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

Jenn M. Coughlan[1,2], John H. Willis[1]

1. *Biological Sciences, Duke University 25 Science Drive, Durham NC*
2. *Biology Department, University of North Carolina, Chapel Hill*

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.*

Number of Figures/tables: 3

Final Word Count: 3000

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

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

Summary

Rationale: Hybrid seed inviability (HSI) is a common reproductive barrier in angiosperms, yet the evolutionary and developmental drivers of HSI remain largely unknown. We test whether conflict between maternal and paternal interests in resource allocation to developing offspring (i.e. parental conflict) are associated with HSI and determine the degree of developmental parallelism between independent incidences of HSI in *Mimulus*.

Methods: We quantified HSI between *M. guttatus* and two clades of *M. decorus* with oppositely asymmetric incompatibilities and surveyed development of hybrid and parental seeds.

Key Results: Crosses between *M. guttatus* and both clades of *M. decorus* show parent-of-origin effects on reciprocal F1 seed development, but in opposing directions. Inviable hybrid seeds exhibit paternal excess phenotypes, wherein endosperm is large and chaotic while viable hybrid seeds produce endosperm cells that are smaller and less prolific (i.e. maternal-excess phenotypes).

Main Conclusions: We find strong parent-of-origin effects on development in reciprocal F1s in multiple incidences of HSI in *Mimulus.* These patterns suggest that parental conflict may be an important force generating HSI in this group, and mismatches between maternal and paternal contributions to developing seeds result in repeatable developmental defects in hybrids.

48

49 ## Introduction

50 The repeatability of speciation is a contentious topic in evolutionary biology. Many pre-
51 zygotic barriers can be attributed to adaptations to the abiotic or biotic environment (Coyne &
52 Orr 2004), and the repeatability of extrinsic reproductive isolation may be directly related to the
53 repeatability of adaptation (i.e. Rundle *et al.* 2000; Rolán-Alvarez *et al.* 2004; Stankowski 2013;
54 Soria-Carrasco *et al.* 2014; Stuart *et al.* 2017, though see Lowry *et al.* 2008). Yet intrinsic post-
55 zygotic reproductive isolation can also manifest canonical hybrid phenotypes (e.g. CMS hybrid
56 sterility in plants- reviewed in Hanson & Bentolila 2004; Chase 2007; Rieseberg & Blackman
57 2010; hybrid necrosis in plant and fungi- reviewed in Bomblies & Weigel 2007), and much less
58 is known about the evolutionary mechanisms that cause this repeatability at the phenotypic level.
59 Moreover, in many systems it is unclear whether these common incompatibility phenotypes are
60 developmentally similar, which can inform our understanding of the evolutionary drivers of
61 incompatibilities. One common hybrid incompatibility in angiosperms is hybrid seed inviability
62 (HSI).

63 Proper seed development is achieved through the effective coordination of three tissues
64 with differing genetic compositions: the maternally derived seed coat, the developing embryo
65 (1m:1p genetic composition), and a nutritive tissue that sustains the embryo until germination-
66 the endosperm. In diploid species, seed development begins with a double fertilization event; one
67 in which the egg cell is fertilized and the developing embryo is formed, while the other involves
68 the fertilization of a diploid central cell that gives rise to a triploid endosperm tissue. The
69 endosperm is thus comprised of 2m:1p genetic composition. This ratio is essential for
70 appropriate seed development, because many genes exhibit parent-of-origin specific expression
71 in the endosperm (i.e. imprinting; Köhler & Weinhofer-Molisch 2010). In *Arabidopsis*, this is
72 caused primarily by DNA methylation and histone methylation (Köhler *et al.* 2012), the later of
73 which are controlled by the Fertilization Independent Seed- Polycomb Group Complex (FIS-PcG
74 complex; Köhler *et al.* 2003; Köhler *et al.* 2005; Gehring *et al.* 2006; Makarevich *et al.* 2006;
75 Köhler & Makarevic 2006; Gehring *et al.* 2009; Hsieh *et al.* 2009; Wolff *et al.* 2011; Waters *et*
76 *al.* 2011; Derkacheva *et al.* 2016). Indeed, imprinting in plants is thought to have evolved as a

77 mechanism to decrease conflict over paternal and maternal interests in endosperm growth (i.e.
78 parental conflict; Trivers 1974; Haig & Westoby 1989; Köhler *et al.* 2010).
79 Conflict between parents can arise in non-monogamous systems, because maternal and
80 paternal interests in resources allocation to offspring differ (Trivers 1974; Haig & Westoby
81 1989). As maternity is guaranteed, selection should favor equal distribution of resources among
82 offspring, but when paternity is variable, selection should favor the evolution of paternally
83 derived alleles that increase resource expenditure on developing offspring (Trivers 1974; Haig &
84 Westoby 1989). If populations have evolved differentially in this co-evolutionary arms race, then
85 mismatches between resource-restrictive maternal alleles and resource-acquiring paternal alleles
86 in hybrids can lead to developmental defects, and in the case of HSI, death. One prediction of
87 parental conflict is that reciprocal F1s should show significantly different developmental
88 trajectories, wherein one direction of the cross exhibits a paternal-excess phenotype, and the
89 other exhibits a maternal-restrictive phenotype (Willi 2013; Raunsgard *et al.* 2018).
90 HSI in inter-ploidy crosses of *Arabidopsis* exhibit these two distinctive endosperm
91 phenotypes (Scott *et al.* 1998), and endosperm defects are the cause of inviability. Both embryo
92 and dosage rescue experiments strongly suggest that in these inter-ploidy crosses, HSI is due
93 specifically to inappropriate development and growth of the endosperm, rather than
94 incompatibilities arising in the embryo (Bushell *et al.* 2003; Josefsson *et al.* 2006; Dilkes *et al.*
95 2008; Erilova *et al.* 2009; Kradolfer *et al.* 2013). Inviability in these cases arises because of de-
96 repression of normally imprinted genes, particularly that of *ADM, SUVH7, PEG2*, and *PEG9*,
97 which are all associated with carbohydrate sequestration and cellularization in normally
98 developing seeds (Kradofler *et al.* 2013; Wolff *et al.* 2015). Overexpression of these alleles
99 causes excessive growth and improper cellularization of the endosperm associated with paternal
100 excess, while maternal excess is often associated with precocious endosperm development (Scott
101 *et al.* 1998; reviewed in Lafon-Placette & Köhler 2016).
102 While HSI is common in diploid species pairs (Tiffin *et al.* 2001), far fewer studies have
103 determined the mechanistic underpinnings of HSI among diploids. In plants with nuclear
104 endosperm development, such as *Arabidopsis* and *Capsella,* HSI mimic inter-ploidy crosses with
105 a delayed or premature endosperm cellularization, depending on the paternal and maternal donor
106 in the cross, and endosperm defects are causative of HSI (Joseffson *et al.* 2006; Burkart-Waco *et*
107 *al.* 2012; Rebernig *et al.* 2015; Lafon-Placette *et al.* 2017l Lafon-Placette *et al.* 2018). In plants

with cellular endosperm development (i.e. *Solanum* and *Mimulus*), HSI is much less explored, although there is evidence to suggest that inappropriate replication of endosperm is at least partially causative of HSI in both *M. nudatus* and *M. guttatus* (Oneal *et al.* 2016), and in wild tomatoes (Lafon-Placette *et al.* 2016; Roth *et al.* 2017).

We previously reported a wide diversity of hybrid seed barriers between *M. guttatus* and its morphological variant, *M. decorus* which confer strong reproductive isolation (Coughlan *et al.* 2018). We found that *M. decorus* is multiple biological species; a diploid northern clade that exhibits strong asymmetric HSI when it is the maternal donor in crosses to *M. guttatus*; a diploid southern clade that exhibits strong asymmetric HSI when it is the paternal donor in crosses to *M. guttatus;* and at least one tetraploid taxon that exhibits variable levels of HSI (Coughlan *et al.* 2018). Because the direction of inviability differs between *M. guttatus* and each clade of diploid *M. decorus*, there must be independent evolution of maternal and paternal alleles causing inviability between northern and southern *M. decorus*, even though they are each other's closest relative (Coughlan *et al.* 2018). HSI between *M. guttatus* and each clade of *M. decorus* thus represents two independent incidences of HSI in relatively recently diverged species. Here, we delve into the developmental basis of HSI using the *Mimulus* as a model. We test whether HSI between different diploid clades of *M. decorus* and *M. guttatus* are associated with similar developmental defects, whether these defects are endosperm related, and whether they are consistent with paternal excess/maternal restriction, as hypothesized by parental conflict. In addition, we assess the level of developmental parallelism of inviable and viable hybrid seeds between these two independent incidences of HSI.

Methods

Growth conditions and plant crossing

Based on previous results, *Mimulus decorus* exhibits two distinct crossing patterns: the northern clade of *M. decorus* produces inviable seeds when they are the maternal plant in crosses with *M. guttatus*, while the southern clade produces inviable seeds predominantly when they are the paternal plant in crosses with *M. guttatus*. To determine the extent of developmental parallelism, we chose a representative diploid population from each crossing type of *M. decorus* (northern clade: *IMP*; southern clade: *Odell Creek*) to cross with an annual population of *M.*

138 *guttatus*- Iron Mountain (*IM*). For each population, we planted seed from 3-4 maternal families
139 or inbred lines. For all plants, seeds were sprinkled onto moist Fafard 4-P soil, left to stratify at
140 4°C for 1 week, then moved to the Duke greenhouses (North Carolina, USA). Plants were
141 transferred within the first 3 days after germination to one plant per 4" pot, then were grown in
142 long days (18h days, 21C day/ 18C nights).

143

144 *Crossing Assays*

145 We performed inter- and intra-specific crosses between both clades of *M. decorus* and *M.*
146 *guttatus* to yield an average of six crosses for each pairwise combination of maternal families
147 between *M. guttatus* and each clade of *M. decorus* (total of 298 crosses). Fruits were allowed to
148 develop until ripe but before dehiscence, then seeds for each cross were counted and categorized
149 as inviable or viable based on morphology for each fruit. Previous works suggests that
150 morphologically assessed viability is a good proxy for germination proclivity (Garner *et al.*
151 2016; Coughlan *et al.* 2018).

152

153 *Microscopy and measuring growth*

154 We sought to survey development of hybrid and parental seeds in two independent
155 incidences of HSI. We crossed plants, as in above, but collected fruits at 4,6,8,10 and 14 Days
156 After Pollination (DAP). Fruits were collected and immediately placed in FAA fixative (2
157 formaldehyde: 1 acetic acid: 10 ethanol). Plants were processed in a similar manner to White &
158 Turner (2012). In brief, after at least 24 hours in fixative, fruits were gradually dehydrated in a
159 Tetrabutyl-Alcohol dehydration series, then mounted in paraffin wax with ~5% gum elemi resin.
160 Paraffin mounted specimens were then sliced to 8 micron ribbons and mounted onto slides. We
161 performed a staining series using Safranin-O and Fast-Green, which stain for nucleic acids and
162 carbohydrates, respectively.
163 We visualized and photographed at least three developing seeds from a single fruit for
164 each cross type and time point combination (40 unique combinations, 120 seeds measured). At
165 least 25 endosperm cell widths were measured per seed using ImageJ (Scheider *et al.* 2012).

Statistical Analyses

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

Results

Post-Mating Reproductive Isolation

All lines of *M. decorus* show consistent patterns of strong, and asymmetric hybrid seed inviability when crossed with *M. guttatus*, but the two genetic clades showed oppositely asymmetric HSI (Figure 1; Odell Creek: X^2= 4598, df=3, p<0.0001; IMP: X^2= 2969, df=3, p<0.0001). Northern *M. decorus* produces entirely inviable seeds when the maternal donor in these crosses, while southern *M. decorus* produces entirely inviable seeds when the paternal donor (and some inviable seeds when the maternal donor; Figure 1; Coughlan *et al.* 2018). We find some evidence of pollen-pistil incompatibilities between *M. guttatus* and southern *M. decorus* (Odell Creek: X^2= 49.33, df=3, p<0.0001; Figure S1), but no evidence of pollen-pistil interactions between *M. guttatus* and northern *M. decorus*, (although we note that IMP has substantially larger ovaries and produces more seeds; X^2= 23.7, df=3, p<0.0001; Figure S1). Inviable hybrid seeds are larger, disc-like, and shriveled, while viable hybrid seeds are smaller (as in Coughlan *et al.* 2018).

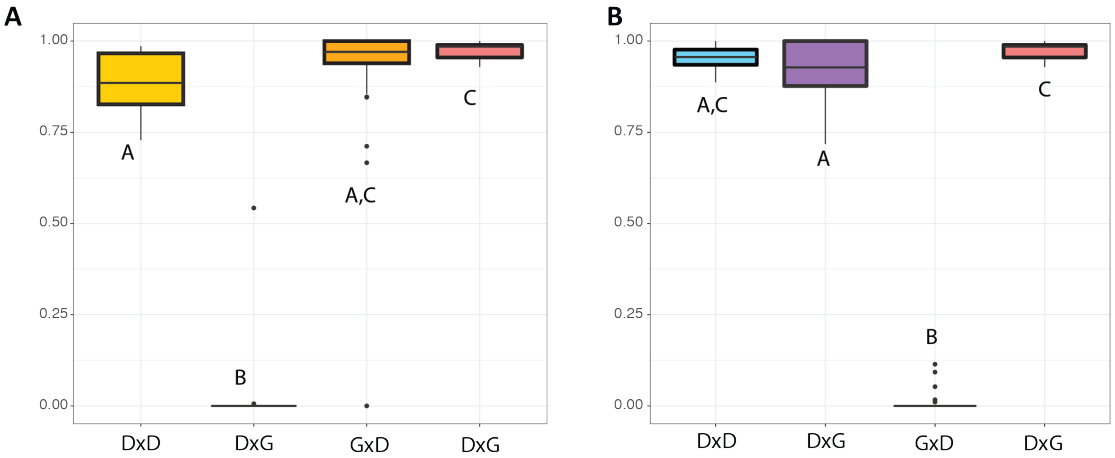


Figure 1: Crosses between *M. guttatus* (G) and each clade of *M. decorus* (D) shows oppositely asymmetric patterns of hybrid seed inviability. Proportion viable seed between three lines of *M. guttatus* (IM) and (A) four maternal families of northern *M. decorus* (IMP) and (B) three maternal families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

Developmental Trajectories

The three parental species exhibit 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 *M. guttatus* displaying a slightly accelerated growth, relative to either southern or northern *M. decorus* (i.e. *M. guttatus* reaches a heart-shaped embryo around 10 DAP, while this occurs in Odell and IMP around 14 DAP; Figure S2; Figure S3). By 14 DAP the embryo comprises most of the embryo sac, with a thin layer of endosperm remaining.

We find tremendous parent-of-origin effects on seed development in crosses between both clades *M. decorus* and *M. guttatus.* Reciprocal F1s from both sets of crosses show distinct developmental trajectories, but hybrid seeds display similar developmental trajectories, depending on seed fate (i.e. whether it will remain viable or not). In both sets of crosses, seeds that remain viable (e.g. IM×IMP and Odell×IM, with the maternal parent listed first) display a precocious development, with small endosperm cells that are quickly degraded by the embryo (Figure 2; Figure 3; Figure S2; Figure S3; Figure S4; Table S1) . By 14 DAP, the only remaining endosperm is a thin layer which surrounds the embryo. Total seed size is also much smaller than

either parent or the reciprocal hybrid (Coughlan *et al.* 2018). In contrast, the hybrid seeds which will eventually become inviable (i.e. IMP×IM and IM×Odell, with the maternal parent listed first) exhibit a chaotic endosperm growth, producing fewer, but larger and more diffuse endosperm cells (Figure 2; Figure 3; Figure S2; Figure S3; Table S1). Embryos in this direction of the cross remain small, relative to both parents and the reciprocal hybrid (Figure 2; Figure S2; Figure S3). By 14 DAP, most embryos were entirely degraded.

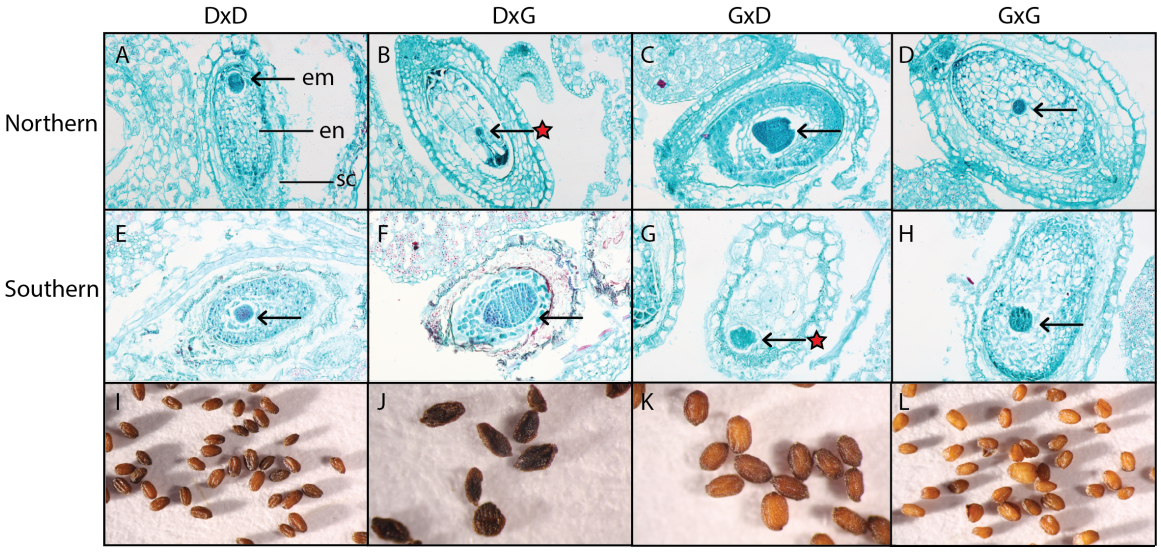


Figure 2: Parent of origin effects on endosperm proliferation between independent incidences of hybrid seed inviability. (A,D,E,H) Developing seeds of *M. guttatus* ('G') and two genetic clades of *M. decorus* ('D') at 8 days after pollination (DAP). Reciprocal F1s between *M. guttatus* and (B,C) the northern clade of *M. decorus* and (F,G) the southern clade of *M. decorus.* Maternal parent is listed first. Tissues are labeled in panel (A): em=embryo, en=endosperm, sc=seed coat. Arrows denote the location of the developing embryo, red stars indicate that these seeds will eventually become inviable. Fully developed seeds of (I) northern *M. decorus,* (L) *M. guttatus,* (J) a representative inviable seed, (K) a representative viable seed.

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 *M. guttatus* and both clades of *M. decorus*. For example, in northern *M. decorus* by *M. guttatus* crosses, where northern *M. decorus* is the maternal donor, the embryo remains comparatively small, and does not appear to grow past the 6 or 8 DAP stages. In contrast, seeds formed from between an *M. guttatus* mother and southern *M. decorus* father often exhibit a developing embryo up until 10 DAP, suggesting that the seed is aborted slightly later in this cross.

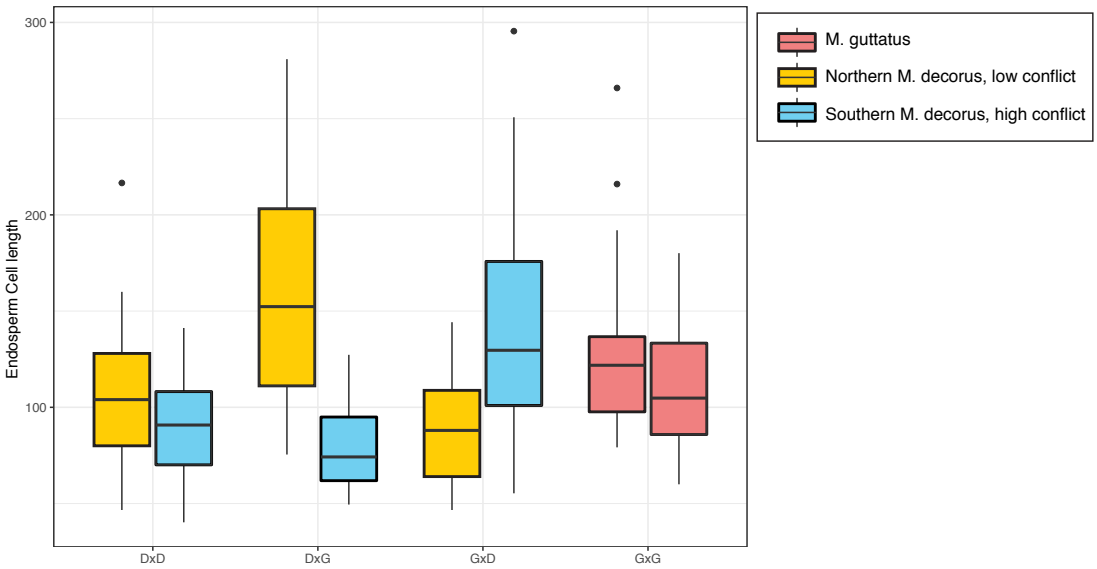


Figure 3: Parent-of-origin effects on endosperm growth are recapitulated at the cellular level. Cell width at 8 Days After Pollination (DAP) for each cross type (maternal parent listed first), for both *M. guttatus* crossed to IMP (yellow) and Odell Creek (blue) are shown. See Supplemental Figure 2 for 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, though both reciprocal F1 seeds show parent-of-origin specific developmental defects.

Discussion

The northern and southern clades of *M. decorus* exhibit strong, and asymmetric reproductive isolation with *M. guttatus*, but in opposite directions of the cross. This observation is true for all maternal family/line combinations tested here (as well as multiple populations tested in Coughlan *et al.* 2018), suggesting that HSI is not geographically restricted, as other intrinsic post-zygotic barriers have been found to be (Sweigart *et al.* 2007; Case & Willis 2008; Martin & Willis 2010; Sicard *et al.* 2015; Barnard-Kubow & Galloway 2017; Zuellig & Sweigart 2018). Total reproductive isolation in these crosses was ~52% for both species pairs, and thus

264 may represent a significant barrier to reproduction. HSI has been shown to be a formidable
265 barrier to reproduction in other systems (i.e. Tiffin *et al.* 2001; Lowry *et al.* 2008), but the
266 developmental causes of this incompatibility have only been quantified for a handful of diploid
267 species pairs. We aimed to determine the developmental trajectories of inviable and viable
268 hybrid seeds in two independent incidences of HSI between close relatives in the *Mimulus*
269 *guttatus* species complex.

270 We show that HSI is strongly associated with developmental defects in endosperm
271 deposition. In inviable directions of the cross, seeds show a paternal-excess phenotype, where
272 endosperm is chaotically distributed, and the cells are few and large. In the viable directions of
273 the cross, seeds show a maternal-restrictive phenotype, where endosperm develops precociously,
274 as witnessed by the general lack of endosperm remaining when seeds are fully developed, as well
275 as the generally smaller size of both the cells and final seeds (Coughlan *et al.* 2018). Overall,
276 these developmental patterns are consistent with a parental conflict model of hybrid inviability,
277 wherein paternal excess is more likely to lead to seed abortion. Unfortunately, given the
278 generally low success rate of the embryo rescues (Coughlan *unpublished*), it remains unclear
279 whether hybrid seed inviability is due strictly to malformation of the endosperm, or whether
280 genetic incompatibilities in the embryo also contribute.

281 Maternal-restriction/paternal-excess endosperm phenotypes have been shown in hybrids
282 between wild species pairs of both *Arabidopsis* and *Capsella*, which have a nuclear-type
283 endosperm development (Rebernig *et al.* 2015; Lafon-Placette *et al.* 2017; Lafon-Placette *et al.*
284 2018). In diploid *Arabidopsis* and *Capsella* crosses, as well as interploidy crosses, endosperm
285 over-proliferation is accompanied by a failure of endosperm cells to cellularize (Scott *et al.*
286 1998; Bushell *et al.* 2003; Rebernig *et al.* 2015; Lafon-Placette *et al.* 2017; Lafon-Placette *et al.*
287 2018), and at least in *Capsella rubella* and *Capsella grandiflora,* paternal excess is associated
288 with inviability, but maternal excess still produces viable seed (Rebernig *et al.* 2015). Findings
289 from species with cellular-type endosperm development (e.g. wild tomatoes and other *Mimulus*
290 species pairs) also show endosperm defects as a major cause of inviability (Roth *et al.* 2017;
291 Oneal *et al.* 2016). However, these defects do not represent defects in rates of cellularization, but
292 rather rates of proliferation, cell size and distribution, and optical density (Oneal *et al.* 2016;
293 Roth *et al.* 2017). Our results are consistent with these findings, where inviable seeds display

294 large, chaotically distributed endosperm cells, but there are fewer of them, suggesting that issues
295 in the regular timing of cell division may be at play.
296 The developmental trajectories of viable versus non-viable hybrid seeds are strikingly
297 similar between crosses of *M. guttatus* and both clades of *M. decorus*, despite the fact that
298 inviability occurs in the opposite cross direction. This degree of developmental parallelism in
299 independent incidences of HSI suggests that similar evolutionary processes may cause HSI
300 within this complex. Given the maternal-restriction/paternal-excess phenotypes that we observe,
301 one hypothesis is parental conflict. Despite considerable developmental parallelism, it remains to
302 be seen how constrained the genetic targets of selection are between these species pairs. In other
303 post-zygotic reproductive barriers, canonical hybrid incompatibilities often share similar genetic
304 bases. For example, hybrid necrosis is caused by interactions involving R genes (reviewed in
305 Bomblies & Weigel 2007), and CMS generally involves PPR genes (Polcentric Peptide Repeat
306 genes; reviewed in Rieseberg & Blackman 2010). Whether this remains true for HSI remains to
307 be seen. A thorough examination of the molecular and developmental genetics of HSI in this
308 system is necessary to answer these questions, and will inform our understanding of the
309 proximate and ultimate causes of HSI.
310

311 **Acknowledgements**

312 We thank members of the Willis lab- particularly Elen Oneal, as well as Miguel Florez and Bob
313 Franks for useful discussions of this project. We are grateful to Melvin Turner for teaching JMC
314 histological techniques, and to Dick White for allowing us access to his lab space and
315 equipment. This project was funded from NSF grants EF-0328636 and EF-0723814 to JHW, a
316 DDIG (DEB- 1501758), ASN Student research award and SSE student research award to JMC.
317 The Duke Graduate School also provided funding to JMC with the Myra and William Waldo
318 Boone fellowship, and financial support was provided from the Duke Biology Department.
319

320 **Data Availability**

321 Data for the manuscript will be made publically available.

322

323 **References**

Barnard-Kubow, K. B., & Galloway, L. F. (2017). Variation in reproductive isolation across a species range. *Ecology and Evolution*, (August), 1–11.

Bomblies, K., & Weigel, D. (2007). Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nature Reviews. Genetics*, *8*(5), 382–393.

Burkart-Waco, D., Josefsson, C., Dilkes, B., Kozloff, N., Torjek, O., Meyer, R., … Comai, L. (2012). Hybrid incompatibility in Arabidopsis is determined by a multiple-locus genetic network. *Plant Physiology*, *158*(2), 801–812.

Bushell, C., Spielman, M., & Scott, R. J. (2003). The basis of natural and artificial postzygotic hybridization barriers in Arabidopsis species. *The Plant Cell*, *15*(6), 1430–1442.

Case, A. L., & Willis, J. H. (2008). Hybrid male sterility in Mimulus (Phrymaceae) is associated with a geographically restricted mitochondrial rearrangement. *Evolution*, *62*(5), 1026–1039.

Chase, C. D. (2007). Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear interactions. *Trends in Genetics*, *23*(2), 81–90.

Coughlan, J.M., Wilson Brown, M, Willis, J.H. 2018. Patterns of hybrid seed inviability in perennials of the Mimulus guttatus sp. complex reveal a potential role of parental conflict in reproductive isolation. *BioRxiv.*

Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sunderland, Massachusett: Sinauer.

Derkacheva, M., Liu, S., Figueiredo, D. D., Gentry, M., Mozgova, I., Nanni, P., … Hennig, L. (2016). H2A deubiquitinases UBP12/13 are part of the Arabidopsis polycomb group protein system. *Nature Plants*, *2*(9), 16126.

Dilkes, B. P., Spielman, M., Weizbauer, R., Watson, B., Burkart-Waco, D., Scott, R. J., & Comai, L. (2008). The Maternally Expressed WRKY Transcription Factor TTG2 Controls Lethality in Interploidy Crosses of Arabidopsis. *PLoS Biology*, *6*(12), e308.

Erilova, A., Brownfield, L., Exner, V., Rosa, M., Twell, D., Scheid, O. M., … Köhler, C. (2009). Imprinting of the Polycomb group gene MEDEA serves as a ploidy sensor in Arabidopsis. *PLoS Genetics*, *5*(9).

Gehring, M., Bubb, K. L., & Henikoff, S. (2009). Extensive Demethylation of Repetitive. *Science*, *324*(June), 1447–1451.

Gehring, M., Huh, J. H., Hsieh, T. F., Penterman, J., Choi, Y., Harada, J. J., … Fischer, R. L. (2006). DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. *Cell*, *124*(3), 495–506.

Haig, D., & Mark Westoby. (1989). Parent-specific gene expression and the triploid endosperm. *American Naturalist*, *134*(1), 147–155.

357 Hanson, M. R., & Bentolila, S. (2004). Interactions of mitochondrial and nuclear genes that
358 affect male gametophyte development. *The Plant Cell Online*, *16*, 154–170.

359 Hsieh, T. (2009). Genome-Wide Demethylation of. *Science*, *1451*(June), 1451–1455.

360 Josefsson, C., Dilkes, B., & Comai, L. (2006). Parent-Dependent Loss of Gene Silencing during
361 Interspecies Hybridization. *Current Biology*, *16*, 1322–1328.

362 Ko, C., Köhler, C., & Weinhofer-Molisch, I. (2010). Mechanisms and evolution of genomic
363 imprinting in plants. *Heredity*, *105*(1), 57–63.

364 Köhler, C., Hennig, L., Spillane, C., Pien, S., Gruissem, W., & Grossniklaus, U. (2003). The
365 Polycomb-group protein MEDEA regulates seed development by controlling expression of
366 the MADS-box gene PHERES1. *Genes and Development*, *17*(12), 1540–1553.

367 Köhler, C., & Makarevich, G. (2006). Epigenetic mechanisms governing seed development in
368 plants. *EMBO Rep*, *7*(12), 1223–1227.

369 Köhler, C., Page, D. R., Gagliardini, V., & Grossniklaus, U. (2005). The Arabidopsis thaliana
370 MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting.
371 *Nature Genetics*, *37*(1), 28–30.

372 Köhler, C., Wolff, P., & Spillane, C. (2012). Epigenetic Mechanisms Underlying Genomic
373 Imprinting in Plants. *Annual Review of Plant Biology*, *63*(1), 331–352.

374 Kradolfer, D., Wolff, P., Jiang, H., Siretskiy, A., & Köhler, C. (2013). An imprinted gene
375 underlies postzygotic reproductive isolation in arabidopsis thaliana. *Developmental Cell*,
376 *26*(5), 525–535.

377 Lafon-Placette, C., Hatorangan, M. R., Steige, K. A., Cornille, A., Lascoux, M., Slotte, T., &
378 Köhler, C. (2018). Paternally expressed imprinted genes associate with hybridization
379 barriers in Capsella. *Nature Plants*.

380 Lafon-Placette, C., Johannessen, I. M., Hornslien, K. S., Ali, M. F., Bjerkan, K. N., Bramsiepe,
381 J., … Köhler, C. (2017). Endosperm-based hybridization barriers explain the pattern of gene
382 flow between *Arabidopsis lyrata* and *Arabidopsis arenosa* in Central Europe. *Proceedings*
383 *of the National Academy of Sciences*, 201615123.

384 Lafon-Placette, C., & Köhler, C. (2016). Endosperm-based postzygotic hybridization barriers:
385 Developmental mechanisms and evolutionary drivers. *Molecular Ecology*, 2620–2629.

386 Lowry, D. B., Modliszewski, J. L., Wright, K. M., Wu, C. A., & Willis, J. H. (2008). The
387 strength and genetic basis of reproductive isolating barriers in flowering plants.
388 *Philosophical Transactions of the Royal Society B*, *363*(1506), 3009–3021.

Makarevich, G., Leroy, O., Akinci, U., Schubert, D., Clarenz, O., Goodrich, J., … Köhler, C. (2006). Different Polycomb group complexes regulate common target genes in Arabidopsis. *EMBO Reports*, *7*(9), 947–952.

Martin, N. H., & Willis, J. H. (2010). Geographical variation in postzygotic isolation and its genetic basis within and between two Mimulus species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3261–3261.

Oneal, E., Willis, J. H., & Franks, R. G. (2016). Disruption of endosperm development is a major cause of hybrid seed inviability between Mimulus guttatus and Mimulus nudatus. *New Phytologist*, *210*, 1107–1120.

Rebernig, C. a., Lafon-Placette, C., Hatorangan, M. R., Slotte, T., & Köhler, C. (2015). Non-reciprocal Interspecies Hybridization Barriers in the Capsella Genus Are Established in the Endosperm. *PLOS Genetics*, *11*(6), e1005295.

Rieseberg, L. H., & Blackman, B. K. (2010). Speciation genes in plants. *Annals of Botany*, *106*(3), 439–455.

Rolán-Alvarez, E., Carballo, M., Galindo, J., Morán, P., Fernández, B., Caballero, A., … Johannesson, K. (2004). Nonallopatric and parallel origin of local reproductive barriers between two snail ecotypes. *Molecular Ecology*, *13*(11), 3415–3424.

Roth, M., Florez-Rueda, A. M., Griesser, S., Paris, M., & Städler, T. (2017). Incidence and developmental timing of endosperm failure in postzygotic isolation between wild tomato lineages. *Annals of Botany*, (January), 1–12.

Rundle, H. D., Nagel, L., Boughman, J. W., & Schluter, D. (2000). Natural Selection and Parellel Speciation in Sympatric Sticklebacks. *Science*, *287*(5451), 306–308.

Schneider, C. a., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675.

Scott, R. J., Spielman, M., Bailey, J., & Dickinson, H. G. (1998). Parent-of-origin effects on seed development in Arabidopsis thaliana. *Development (Cambridge, England)*, *125*(October 2014), 3329–3341.

Sicard, A., Kappel, C., Josephs, E. B., Lee, Y. W., Marona, C., Stinchcombe, J. R., … Lenhard, M. (2015). Divergent sorting of a balanced ancestral polymorphism underlies the establishment of gene-flow barriers in Capsella. *Nature Communications*, *6*, 7960.

Soria-Carrasco, V., Gompert, Z., Comeault, A. A., Farkas, T. E., Parchman, T. L., Johnson, S. J., … Nosil, P. (2014). Stick insect genomes reveal natural selection's role in parallel speciation. *Science*, *344*(May), 1497–1502.

Stankowski, S. (2013). Ecological speciation in an island snail : evidence for the parallel evolution of a novel ecotype and maintenance by ecologically dependent postzygotic isolation. *Animal Biology*, 2726–2741.

Stuart, Y. E., Veen, T., Weber, J. N., Hanson, D., Ravinet, M., Lohman, B. K., … Bolnick, D. I. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature Ecology and Evolution*, *1*(6), 1–7.

Sweigart, A. L., Mason, A. R., & Willis, J. H. (2007). Natural variation for a hybrid incompatibility between two species of mimulus. *Evolution*, *61*(1), 141–151.

Tiffin, P., Olson, M. S., & Moyle, L. C. (2001). Asymmetrical crossing barriers in angiosperms. *Proceedings. Biological Sciences / The Royal Society*, *268*(1469), 861–867.

Trivers, R. L. (1974). Parent-Offspring Conflict. *American Zoology*, *14*(1), 249–264.

Waters, A. J., Makarevitch, I., Eichten, S. R., Swanson-Wagner, R. A., Yeh, C.-T., Xu, W., … Springer, N. M. (2011). Parent-of-Origin Effects on Gene Expression and DNA Methylation in the Maize Endosperm. *The Plant Cell*, *23*(12), 4221–4233.

White, R. A., & Turner, M. D. (2012). The Anatomy and Occurrence of Foliar Nectaries in Cyathea (Cyatheaceae). *American Fern Journal*, *102*(2), 91–113.

Wolff, P., Jiang, H., Wang, G., Santos-González, J., & Köhler, C. (2015). Paternally expressed imprinted genes establish postzygotic hybridization barriers in *Arabidopsis thaliana*. *ELife*, *4*, 1–14.

Wolff, P., Weinhofer, I., Seguin, J., Roszak, P., Beisel, C., Donoghue, M. T. a, … Köhler, C. (2011). High-Resolution analysis of parent-of-origin allelic expression in the Arabidopsis endosperm. *PLoS Genetics*, *7*(6).

Zuellig, M. P., & Sweigart, A. L. (2018). A two-locus hybrid incompatibility is widespread, polymorphic, and active in natural populations of Mimulus. *Evolution*, 1–12.

Figures

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

457

458 Figure 2: Parent of origin effects on endosperm proliferation between independent incidences of
459 hybrid seed inviability. (A,D,E,H) Developing seeds of *M. guttatus* ('G') and two genetic clades
460 of *M. decorus* ('D') at 8 days after pollination (DAP). Reciprocal F1s between *M. guttatus* and
461 (B,C) the northern clade of *M. decorus* and (F,G) the southern clade of *M. decorus.* Maternal
462 parent is listed first. Tissues are labeled in panel (A): em=embryo, en=endosperm, sc=seed coat.
463 Arrows denote the location of the developing embryo, red stars indicate that these seeds will
464 eventually become inviable. Fully developed seeds of (I) northern *M. decorus,* (L) *M. guttatus,*
465 (J) a representative inviable seed, (K) a representative viable seed.

466

467 Figure 3: Parent-of-origin effects on endosperm growth are recapitulated at the cellular level.
468 Cell width at 8 Days After Pollination (DAP) for each cross type (maternal parent listed first),
469 for both *M. guttatus* crossed to IMP (yellow) and Odell Creek (blue) are shown. See
470 Supplemental Figure 2 for all sampled time points.

471
472
473
474
475
476

477 **Supplemental Figures**

478

479 **Table S1: Mixed model for type III ANOVAs for seed cell size across developmental time**
480 **for IMP and Odell Creek crossed reciprocally to IM62. Cross Type= both reciprocal F1**
481 **crosses and self-fertilizations from each species treated as a separate level; treated as a**
482 **fixed effect), DAP= Days After Pollination (fixed effect). Seed replicate was treated as a**
483 **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

484

485

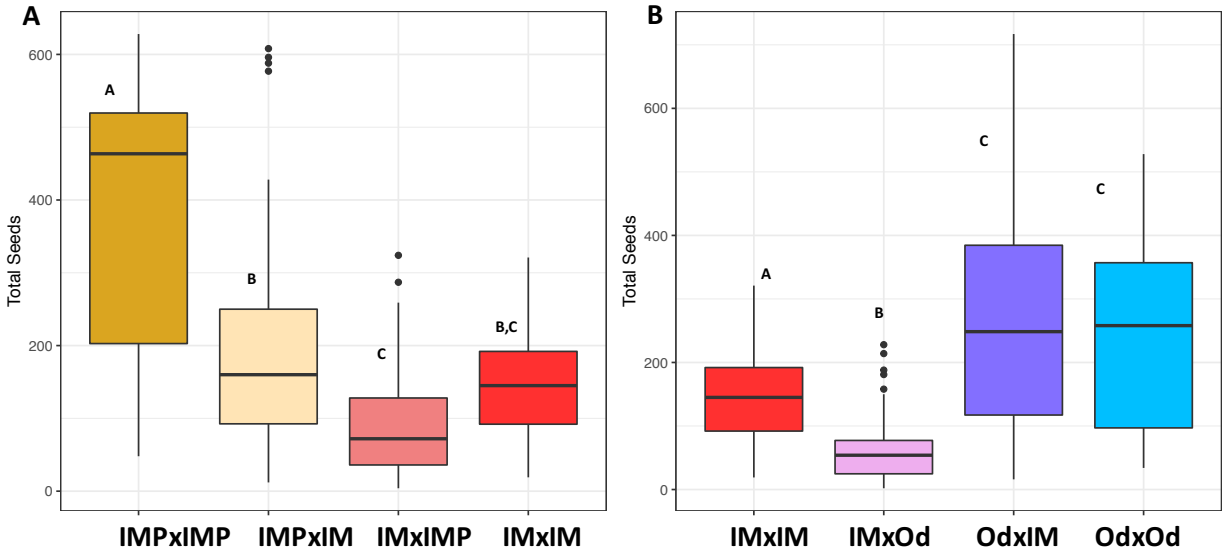
486

487

488

489

490



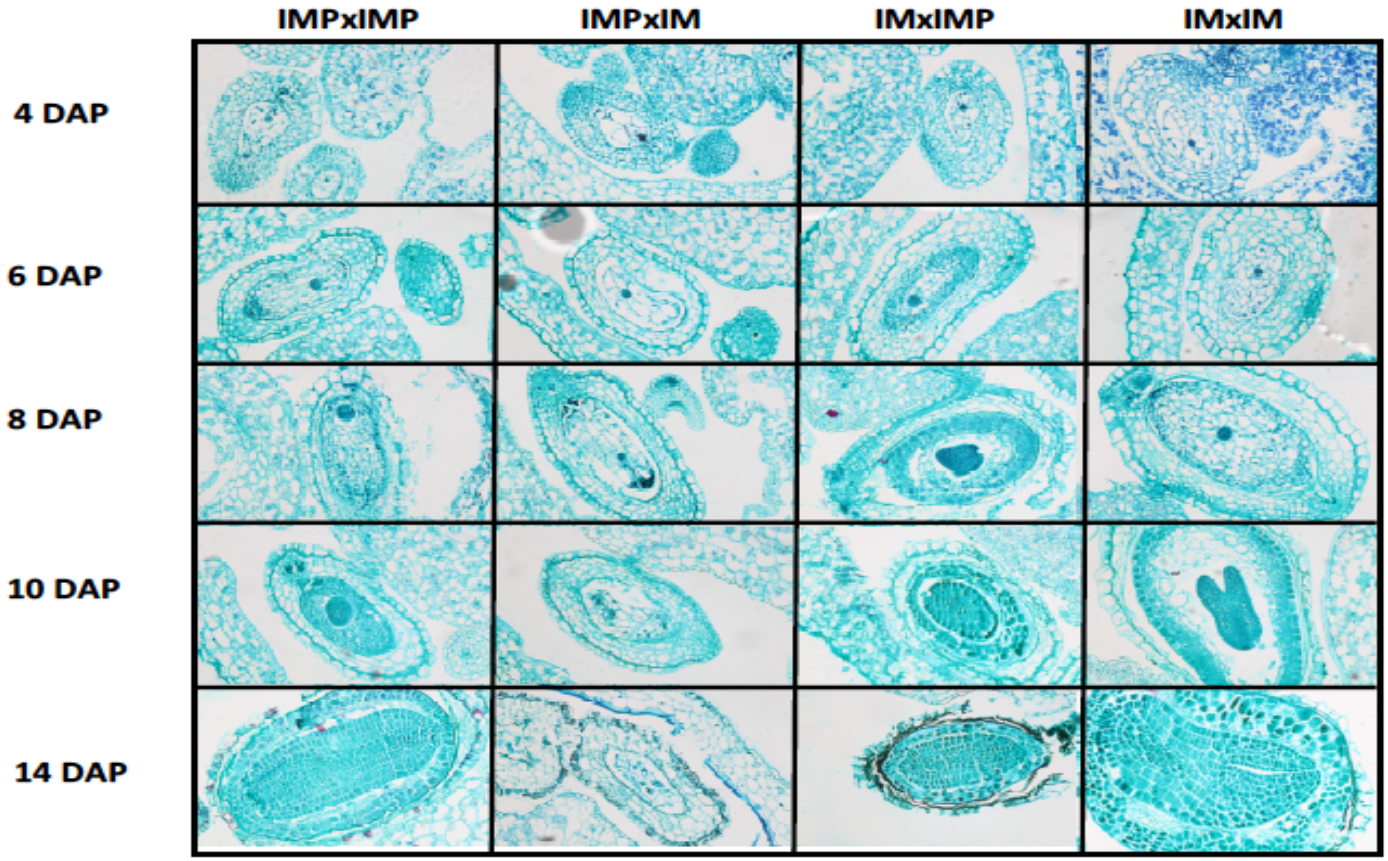
491

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

495

496

497



498

499 **Figure S2: Reciprocal hybrids between *M. guttatus* and northern *M. decorus* show significant parent-of-origin effects on**
500 **endosperm growth. Developing seeds between IM62 x IMP at 4, 6, 8, 10, and 14 Days After Pollination (DAP) are shown.**
501 **Maternal parent is listed first.**

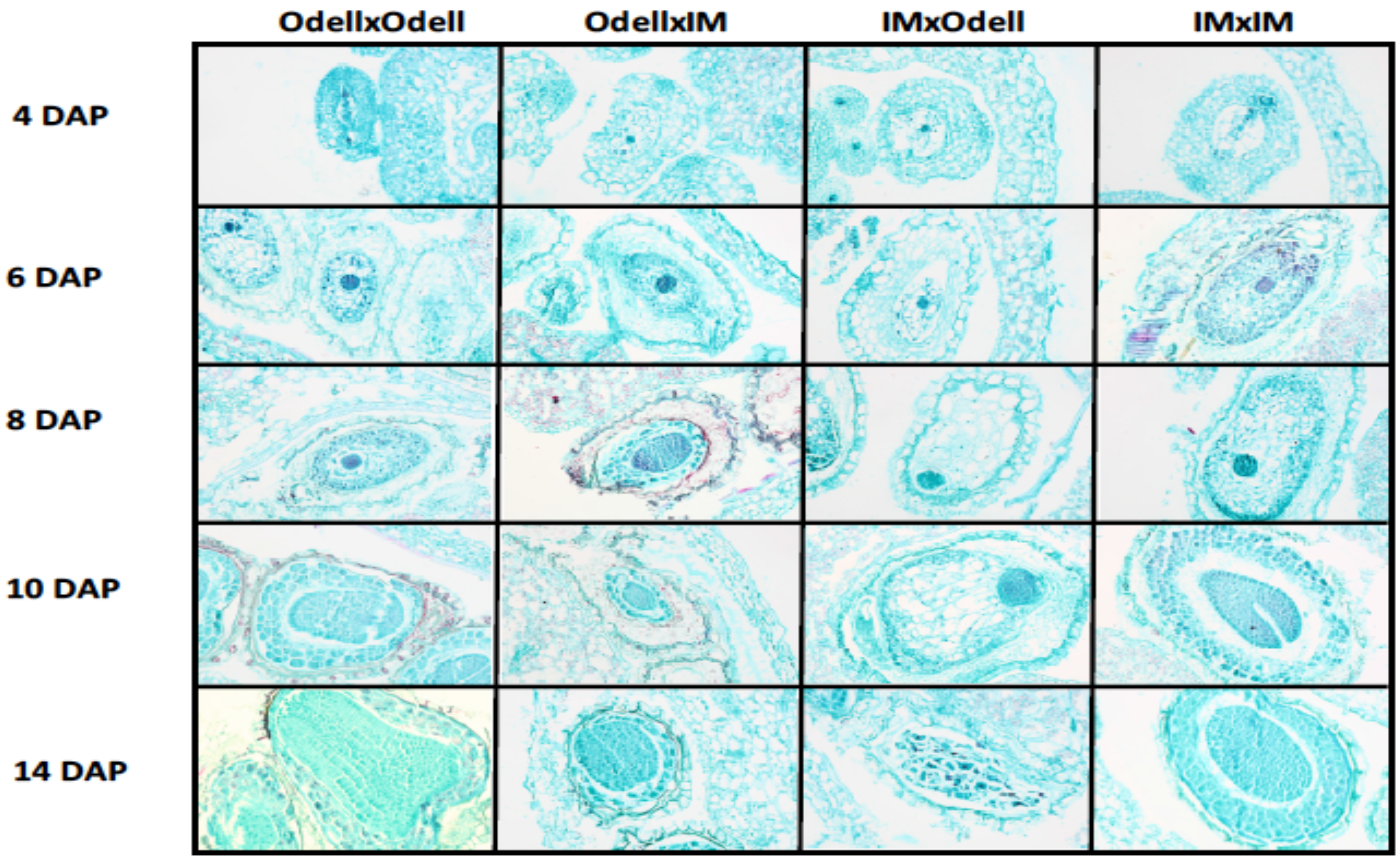
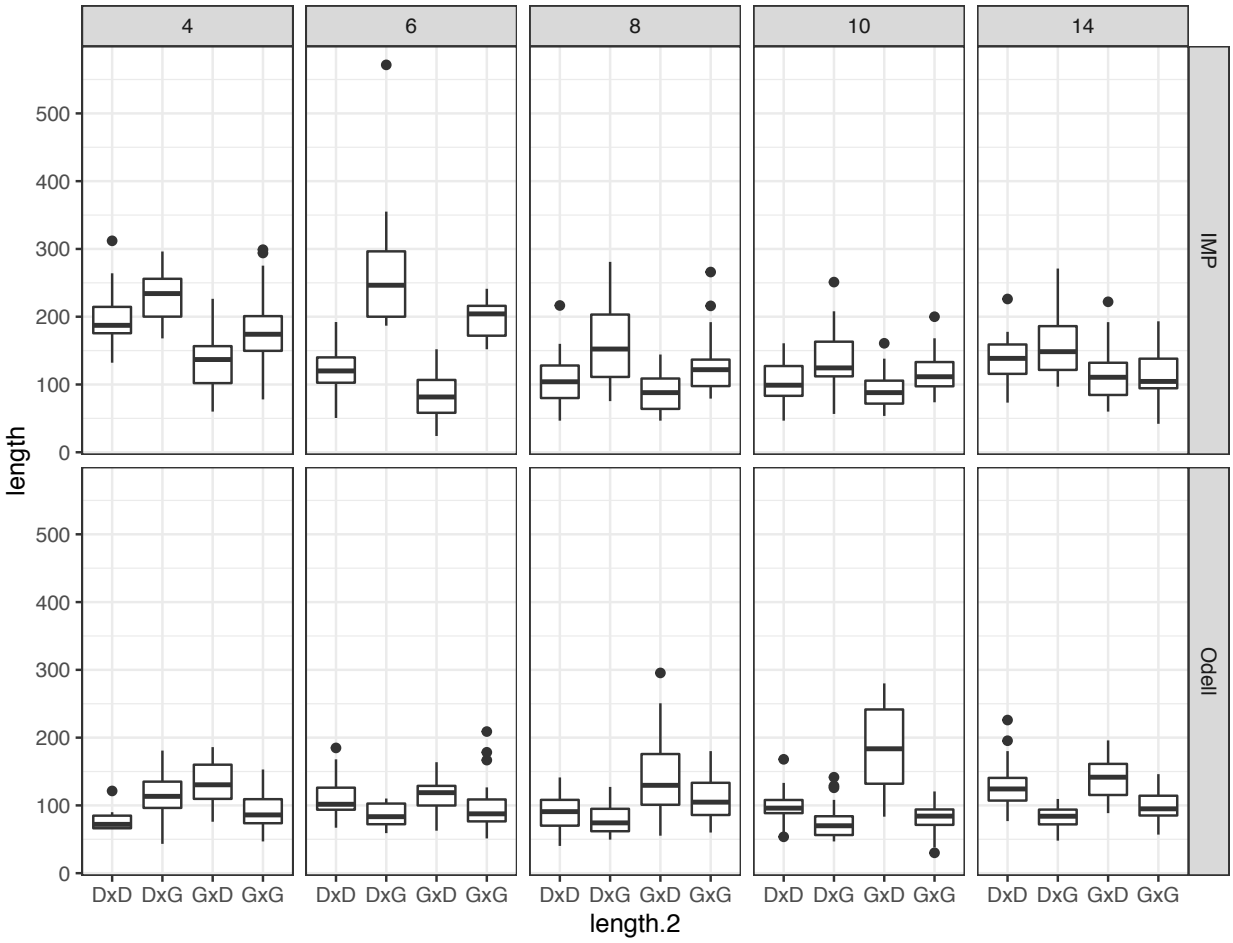


Figure S3: Reciprocal hybrids between *M. guttatus* and southern *M. decorus* show significant parent-of-origin effects on endosperm growth. Developing seeds between IM62 x Odell Creek at 4, 6, 8, 10, and 14 Days After Pollination (DAP) are shown. Maternal parent is listed first.

506



507

508 **Figure S4: Cell lengths across all cross types for 5 developmental stages for both IMxIMP**

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

510

511