1   **Indirect effects of parental conflict on conspecific offspring development**

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7   *Key Words:* Endosperm, Hybridization, Hybrid Seed Inviability, Resource Competition,

8   Reproductive Isolation, Secondary Contact

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10   *Running head: Hybridization influences intraspecific seed development*

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12   *Total Words of Main Text:* 2895

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14   *Number of Figures:* 2

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16   *Author Contributions:* JMC conceived of this project, performed all experiments, analyzed the

17   data and wrote the paper.

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Abstract

Hybrid Seed Inviability (HSI) is a common barrier in angiosperms. Recent work suggests that the rapid evolution of HSI may, in part, be due to conflict between maternal and paternal optima for resource allocation to developing offspring (i.e. parental conflict). However, parental conflict requires that paternally-derived resource acquiring alleles impose a maternal cost. I test this requirement using three closely related species in the *Mimulus guttatus* species complex that exhibit significant HSI and differ in their inferred histories of parental conflict. I show that the presence of hybrid seeds significantly affects conspecific seed size for almost all crosses, such that conspecific seeds are smaller after developing with hybrids from fathers with a stronger history of conflict, and larger after developing with hybrids from fathers with a weaker history of conflict. This work demonstrates a cost of paternally-derived alleles, and also has implications for species fitness in secondary contact.

Introduction

54   A fundamental source of conflict in viviparous organisms stems from differences

55   between maternal and paternal optima for how many resources to allocate to developing

56   offspring (i.e. parental conflict (Trivers 1974; Charnov 1979; Haig and Westoby 1989)). This is

57   because in non-monogamous systems while maternity is guaranteed, fathers are not equally

58   related to all offspring produced within a brood, and thus selection can favor the evolution of

59   paternally-derived, resource acquiring alleles that come at a cost to either mothers directly or

60   indirectly by influencing other developing offspring in that brood (e.g. 'greedy alleles'; (Trivers

61   1974; Charnov 1979; Haig and Westoby 1989; Haig 1997; Wilkins and Haig 2001; Brandvain

62   2010)). Consequently, selection will then favor maternally-derived, resource repressive alleles,

63   and a co-evolutionary arms race may subsequently evolve (Trivers 1974; Charnov 1979; Haig

64   and Westoby 1989; Haig 1997; Wilkins and Haig 2001; Brandvain 2010). As such, parental

65   conflict theory predicts that the severity of parental conflict is a reflection of the variance in

66   paternity within broods (Queller 1984; Brandvain and Haig 2005; Brandvain et al. 2011; Willi

67   2013; Raunsgard et al. 2018). Parental conflict may be particularly important in systems where

68   nutrients are partitioned directly, dynamically, and post-fertilization from maternal parents to

69   developing offspring *in utero* via an intermediary tissue, such as placental or endosperm (Haig

70   and Westoby 1989; Moore and Haig 1991; Zeh and Zeh 2000).

71   In seed plants, the endosperm is a nutritive tissue that is essential for proper embryo

72   development and is analogous to the placenta in mammals. Endosperm arises via the

73   fertilization of the central cell; a di-haploid structure within the megagametophyte, resulting in a

74   triploid tissue that is 2 maternal genomes: 1 paternal genome. The balance of 2m:1p in the

75   endosperm is crucial for its development, as many genes which are essential for proper

76   endosperm development are imprinted (i.e. genes are expressed based on whether they are

77   maternally or paternally derived), the balance of which allows development to proceed normally

78   (Scott et al. 1998; Köhler and Weinhofer-Molisch 2010). Much research using interploidy

79   crosses has demonstrated that an overexpression of maternally expressed or paternally

80   expressed genes results in canonical developmental defects across multiple plant systems and

81   endosperm developmental programs (Scott et al. 1998; Kradolfer et al. 2013; Wolff et al. 2015;

82   Lafon-Placette and Köhler 2016; Lafon-Placette et al. 2017; Morgan et al. 2021). Strikingly,

83   excess of maternal or paternal expression results in growth-repressive or growth-excessive

84   phenotypes, reminiscent of predictions of parental conflict. If these endosperm defects are

85   severe enough they can cause embryo death, and as such seed inviability is thought to be a

86   crucial reproductive barrier between plants of different ploidy levels (often referred to as 'Triploid

87   Block'; (Comai 2005; Köhler et al. 2010, 2021; Sutherland and Galloway 2017; Morgan et al.

88   2021)).

89   Yet, Hybrid Seed Inviability (HSI) is also common in diploid plant systems and largely

90   results from parent-of-origin specific growth defects in the endosperm (Brandvain and Haig

91   2005; Lowry et al. 2008; Briscoe Runquist et al. 2014; Rebernig et al. 2015; Garner et al. 2016;

92   Lafon-Placette and Köhler 2016; Oneal et al. 2016; Lafon-Placette et al. 2017, 2018; Roth et al.

93   2018*b*, 2019; Coughlan et al. 2020*b*; Sandstedt et al. 2020; Gustafsson et al. 2021; İltaş et al.

94   2021). These patterns, while strikingly similar to the defects exemplified in interploidy crosses

95   (Lafon-Placette and Köhler 2016; Lafon-Placette et al. 2017; Städler et al. 2021), cannot be

96   explained by genome-wide imbalances of maternal:paternal gene expression in the endosperm,

97   and must involve the evolution of paternal-excessive and maternal-repressive alleles. The

98   observation that interspecific diploid crosses often mirror inter-ploidy crosses has sparked a

99   conceptual framework to categorize diploid taxa according to the extent of maternal-repression

100   and paternal-excess that they exhibit when crossed to other diploids, referred to as their

101   Endosperm Balance Number (EBN;(Johnston et al. 1980; Katsiotis et al. 1995; Carputo et al.

102   1999; Johnston and Hanneman 1999; Lafon-Placette and Köhler 2016; Lafon-Placette et al.

103   2018; Städler et al. 2021)), or genome strength (Brandvain and Haig 2018). Differences

104   between taxa in EBNs are thought to reflect different histories of parental conflict (Lafon-

105   Placette et al. 2018; Raunsgard et al. 2018; Coughlan et al. 2020*b*; Städler et al. 2021).

106   Although its role in underlying HSI is garnering much current support (Lafon-Placette et

107   al. 2018; Coughlan et al. 2020*b*; İltaş et al. 2021), parental conflict may also play a secondary

108   role in speciation in the context of secondary contact. Hybridization between closely related

109   species that vary in EBNs can not only result in the loss of gametes (i.e. reproductive

110   interference), but when hybrids are formed in a brood that also contains conspecific offspring,

111   differences in the competitive ability between hybrid and conspecific siblings borne through

112   differences in paternally-derived resource acquiring alleles may affect conspecific size, and

113   consequently offspring fitness. In seed plants, seed size is often a proxy for various fitness

114   components (such as the probability of germination, seedling size, and the number of flowers

115   produced (Stanton 1984; Krannitz et al. 1991; Simons and Johnston 2000; Gómez 2004)).

116   Thus, competition for limited maternal resources between hybrid and intraspecific siblings may

117   have substantial implications for intraspecific fitness in secondary contact zones.

118   Here I use a model organism for ecology, evolution, and genetics; the *Mimulus guttatus*

119   species complex to address if conspecific seed size varies when seeds are grown only with

120   other conspecific siblings versus hybrid siblings from sires with different EBNs. Previously, I

121   have shown that *M. guttatus* and a closely related species, *M. decorus,* are reproductively

122   isolated by HSI, and patterns of HSI support a role for parental conflict (Coughlan et al. 2020*b*).

123   Namely, hybrid seeds exhibit parent-of-origin specific growth defects (maternal-repressive and

124   paternal-excess phenotypes) that are associated with parent-of-origin specific endosperm

125   defects (Coughlan et al. 2020*b*). *Mimulus decorus* comprises two distinct diploid lineages; one

126   that exhibits substantially lower EBN than *M. guttatus* and one that exhibits a higher EBN than

127   *M. guttatus*, despite a relatively recent divergence time (roughly 230kya; (Coughlan et al.

128   2020*b*)). Here, I leverage this diversity of EBNs in this group to assess indirect growth effects in

129   conspecific seeds when these seeds are grown alongside hybrid siblings that vary in their

130   father's EBN. This work is one of the first to provide an explicit test of the cost of paternally

131   derived, resource acquiring alleles; a central prediction of parental conflict. Moreover, the results

132   of this experiment have implications for secondary contact between species that differ in EBN.

133

134   **Materials & Methods**

135   *Plant materials and crosses*

136   I grew between 6-16 replicate lines of a single genotype for each of *M. guttatus* (*IM62*),

137   Southern *M. decorus* (*OD11*), and Northern *M. decorus* (*HWY15D*). Previous work using

138   population genomics and experimental crosses suggests that Northern *M. decorus* produces

139   relatively weak dams and sires (i.e. has a low EBN), and is predicted to have experienced the

140   weakest history of parental conflict, while Southern *M. decorus* produces relatively strong dams

141   and sires (i.e. a high EBN), and is predicted to have experienced the strongest history of

142   parental conflict (Coughlan et al. 2020*b*). *Mimulus guttatus* exhibits an EBN intermediate to the

143   two genetic lineages of *M. decorus* (i.e. stronger than Northern *M. decorus,* but weaker than

144   Southern *M. decorus*; *(Coughlan et al. 2020b)*)*.* I first cold and dark stratified seeds in water for

145   one week, then dispersed them on moist Fafard 4P soil (SunGro Horticultural Inc) in the

146   University of North Carolina at Chapel Hill greenhouses. I transferred early germinants to

147   individual 2 1/2" pots and grew plants in warm, long day conditions (16hours of light/ 20C).

148   I emasculated individual flowers at least one day before flowers would have naturally

149   opened and well before natural pollen dehiscence. All species used in this experiment have a

150   highly outcrossing morphology and very rarely (if ever) set autogamous selfed seed in the

151   greenhouse (Coughlan and Willis 2019; Coughlan et al. 2020*b*). In accordance with this

152   observation, no unpollinated stigma bore a fruit during the course of this experiment. For each

153   species, stigmas were pollinated with either pure self pollen or a mixture of self pollen and

154   pollen from another species. For both pollination types, anthers were manually dehisced on a

155   glass slide, then I manually transferred pollen to an open stigma using fine forceps. For the

6

156   mixed pollination treatment, anthers were manually dehisced until a roughly equal quantity of

157   pollen from each species was available, then pollen samples were thoroughly mixed with fine

158   forceps. The exact ratio of pollen from each parent is unlikely to have been equal for every

159   cross, and indeed fertilization rates between intra- and inter-specific fathers did significantly

160   differ for may fruits, which may reflect departures from a 1:1 ratio of pollen genotypes or

161   differences in competitive ability of pollen genotypes (see Table S2). Nonetheless, the aim of

162   this work is to assess if the presence of hybrid siblings influences intraspecific seed size, and as

163   such an exact 1:1 ratio of paternal genotypes in the pollen is unnecessary. I collected fully

164   ripened fruits shortly before natural dehiscence. All fruits were dried at ambient temperatures for

165   at least one week before seeds were counted, categorized, and measured. In total, an average

166   of 26.5 fruits (range: 20-31 fruits) were quantified for each species and cross type combination

167   (see Figure 1 for overview of experimental design).

168

169   *Seed Quantification*

170   For each cross, all fertilized seeds were counted and quantified for viability based on

171   morphology, with shriveled, disc-like, or concave seeds scored as inviable. Morphology has

172   previously been shown to be a good indicator of viability in *Mimulus* (Garner et al. 2016;

173   Coughlan et al. 2020*b*). In four of the six mixed pollen treatments, hybrids are almost always

174   inviable (<<<0.1% viability. In contrast, selfed crosses show generally low rates of inviability

175   (~95% viable); (Coughlan et al. 2020*b*)). We therefore assumed that any viable seed in these

176   four mixed pollination crosses were likely to be a product of self fertilization. In two of the six

177   mixed-pollen crosses, hybrid seeds are generally viable, though noticeably smaller and produce

178   much darker seed coats than selfed seed (*M. guttatus* mothers x Northern *M. decorus* pollen

179   donors and Southern *M. decorus* mothers x *M. guttatus* pollen donors). In the case of Southern

180   *M. decorus,* crosses in which pollen was mixed with *M. guttatus* yielded a generally high level of

181   fruit failure, and thus this cross type was excluded from subsequent analysis. In the case of

182   crosses involving *M. guttatus* mothers with a mixture of *M. guttatus* and Northern *M. decorus*

183   pollen, a subset of putative self-fertilized and putative hybrid seeds were grown to confirm that

184   designations based on seed phenotype accurately indicated paternal genotype. All seeds which

185   were presumed to be self fertilized were indeed conspecific seeds, and only 2/86 presumed

186   hybrids were likely conspecific seeds (~2.3% error rate; Figure S1). I then measured the area of

187   all viable, putatively non-hybrid seeds using ImageJ (Schneider et al. 2012) for the remaining 8

188   cross type/maternal parent combinations. In total, this resulted in an average of 29, 55, and 128

189   seeds measured per fruit, for crosses involving Southern *M. decorus*, *M. guttatus*, and Northern

190   *M. decorus* as the maternal donor, respectively. In total, I measured 16,199 seeds across 215

191   fruits for all 8 experimental cross types.
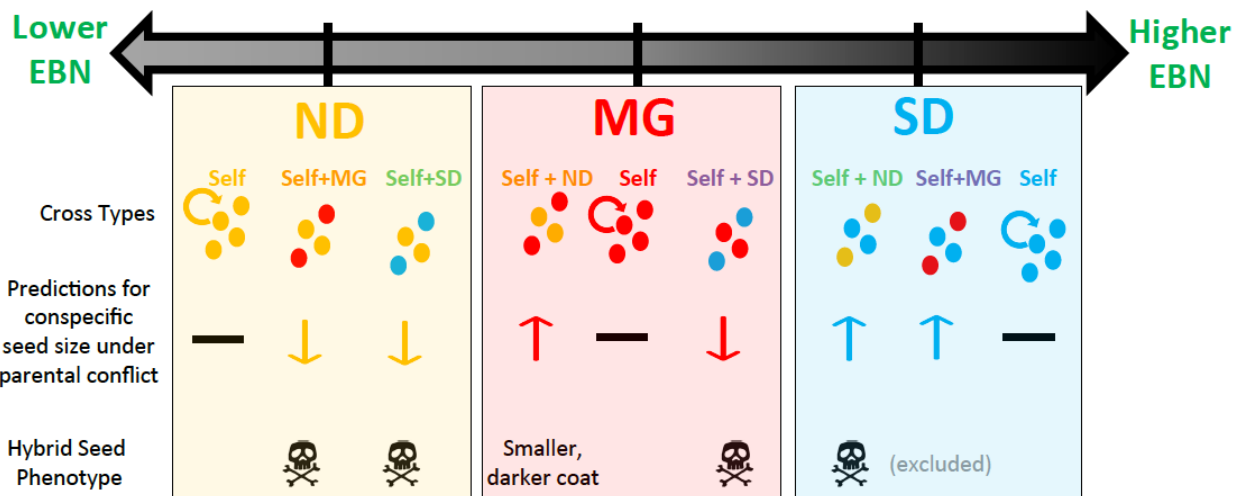
192

193   **Figure 1: Overview of the experimental design.** This experiment leveraged natural variation
194   in EBN across three species (ND= Northern *M. decorus*, MG= *M. guttatus*, SD= Southern *M.
195   decorus*; outlined in (Coughlan et al. 2020*b*)). To compare conspecific seed size when
196   conspecific seeds developed along full siblings versus half siblings whose sires vary in EBN,
197   nine types of crosses were completed: each species was self fertilized, or fertilized with a
198   mixture of self-pollen and pollen from another species. After excluding putative hybrid seeds
199   based on diagnostic phenotypes (hybrids having inviable or much smaller and darker seeds;
200   see Figure S1 for confirmation), all conspecific seeds for each cross type were measured for
201   total area. Prediction based on parental conflict theory are outlined: conspecific seeds
202   developing with hybrid siblings sired by a higher EBN father should be smaller (exemplified by
203   both mixed crosses involving Northern *M. decorus* as the dam), while conspecific seeds

204 developing alongside hybrid siblings sired by a lower EBN father should be larger (exemplified
205 by both mixed crosses involving Southern *M. decorus* as the dam).
206

207 *Analyses*

208 To assess if the presence of hybrid siblings affected the size of intraspecific seeds, I used a

209 linear mixed model with log(seed area) as the response variable and cross type (e.g. pure self-

210 fertilized, mixed pollen with species 1 and mixed pollen with species 2 as the levels), and the

211 total number of seeds per fruit as fixed effects. As the same plant was used for multiple crosses,

212 and multiple seeds were measured from a single fruit, I also included maternal replicate and fruit

213 replicate as random effects. The significance of the fixed effects were assessed by a Type III

214 Wald χ^2 test using the *lme4* and *car* packages in R (Bates et al. 2012; Fox and Weisberg 2018).

215 Significance between cross types were assessed using the *emmeans* package in R (Russell

216 2019). These analyses were performed for each maternal genotype separately.

217

218 **Results**

219 *The presence of hybrid seeds affects intraspecific seed size*

220 The presence of hybrid seeds significantly affected conspecific seed size in almost all

221 crosses (Figure 2, Table S1). The direction of these effects depended on the type of hybrids that

222 conspecific seeds developed alongside (Figure 2). For *M. guttatus*, conspecific seeds were

223 smaller when they developed along hybrids sired by a higher EBN pollen donor (i.e. Southern

224 *M. decorus*), and larger when they developed alongside hybrid siblings sired by a lower EBN

225 pollen donor (i.e. Northern *M. decorus*; Figure 2). Similarly, for Southern *M. decorus*, conspecific

226 seeds were larger when they developed alongside hybrid siblings sired by a lower EBN pollen

227 donor (i.e. Northern *M. decorus*; Figure 2). For Northern *M. decorus*, conspecific seeds were

228 significantly smaller when developing alongside hybrid siblings sired by the highest EBN pollen

229 donor in this experiment (i.e. Southern *M. decorus*; Figure 2), although there is no significant

230   difference in intraspecific seed size between fruits containing only pure intraspecific selfs and

231   fruits containing both intraspecific selfs and hybrid siblings sired by *M. guttatus* (which has a

232   higher EBN than Northern *M. decorus*, but a lower EBN that Southern *M. decorus*). Overall,

233   these effects are relatively small, for example intraspecific *M. guttatus* seeds are ~5% larger

234   when developing alongside hybrids from a lower EBN father and ~7% smaller when developing

235   alongside hybrids from a higher EBN father (see Figure 2 for all estimated effect sizes based on

236   estimated marginal means).

237

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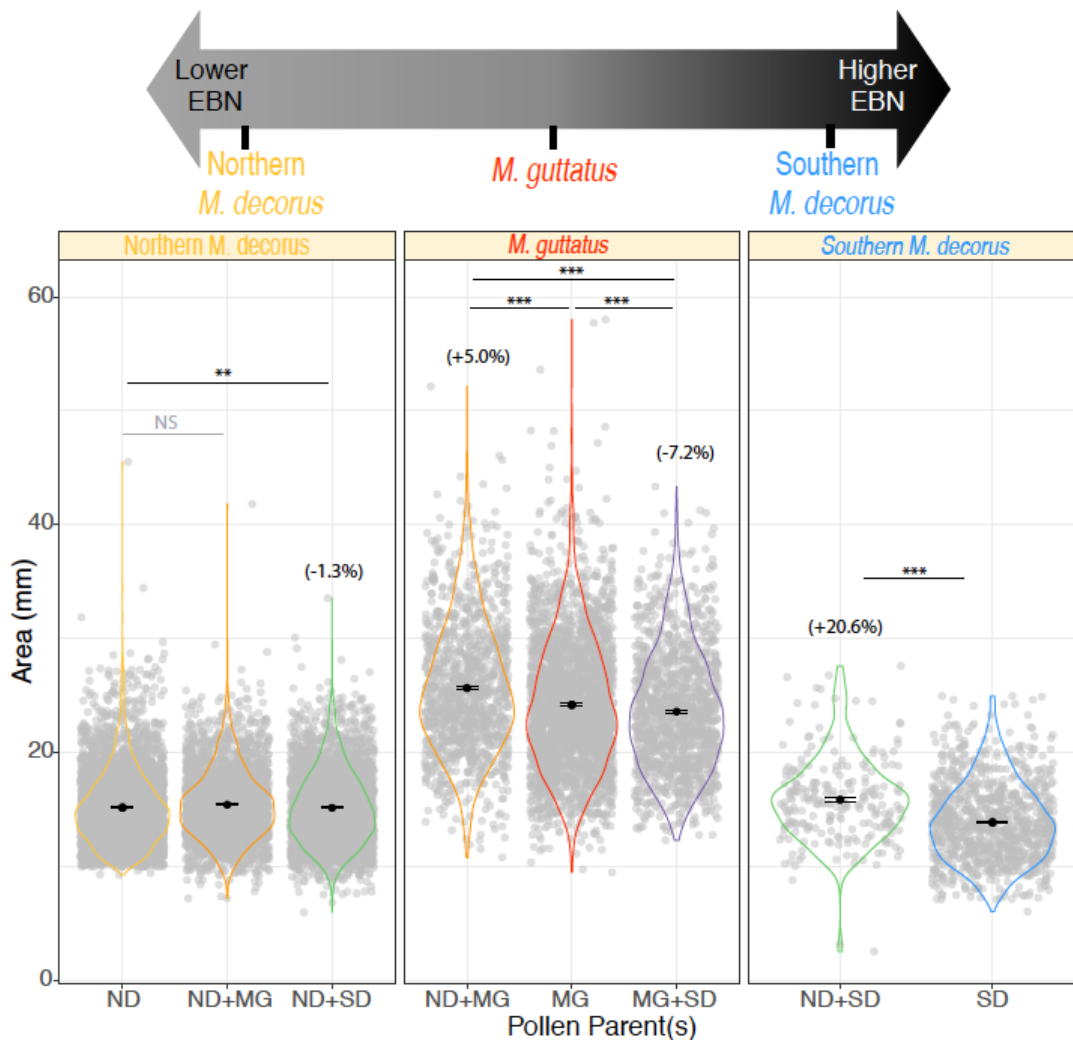
Figure 2: Intraspecific seed size differs between developmental contexts. For each
species, either pure conspecific (self) pollen or a mixture of con- and interspecific pollen was
applied to receptive stigmas, then the resultant conspecific seeds were measured for total area

242   (in mm). Each panel depicts seeds produced from each genotype as the maternal parent, while
243   colors of the violins indicate the pollen donor(s) (note: while only conspecific seeds were
244   measured, for mixed pollination crosses I use an intermediate color between the two pollen
245   donors for contrast to pure conspecific crosses). Filled, solid black points represent the means
246   and standard errors, while translucent, grey points are all raw data. For crosses that show a
247   significant difference in conspecific seed size, the effect size relative to conspecific seeds that
248   developed alongside full siblings as estimated from the *emmeans* package in R (Russell 2019)
249   are shown in brackets. Significant differences were determined using a linear mixed effect
250   model (See Table S1 for output). Note that for Southern *M. decorus* fruits generally failed when
251   fertilized with a combination of self-pollen and pollen from *M. guttatus*, so this cross was
252   excluded from further analysis.
253

Discussion

255   Here I show that conspecific seed size is influenced by the presence of hybrid siblings

256   during development. These results are consistent with the idea that paternally-derived, resource

257   acquiring alleles are costly to less resource-competitive siblings developing in the same brood

258   (and in turn, to maternal parents). Although these maternal costs are a central prediction of

259   parental conflict theory, very few studies have empirically demonstrated them. To my

260   knowledge, the only other study to show these costs utilized variation between maize

261   accessions for paternally-derived resource acquiring alleles to show that seed size differed

262   when half-siblings whose fathers differed in their competitive ability developed alongside one

263   another relative to when the same genotypes developed alongside full siblings (Cailleau et al.

264   2018). Here, I leverage proposed differences in EBN between recently diverged taxa to illustrate

265   a similar phenomenon.

266   While a growing body of evidence has highlighted the role of parental conflict in the

267   origins of reproductive isolation (namely HSI, and early-onset hybrid inviability in mammalian

268   systems; (Vrana et al. 1998, 2000; Brekke and Good 2014; Brekke et al. 2016, 2021; Oneal et

269   al. 2016; Lafon-Placette et al. 2018; Roth et al. 2018*a*; Coughlan et al. 2020*b*; Sandstedt et al.

270   2020; Arévalo et al. 2021)), the work presented here highlights a secondary role of parental

271   conflict in speciation: hybridization between species that vary in their histories of parental

272   conflict may result in indirect effects to intraspecific offspring development, in the event that

11

273   intraspecific offspring develop alongside hybrids. In this system, *M. guttatus* co-occurs with both

274   Northern and Southern *M. decorus* for large portions of their range (JMC personal obs;

275   (Coughlan et al. 2020*a*)), and are thought to routinely hybridize and introgress (JMC

276   unpublished data; (Puzey et al. 2017)). Although seed size differences have been shown to

277   influence several components of fitness in other systems (germination probability, seedling

278   survivorship, flower production; (Stanton 1984; Simons and Johnston 2000; Gómez 2004)),

279   these effects are likely to be context specific (for example, based on the competitive

280   environment; (Stanton 1984)), and substantial fieldwork and further experimentation is required

281   to quantify fitness effects in this system. However, one intriguing finding of this work is that the

282   consequences of hybridization for intraspecific seed development are not always costs. If

283   hybridization with fathers with lower EBNs consistently results in slightly larger conspecific

284   seeds, this hybridization might actually result in a fitness benefit to individual seeds. However,

285   this potential benefit is likely a very small one, and moreover hybridization likely still results in an

286   overall cost to maternal parents and a loss of inclusive fitness to individual seeds, given the loss

287   of gametes to inviable hybrid offspring. Depending on the rates of hybridization and the

288   competitive environment in which seeds (or in the case of mammals, young offspring) find

289   themselves, this work may have significant implications for the dynamics of hybridization and

290   coexistence of species that vary in their histories of conflict and find themselves in secondary

291   contact.

292

293   **Data Availability**

294   All raw data are available under the Dryad submission https://doi.org/10.5061/dryad.m905qfv2d.
295   Summaries of all crosses are included in Supplementary Table 2 of this manuscript.
296

297   **Acknowledgements**

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Supplemental Figures
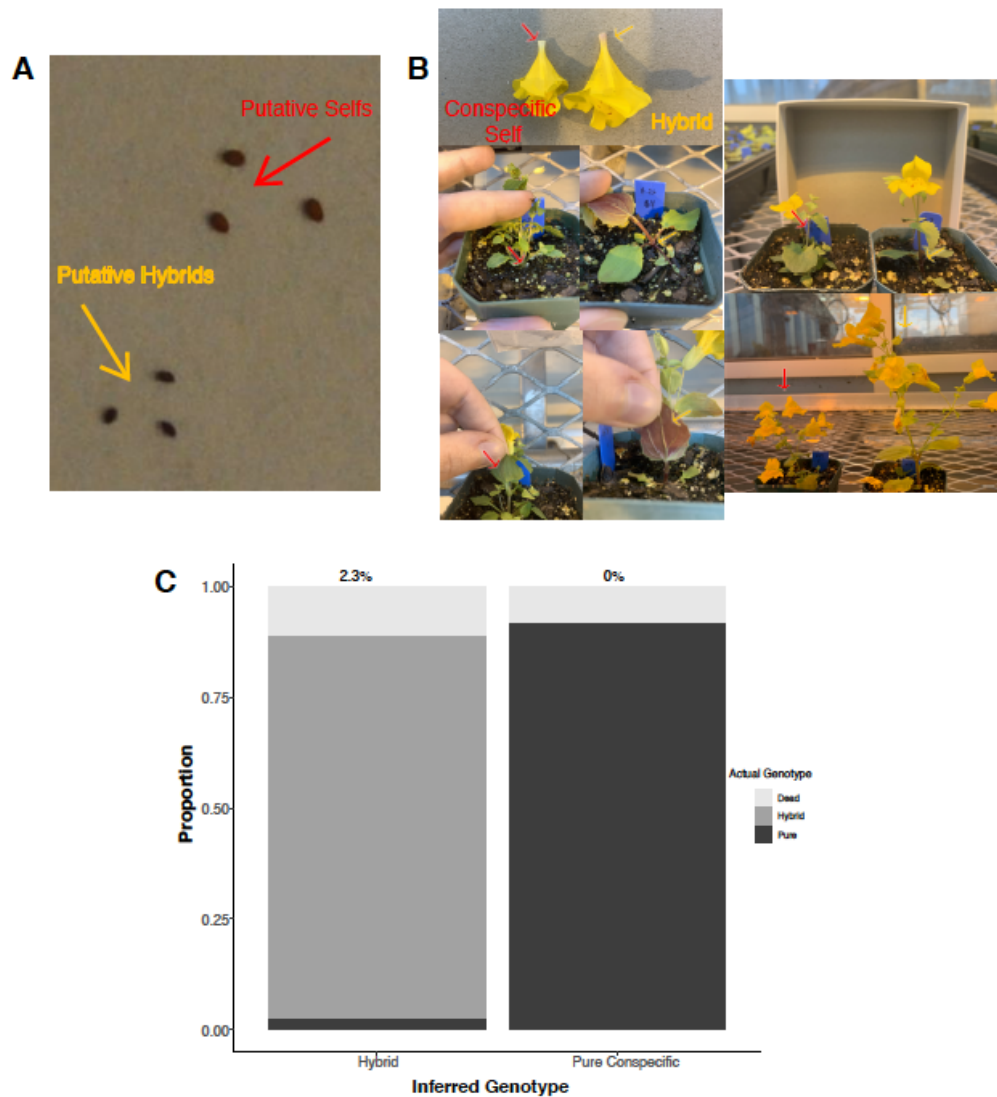
Figure S1: Confirmation of paternal genotypes for mixed pollination crosses involving *M. guttatus* as the dam and mixed pollen from *M. guttatus* and Northern *M. decorus*. (A) Putative hybrids and putative self-fertilized seeds exhibit distinct seed characteristics; hybrids are smaller and have much darker seed coats. To confirm paternal genotype, a subset of putative selfs and putative hybrids were grown. Plants differ in a number of diagnostic characters (selfs on the left, hybrids on the right), including: presence anthocyanin pigmentation in the base of the corolla in hybrids (top left), the presence of stolons in hybrids (middle left), the presence of anthocyanin pigmentation on the underside of the leaf in hybrids (bottom left), stems with anthocyanin pigmentation in hybrids (top right), and overall size; both of flowers and total plant, wherein hybrids are larger (bottom right). (C) Based on this set of characters, there were no hybrids that were designated as selfed seed and only ~2% of seeds designated as hybrids were in fact conspecific seeds.

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488 **Supplemental Tables**

489 **Table S1:** LMER outputs for each species as maternal parent. In all cases, log(seed area) was

490 the response variable, with Cross Type (pure-self pollinated, mixed pollen with species 1, mixed

491 pollen with species 2 as the levels), and the total number of seeds per fruit as fixed effects.

492 Maternal plant replicate and fruit replicate were included as random effects. Significance of fixed

493 effects was determined using Type III Wald χ^2 test using the lme4 and car packages in R

494 (Bates et al. 2012; Fox and Weisberg 2018).

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Norther *M. decorus* (Hwy15D)			
Response	***F***	**DF**	**p**
Intercept	10620.635	1	<0.001***
Cross Type	10.017	2	0.0067**
Total Num. of seeds	156.698	1	<0.001***
M. guttatus (IM62)			
Intercept	15122.371	1	< 0.0001***
Cross Type	124.602	2	< 0.0001***
Total Num. of seeds	33.538	1	< 0.0001***
Southern *M. decorus* (OD11)			
Intercept	3891.8363	1	< 0.0001***
Cross Type	88.9300	2	< 0.0001***
Total Num. of seeds	7.0686	1	0.0078**

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502 **Table S2:** Number of seeds produced, proportion inviable, and average seed size per fruit for all

503 crosses. **Mat** = Maternal genotype; **Mat.Rep**= individual replicate of that genotype; **Pat**=

504 Paternal donor(s); **CR**= Cross Replicate (fruit replicate for each Maternal/Paternal combination);

505 **Viable**= Number of viable seeds; **Inviable**= Number of inviable seeds; **Total**= total number of

506 seeds produced per fruit; **Prop**= proportion of inviable seeds; **AA**= average seed area (mm),

507 **Sig**= is there a significant deviation from 1:1 pollination of the two paternal genotypes (SIG=

508 significant deviation, NS= Not-significant, NA= not applicable (i.e. only one paternal donor))).

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Mat	Mat.Rep	Pat	CR	Viable	Inviable	Total	Prop	AA	Sig?
IM62	4	OD+IM	1	20	8	28	0.2857	21.6246	NS
IM62	14	OD+IM	2	90	0	90	0.0000	18.5862	SIG
IM62	0	OD+IM	3	3	7	10	0.7000	31.9987	NS
IM62	12	OD+IM	4	15	8	23	0.3478	29.3106	NS
IM62	0	OD+IM	5	117	15	132	0.1136	21.2291	SIG
IM62	0	OD+IM	6	62	9	71	0.1268	25.6564	SIG
IM62	0	OD+IM	7	48	62	110	0.5636	22.2014	NS
IM62	13	OD+IM	8	28	15	43	0.3488	24.1408	NS
IM62	6	OD+IM	9	28	25	53	0.4717	23.7778	NS
IM62	12	OD+IM	10	39	47	86	0.5465	24.0505	NS
IM62	5	OD+IM	11	34	46	80	0.5750	25.1875	NS
IM62	14	OD+IM	12	18	53	71	0.7465	21.0091	SIG
IM62	13	OD+IM	13	38	23	61	0.3770	26.2518	NS

IM62	13	OD+IM	14	7	37	44	0.8409	24.7432	SIG
IM62	2	OD+IM	15	14	23	37	0.6216	24.0467	NS
IM62	0	OD+IM	16	11	7	18	0.3889	20.1029	NS
IM62	3	OD+IM	17	6	10	16	0.6250	29.2899	NS
IM62	0	OD+IM	18	24	6	30	0.2000	24.5210	NS
IM62	7	OD+IM	19	47	14	61	0.2295	23.1699	SIG
IM62	15	OD+IM	20	26	11	37	0.2973	22.3076	NS
IM62	6	OD+IM	21	41	23	64	0.3594	25.3321	NS
IM62	6	OD+IM	22	6	0	6	0.0000	25.4753	NS
IM62	9	OD+IM	23	112	8	120	0.0667	23.5290	SIG
IM62	8	OD+IM	24	11	12	23	0.5217	23.7891	NS
IM62	4	OD+IM	25	5	25	30	0.8333	27.2043	NS
IM62	0	OD+IM	26	44	11	55	0.2000	24.6987	SIG
IM62	0	OD+IM	27	92	10	102	0.0980	25.9432	SIG
IM62	0	OD+IM	28	48	20	68	0.2941	22.2567	NS
IM62	0	OD+IM	29	38	19	57	0.3333	29.3798	NS
IM62	0	OD+IM	30	41	36	77	0.4675	26.0528	NS
HWY15D	6	IM62+HWY	1	182	157	339	0.4631	13.7714	NS
HWY15D	4	IM62+HWY	2	42	96	138	0.6957	16.8653	SIG
HWY15D	6	IM62+HWY	3	91	54	145	0.3724	17.2701	NS
HWY15D	4	IM62+HWY	4	139	144	283	0.5088	15.3169	NS
HWY15D	4	IM62+HWY	5	84	160	244	0.6557	15.4467	SIG
HWY15D	6	IM62+HWY	6	58	61	119	0.5126	17.5574	NS

HWY15D	6	IM62+HWY	7	65	99	164	0.6037	15.2325	NS
HWY15D	6	IM62+HWY	8	72	61	133	0.4586	16.7600	NS
HWY15D	6	IM62+HWY	9	101	110	211	0.5213	16.2864	NS
HWY15D	4	IM62+HWY	10	63	134	197	0.6802	17.3873	SIG
HWY15D	4	IM62+HWY	11	44	122	166	0.7349	17.8447	SIG
HWY15D	4	IM62+HWY	12	30	79	109	0.7248	18.8440	SIG
HWY15D	6	IM62+HWY	13	22	23	45	0.5111	15.9900	NS
HWY15D	6	IM62+HWY	14	141	105	246	0.4268	14.9731	NS
HWY15D	6	IM62+HWY	15	58	36	94	0.3830	16.2463	NS
HWY15D	7	IM62+HWY	16	149	94	243	0.3868	17.2735	NS
HWY15D	5	IM62+HWY	17	96	135	231	0.5844	13.7065	NS
HWY15D	5	IM62+HWY	18	61	108	169	0.6391	14.1027	NS
HWY15D	7	IM62+HWY	19	80	62	142	0.4366	17.4737	NS
HWY15D	7	IM62+HWY	20	46	32	78	0.4103	16.4966	NS
HWY15D	7	IM62+HWY	21	124	72	196	0.3673	16.3268	NS
HWY15D	7	IM62+HWY	22	15	57	72	0.7917	16.2255	SIG
HWY15D	4	IM62+HWY	23	82	163	245	0.6653	15.9680	SIG
HWY15D	6	IM62+HWY	24	132	52	184	0.2826	13.3957	SIG
HWY15D	6	IM62+HWY	25	214	137	351	0.3903	13.7087	SIG
HWY15D	4	IM62+HWY	26	37	141	178	0.7921	18.2464	SIG
HWY15D	4	IM62+HWY	27	10	12	22	0.5455	21.0723	NS
HWY15D	6	IM62+HWY	28	36	43	79	0.5443	13.8636	NS
HWY15D	4	IM62+HWY	29	49	40	89	0.4494	16.5796	NS

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HWY15D	6	IM62+HWY	30	99	98	197	0.4975	13.4846	NS
HWY15D	6	OD+HWY15	1	166	65	231	0.2814	14.7041	SIG
HWY15D	6	OD+HWY15	2	229	145	374	0.3877	14.6194	SIG
HWY15D	6	OD+HWY15	3	276	152	428	0.3551	NA	SIG
HWY15D	6	OD+HWY15	4	206	110	316	0.3481	14.5547	SIG
HWY15D	4	OD+HWY15	5	30	90	120	0.7500	16.4631	SIG
HWY15D	4	OD+HWY15	6	92	88	180	0.4889	17.2475	NS
HWY15D	6	OD+HWY15	7	48	32	80	0.4000	17.5165	NS
HWY15D	6	OD+HWY15	8	183	78	261	0.2989	15.0614	SIG
HWY15D	4	OD+HWY15	9	19	19	38	0.5000	20.1781	NS
HWY15D	4	OD+HWY15	10	217	161	378	0.4259	14.3388	NS
HWY15D	4	OD+HWY15	11	231	162	393	0.4122	13.8283	NS
HWY15D	4	OD+HWY15	12	121	71	192	0.3698	15.8368	NS
HWY15D	6	OD+HWY15	13	64	57	121	0.4711	14.9187	NS
HWY15D	6	OD+HWY15	14	29	32	61	0.5246	15.5215	NS
HWY15D	6	OD+HWY15	15	37	12	49	0.2449	17.8154	NS
HWY15D	6	OD+HWY15	16	26	57	83	0.6867	14.6550	NS
HWY15D	4	OD+HWY15	17	265	96	361	0.2659	15.5190	SIG
HWY15D	4	OD+HWY15	18	313	132	445	0.2966	14.8546	SIG
HWY15D	6	OD+HWY15	19	129	107	236	0.4534	16.2029	NS
HWY15D	5	OD+HWY15	20	10	39	49	0.7959	17.1143	SIG
HWY15D	5	OD+HWY15	21	126	166	292	0.5685	14.4543	NS
HWY15D	5	OD+HWY15	22	64	134	198	0.6768	13.1848	SIG

HWY15D	6	OD+HWY15	23	126	96	222	0.4324	15.9587	NS
HWY15D	6	OD+HWY15	24	88	72	160	0.4500	19.0036	NS
HWY15D	4	OD+HWY15	25	53	72	125	0.5760	15.0604	NS
OD11	3	HWY15+OD	1	64	104	168	0.6190	14.1142	NS
OD11	3	HWY15+OD	2	6	32	38	0.8421	15.3353	SIG
OD11	3	HWY15+OD	3	16	110	126	0.8730	15.9940	SIG
OD11	5	HWY15+OD	4	12	95	107	0.8879	15.9141	SIG
OD11	5	HWY15+OD	5	6	121	127	0.9528	14.3608	SIG
OD11	5	HWY15+OD	6	8	35	43	0.8140	14.8058	SIG
OD11	5	HWY15+OD	7	8	97	105	0.9238	14.8111	SIG
OD11	5	HWY15+OD	8	12	154	166	0.9277	15.4242	SIG
OD11	5	HWY15+OD	9	4	67	71	0.9437	15.9485	SIG
OD11	3	HWY15+OD	10	23	137	160	0.8563	17.6589	SIG
OD11	3	HWY15+OD	11	13	36	49	0.7347	19.9525	NS
OD11	3	HWY15+OD	12	2	19	21	0.9048	14.7240	SIG
OD11	5	HWY15+OD	13	20	74	94	0.7872	15.2884	SIG
OD11	5	HWY15+OD	14	8	61	69	0.8841	17.9506	SIG
OD11	3	HWY15+OD	15	7	20	27	0.7407	15.7819	NS
OD11	5	HWY15+OD	16	7	53	60	0.8833	15.3839	SIG
OD11	3	HWY15+OD	17	10	74	84	0.8810	17.4214	SIG
OD11	5	HWY15+OD	18	2	54	56	0.9643	18.0635	SIG
OD11	5	HWY15+OD	19	2	51	53	0.9623	15.7985	SIG
OD11	5	HWY15+OD	20	6	26	32	0.8125	18.4395	NS

OD11	3	HWY15+OD	21	4	11	36	0.3056	19.8088	NS
OD11	3	HWY15+OD	22	0	11	11	1.0000		NS
IM62	0	IM62+HWY	1	67	108	175	0.6171	25.0784	NS
IM62	7	IM62+HWY	2	34	26	60	0.4333	15.3029	NS
IM62	9	IM62+HWY	3	64	41	105	0.3905	23.5853	NS
IM62	15	IM62+HWY	4	117	73	190	0.3842	22.9275	NS
IM62	8	IM62+HWY	5	42	51	93	0.5484	21.2626	NS
IM62	5	IM62+HWY	6	5	11	16	0.6875	23.9093	NS
IM62	12	IM62+HWY	7	56	35	91	0.3846	23.5435	NS
IM62	9	IM62+HWY	8	7	18	25	0.7200	28.8460	NS
IM62	15	IM62+HWY	9	6	19	25	0.7600	27.4792	NS
IM62	5	IM62+HWY	10	33	35	68	0.5147	28.8596	NS
IM62	5	IM62+HWY	11	43	121	164	0.7378	28.8578	SIG
IM62	12	IM62+HWY	12	67	157	224	0.7009	21.8156	SIG
IM62	11	IM62+HWY	13	36	34	70	0.4857	25.0363	NS
IM62	12	IM62+HWY	14	41	61	102	0.5980	30.0482	NS
IM62	0	IM62+HWY	15	8	22	30	0.7333	22.3832	NS
IM62	0	IM62+HWY	16	27	79	106	0.7453	26.9391	SIG
IM62	0	IM62+HWY	17	18	17	35	0.4857	34.5770	NS
IM62	0	IM62+HWY	18	34	67	101	0.6634	32.6350	NS
IM62	0	IM62+HWY	19	42	40	82	0.4878	28.8821	NS
IM62	5	IM62+HWY	20	54	108	162	0.6667	23.8263	SIG
IM62	5	IM62+HWY	21	22	21	43	0.4884	28.3003	NS

IM62	5	IM62+HWY	22	19	53	72	0.7361	29.2312	SIG
IM62	4	IM62+HWY	23	30	86	116	0.7414	30.3648	SIG
IM62	2	IM62+HWY	24	28	22	50	0.4400	28.9807	NS
IM62	13	IM62+HWY	25	42	43	85	0.5059	30.1018	NS
IM62	13	IM62+HWY	26	43	47	90	0.5222	32.1721	NS
IM62	9	IM62+HWY	27	7	29	36	0.8056	24.5183	NS
IM62	0	IM62+HWY	28	77	67	144	0.4653	22.7292	NS
IM62	12	IM62+HWY	29	27	34	61	0.5574	21.2397	NS
IM62	12	IM62+HWY	30	48	53	101	0.5248	24.7898	NS
OD11	5	IM62+OD	1	1	27	28	0.9643		SIG
OD11	2	IM62+OD	2	8	84	92	0.9130		SIG
OD11	3	IM62+OD	3	13	87	100	0.8700		NA
IM62	0	IM62	1	164	8	172	0.0465	21.5154	NA
IM62	7	IM62	2	69	10	79	0.1266	26.9359	NA
IM62	16	IM62	3	154	8	162	0.0494	22.1432	NA
IM62	0	IM62	4	115	5	120	0.0417	25.9444	NA
IM62	0	IM62	5	24	9	33	0.2727	23.4533	NA
IM62	7	IM62	6	136	5	141	0.0355	17.7310	NA
IM62	16	IM62	7	140	4	144	0.0278	22.7858	NA
IM62	11	IM62	8	120	3	123	0.0244	26.9818	NA
IM62	10	IM62	9	101	3	104	0.0288	26.8373	NA
IM62	13	IM62	10	58	2	60	0.0333	32.8099	NA
IM62	13	IM62	11	51	5	56	0.0893	35.3827	NA

IM62	11	IM62	12	98	3	101	0.0297	22.9827	NA
IM62	6	IM62	13	96	4	100	0.0400	24.3869	NA
IM62	6	IM62	14	50	1	51	0.0196	27.9854	NA
IM62	6	IM62	15	109	0	109	0.0000	24.8258	NA
IM62	6	IM62	16	55	3	58	0.0517	27.3090	NA
IM62	6	IM62	17	83	0	83	0.0000	25.7117	NA
IM62	4	IM62	18	44	1	45	0.0222	27.6953	NA
IM62	6	IM62	19	90	3	93	0.0323	19.6464	NA
IM62	13	IM62	20	25	2	27	0.0741	28.5040	NA
IM62	16	IM62	21	112	14	126	0.1111	21.8118	NA
IM62	7	IM62	22	59	4	63	0.0635	20.1915	NA
IM62	10	IM62	23	74	2	76	0.0263	27.7943	NA
IM62	10	IM62	24	95	1	96	0.0104	22.5340	NA
IM62	9	IM62	25	92	0	92	0.0000	19.2019	NA
IM62	9	IM62	26	146	4	150	0.0267	25.5663	NA
IM62	9	IM62	27	84	5	89	0.0562	29.6586	NA
IM62	16	IM62	28	157	2	159	0.0126	25.3686	NA
IM62	16	IM62	29	26	6	32	0.1875	26.4208	NA
IM62	16	IM62	30	81	4	85	0.0471	22.0754	NA
IM62	11	IM62	31	131	8	139	0.0576	20.5657	NA
HWY15D	6	HWY15	1	152	22	174	0.1264	13.8366	NA
HWY15D	6	HWY15	2	214	17	231	0.0736	13.6000	NA
HWY15D	4	HWY15	3	15	3	18	0.1667	20.6085	NA

HWY15D	5	HWY15	4	268	29	297	0.0976	15.1823	NA
HWY15D	6	HWY15	5	50	9	59	0.1525	18.2932	NA
HWY15D	6	HWY15	6	42	8	50	0.1600	21.6989	NA
HWY15D	4	HWY15	7	279	27	306	0.0882	16.3628	NA
HWY15D	4	HWY15	8	186	24	210	0.1143	15.4090	NA
HWY15D	6	HWY15	9	218	28	246	0.1138	15.2564	NA
HWY15D	6	HWY15	10	180	21	201	0.1045	15.5260	NA
HWY15D	6	HWY15	11	174	34	208	0.1635	13.7842	NA
HWY15D	7	HWY15	12	153	18	171	0.1053	16.4726	NA
HWY15D	4	HWY15	13	155	13	168	0.0774	17.1155	NA
HWY15D	4	HWY15	14	208	21	229	0.0917	13.8453	NA
HWY15D	4	HWY15	15	366	12	378	0.0317	14.6420	NA
HWY15D	4	HWY15	16	261	32	293	0.1092	14.0407	NA
HWY15D	6	HWY15	17	87	18	105	0.1714	14.8045	NA
HWY15D	4	HWY15	18	349	27	376	0.0718	14.7766	NA
HWY15D	4	HWY15	19	274	15	289	0.0519	16.2413	NA
HWY15D	6	HWY15	20	222	34	256	0.1328	16.8549	NA
HWY15D	6	HWY15	21	242	24	266	0.0902	15.4280	NA
HWY15D	6	HWY15	22	49	9	58	0.1552	21.0781	NA
HWY15D	6	HWY15	23	30	16	46	0.3478	20.7791	NA
HWY15D	6	HWY15	24	322	9	331	0.0272	14.9536	NA
OD11	3	OD11	1	87	34	121	0.2810	11.4002	NA
OD11	3	OD11	2	104	32	136	0.2353	12.1900	NA

OD11	5	OD11	3	113	54	167	0.3234	16.5155	NA
OD11	5	OD11	4	137	44	181	0.2431	16.2901	NA
OD11	5	OD11	5	143	66	209	0.3158	15.7383	NA
OD11	5	OD11	6	16	8	24	0.3333	13.2572	NA
OD11	3	OD11	7	162	73	235	0.3106	13.5372	NA
OD11	3	OD11	8	15	5	20	0.2500	15.1527	NA
OD11	3	OD11	9	68	13	81	0.1605	13.1667	NA
OD11	3	OD11	10	50	16	66	0.2424	11.1004	NA
OD11	3	OD11	11	89	14	103	0.1359	12.2546	NA
OD11	3	OD11	12	88	18	106	0.1698	15.5921	NA
OD11	5	OD11	13	6	4	10	0.4000	14.5205	NA
OD11	5	OD11	14	78	43	121	0.3554	13.6518	NA
OD11	2	OD11	15	64	54	118	0.4576	15.6383	NA
OD11	2	OD11	16	76	34	110	0.3091	12.7179	NA
OD11	2	OD11	17	75	35	110	0.3182	13.2970	NA
OD11	3	OD11	18	104	52	156	0.3333	14.4804	NA
OD11	3	OD11	19	97	65	162	0.4012	12.8145	NA
OD11	3	OD11	20	35	21	56	0.3750	11.5924	NA

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