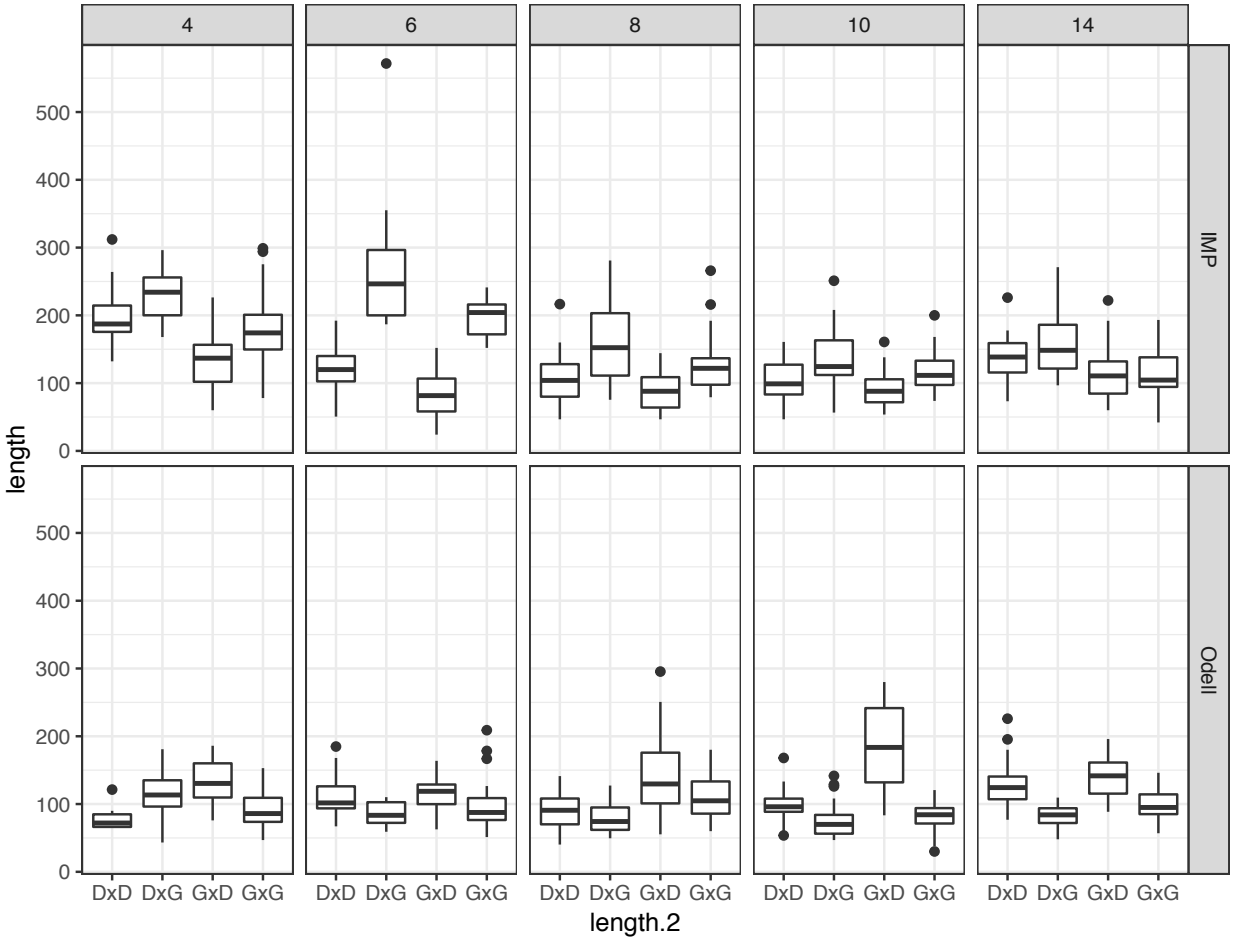


Figure S1: Total number of seeds in crosses for (A) three lines from IM and four maternal families of IMP selfed and crossed reciprocally and (B) three lines of IM and three maternal families of Odell Creek (Od) selfed and crossed reciprocally.



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536 **Figure S2: Cell lengths across all cross types for 5 developmental stages for both IMxIMP**

537 **(top) and IMxOdell (bottom) crosses. Panels from left to right are each DAP.**