Intersexual conflict over seed size is stronger in more outcrossed populations of a mixed-mating plant

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Abstract

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16 In polyandrous species, fathers benefit from attracting greater maternal investment towards
17 their offspring at the expense of the offspring of other males, while mothers should usually
18 allocate resources equally among offspring. This conflict can lead to an evolutionary arms
19 race between the sexes, manifested through antagonistic genes whose expression in offspring
20 depends upon the parent of origin. The arms race may involve an increase in the strength of
21 maternally versus paternally derived alleles engaged in a 'tug-of-war' over maternal
22 provisioning, or repeated 'recognition-avoidance' co-evolution where growth-enhancing
23 paternally derived alleles evolve to escape recognition by maternal genes targeted to suppress
24 their effect. Here, we develop predictions to distinguish between these two mechanisms when
25 considering crosses among populations that have reached different equilibria in this
26 intersexual arms race. We test these predictions using crosses within and among populations
27 of *Dalechampia scandens* (Euphorbiaceae) that presumably have experienced different
28 intensities of intersexual conflict, as inferred from their historical differences in mating
29 system. In crosses where the paternal population was more outcrossed than the maternal
30 population, hybrid seeds were larger than those normally produced in the maternal
31 population, whereas when the maternal population was more outcrossed, hybrid seeds were
32 smaller than normal. These results confirm the importance of mating systems in determining
33 the intensity of intersexual conflict over maternal investment and provide strong support for a
34 tug-of-war mechanism operating in this conflict. They also yield clear predictions for the
35 fitness consequences of gene flow among populations with different mating histories.
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37 Keywords: *Dalechampia,* inter-locus contest evolution, kinship genomic imprinting, multiple
38 paternity, parent-offspring conflict, sexual conflict.
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Significance

41 Intersexual conflict over maternal resource allocation to offspring can lead to the evolution of
42 imprinted genes with parent-of-origin-specific expression. However, the precise mechanism
43 involved in the evolution of such imprinted genes is less well understood, and few clear
44 predictions have been presented. We resolve this issue and, using different populations of a
45 mixed-mating plant, we demonstrate that more outcrossed paternal populations produce larger
46 seeds when crossed with less outcrossed maternal populations, and *vice versa*. This provides
47 the first clear support for a 'tug-of-war' mechanism operating between maternally and

48 paternally imprinted genes. Such a mechanism can have important consequences for local

49 adaptation in offspring size in the presence of gene flow between populations with different

50 mating systems.

51 \body

Introduction

53 Females of many species frequently mate with several males (polyandry), thus opening an

54 arena for intersexual conflict over the allocation of maternal resources (1-3). Indeed, while

55 mothers maximise their fitness by allocating resources equally among offspring (4), fathers

56 will increase their fitness by causing more maternal resources to be invested in their own

57 offspring, at the expense of offspring sired by other males (3, 5-12). Consequently, selection

58 should favour paternally derived alleles that increase nutrient demands on the mother when

59 expressed in offspring (10, 12) and also maternal mechanisms that counteract the effects of

60 paternally derives alleles in order to ensure an equal allocation of resources among offspring,

61 thus avoiding the commitment of resources beyond the maternal optimum (13, 14). This

62 conflict of interests should result in an evolutionary arms race between sexes over maternal

63 investment in offspring (2, 13-15).

64   This intersexual arms race can be manifested through imprinted genes with differential

65 expression depending on the parent of origin (12, 16). Although the importance of such

66 imprinted genes has been demonstrated both during endosperm development in angiosperm

67 seeds (17) and in placental activity in mammals (18), the exact mechanism by which these

68 genes interact to control maternal allocation remains debated. Two genetic mechanisms,

69 which we refer to as 'tug-of-war' and 'recognition-avoidance', have been proposed to explain

70 the action of imprinted genes in the arms race between the sexes over maternal investment

71 (19). Here, we provide novel predictions for distinguishing between these two mechanisms

72 when considering the phenotype of offspring produced by crosses among populations with

73 different levels of intersexual conflict over maternal provisioning. We then test these

74 predictions experimentally by using crosses among natural populations of a mixed-mating

75 plant species. Working with natural populations allow us to interpret our results in the broad

76 context of local adaptation.

77   The 'tug-of-war' mechanism describes a system where alleles at loci promoting

78 offspring growth are expressed in the offspring when paternally derived and silenced when

79 maternally derived, while alleles at growth-suppressing loci are expressed when maternally

80 derived and silenced when paternally derived (7, 10-13). This mechanism can thus be

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81 described as a tug of war between maternally and paternally derived alleles over maternal

82 investment, with coevolution leading to an escalation of the number and/or strength of these

83 genes (20).

84 In the 'recognition-avoidance' mechanism, genes in maternal tissues surrounding the

85 developing embryo, or maternally expressed alleles in the offspring, may have evolved to

86 recognise and control the effects of paternally derived growth-enhancing alleles (14, 19). In

87 such a case, the arms race between the sexes will lead to the evolution of recognition-

88 avoidance tactics, analogous to host-parasite coevolution (21), where paternally derived

89 growth-enhancing alleles evolve to escape recognition and hence bypass maternal control,

90 while maternal or maternally derived alleles evolve to recognise new growth-promoting

91 alleles (19). An important difference between these two mechanisms is that maternal (or

92 maternally expressed) alleles involved in the coevolutionary process should directly influence

93 resource allocation in the tug-of-war system, while their effect in the recognition-avoidance

94 system is only indirect through controlling the effects of paternally expressed growth-

95 promoting genes with which they have coevolved.

96 At the population level, the intensity of intersexual conflict depends on the degree of

97 relatedness among the offspring from a given mother, which in turn depends on the

98 frequencies of multiple paternity and outcrossing (22). Populations that have historically

99 experienced different intensities of intersexual conflict over offspring size through differences

100 in mating system should either have reached different tug-of-war equilibria or gone to fixation

101 for different recognition-avoidance alleles (22-26). Consequently, crosses between

102 populations that have experienced different intensities of intersexual conflict should perturb

103 the genetic mechanisms that regulate offspring size. Here, we argue that the direction and

104 magnitude of the difference in offspring size resulting from this genetic perturbation will

105 depend on the genetic mechanism involved in the coevolutionary process, and we derive

106 specific predictions allowing us to distinguish between the two mechanisms.

107 With the tug-of-war mechanism, the divergence in offspring size will depend upon the

108 relative strength of the growth-promoting and growth-suppressing alleles derived from the

109 parental populations. Alleles derived from populations with histories of more-intense

110 intersexual conflict are expected to 'overpower' alleles from populations with histories of

111 less-intense conflict (22, 23, 27, 28). Consequently, (prediction 1) crosses involving a

112 maternal population with a history of more-intense conflict than the paternal population

113 should produce offspring smaller than the average offspring within the maternal population

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114   (growth-suppressing alleles are stronger than growth-enhancing alleles), while crosses in the

115   opposite direction should produce larger offspring (29; Fig. 1a).

116   Because any intersexual arms race operating through a recognition-avoidance

117   mechanism will be driven by males evolving new tactics to bypass maternal control, we

118   expect the alleles involved in such a mechanism to be population specific (14, 19).

119   Consequently, between-population crosses may lead to the failure of maternal (or maternally

120   derived) alleles to recognise 'foreign' paternally derived alleles and thus failure to control

121   their effects on offspring size (19, 25). In the absence of maternal regulation, inter-population

122   crosses are expected to systematically produce larger offspring than the average offspring

123   within the maternal population (prediction 2, Fig. 1b), unless the paternal population has a

124   long history of strict monogamy or self-fertilisation (i.e. with no intersexual conflict over

125   maternal investment). In the latter case, hybrid offspring size should be similar to the average

126   offspring size within the maternal population, because selfish paternal alleles are not expected

127   to have evolved in completely monogamous or selfing populations (22).

128   These two predictions can be distinguished by the following features. With a tug-of-

129   war mechanism the difference in size between normal and hybrid offspring should be best

130   explained by the relative intensity of intersexual conflict in the paternal and maternal

131   population. In contrast, with a recognition-avoidance mechanism we do not expect the

132   intensity of intersexual conflict in the maternal population to affect the difference in hybrid

133   offspring size. Instead, the magnitude of this difference in offspring size should be explained

134   by the intensity of intersexual conflict in the paternal population alone. Consequently, with a

135   recognition-avoidance mechanism, we do not expect inter-population crosses to produce

136   offspring smaller than the average offspring normally produced in the maternal population

137   (Fig. 1b). Even if the evolution of offspring size regulation results from a combination of the

138   two mechanisms, the occurrence of such smaller hybrids remains exclusively associated with

139   the tug-of-war mechanism.

140   Plant mating systems are extremely variable, ranging from functional asexuality to

141   enforced outcrossing through self-incompatibility (30). More than 40% of plant species

142   exhibit mixed mating systems, wherein progeny are produced by a mixture of selfing and

143   outcrossing (31). Populations of mixed-mating species often differ widely in outcrossing rates

144   (32) and hence in the intensity of intersexual conflict over maternal investment. Thus, plants

145   provide ideal experimental systems for testing predictions of intersexual conflict-theory.

146   Furthermore, offspring size (seed size) in plants has important fitness consequences because it

147   affects dispersal, germination and seedling establishment (33-39).

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148   Most previous research assessing seed size in inter-population crosses in plants seems

149 to support the idea of intersexual conflict over maternal investment, but few studies have

150 explicitly considered the role of the mating system (22). Furthermore, the relative importance

151 of the two genetic mechanisms (tug-of-war vs. recognition avoidance) in this conflict has

152 rarely been studied, although this knowledge is essential if we want to predict the outcome of

153 inter-population hybridisation. A notable exception is the study by Willi (19) in which

154 support was found for both tug-of-war and recognition-avoidance mechanisms in crosses

155 between predominantly selfing and predominantly outcrossing populations of *Arabidopsis*

156 *lyrata*. Given the prevalence of mixed-mating plant species, studies on such species are

157 crucial to achieve a more general understanding of the effect of mating systems on the

158 evolution of intersexual conflict in natural populations. Importantly, the observation of a

159 mating-system effect in crosses between populations with subtle differences in mating

160 system, would underscore the importance of the mating system in the evolution of this

161 conflict. Finally, the predictions we present are general and can be applied to any organism

162 where regulation of offspring size depends on genomic imprinting and where populations

163 vary in the intensity of intersexual conflict over maternal provisioning, via for example,

164 variation in multiple-paternity rates.

165   Here, we assess the role of mating systems in the evolution of intersexual conflict over

166 seed size and test which of the two genetic mechanisms (tug-of-war vs. recognition-

167 avoidance) mediate this conflict. To this end, we analysed two independent datasets obtained

168 from crosses within and between populations of the mixed-mating plant *Dalechampia*

169 *scandens* (Euphorbiaceae) (Fig. 2). The first dataset was obtained from crosses among nine

170 populations spanning a wide range of inferred mating systems (Table S1). Because the

171 number of crosses performed between each pair of populations was rather small and the

172 design somewhat unbalanced (Table S2), we obtained a second dataset from a controlled full-

173 diallel crossing-design using four populations (Table S3 and S4). Populations of *D. scandens*

174 differ in their rate of outcrossing, and because pollinators visit several plants per foraging

175 bout (40), the probability of multiple paternity, both within and among fruits, is likely to

176 increase with outcrossing rate. In this system, population-mean herkogamy (i.e. the spatial

177 separation of male and female structures in the inflorescence) correlates positively with

178 outcrossing rate ($r = 0.93$, $n = 4$ populations), pollination reliability, and genetic diversity

179 (41). We therefore used the population-mean herkogamy as a proxy for variation in mating

180 system, and hence the intensity of intersexual conflict in each population. Importantly, while

181 outcrossing rates are known to fluctuate between years (42), mean herkogamy is an evolved

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character, presumably representing the long-term outcome of selection for or against selfing, depending on the long-term average reliability of pollination (43). We estimated the effects of the relative outcrossing rate of the two parental populations and the outcrossing rate of the paternal population alone on inter-population hybrid seed size to test the first and second prediction, respectively. The effect on hybrid seed size was estimated as the percent difference in seed diameter between hybrid seeds and the mean diameter of seeds produced in the maternal population, to account for the maternal effects commonly observed for this trait (44-48).

Results and Discussion

We found no indications of incompatibilities between populations (Table S9). In both datasets, size differences between seeds resulting from inter-population crosses and those resulting from within-population (maternal) outcrossing were best explained by the relative outcrossing rates of the two parental populations (Fig. 3a and c; Table S5). Hybrid seeds were larger when the inferred historical outcrossing rate of the paternal population exceeded the inferred historical outcrossing rate of the maternal population, and smaller in the opposite case. In the dataset including crosses among nine populations of *D. scandens* with a broad range of inferred outcrossing rates (herkogamy ranging from 0.33 to 3.40 mm, Table S1), hybrid relative seed size increased by $1.34 \pm 0.46\%$ (mean $\pm$ SE) per unit change in the relative outcrossing rate of the parental populations (Fig. 3a). In contrast, the increase with the outcrossing rate of the paternal population alone was weakly supported statistically (Fig. 3b; $\beta = 1.29 \pm 0.66$, Table S6b). This pattern was confirmed by the results from the diallel crosses among four populations. Although these populations exhibited a smaller range of inferred outcrossing rates (herkogamy ranging from 2.61 to 3.40 mm, Table S3), we observed a clear increase in hybrid relative seed size per unit change in the relative outcrossing rate ($\beta = 3.28 \pm 1.19$; Fig. 3c; Table S5), while the effect of the outcrossing rate of the paternal population on hybrid seed size was limited and weakly supported statistically ($\beta = 0.81 \pm 0.49$; Fig. 3d, Table S6b). Finally, between-population crosses did not systematically produce larger seeds compared to the within-population crosses, and this was true in the broad dataset (contrast = $0.45 \pm 0.57\%$; Table S6b) and in the diallel dataset (contrast = $0.11 \pm 0.30\%$; Table S6b).

Overall, these results strongly support the hypothesis that the intensity of intersexual conflict over maternal investment in seeds increases with outcrossing rate in this self-compatible, perennial plant species. More specifically, the results support the tug-of-war

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215   hypothesis, wherein uniparentally expressed genes with opposite effects on seed growth have

216   coevolved within populations. Although differences in measured seed diameter between

217   hybrid and within-population seeds were small (ca. 5% and 2% in the first and second dataset,

218   respectively, Fig. 3a and c), these translate into ca. 14% and 6% differences in seed mass,

219   respectively.

220   As expected from previous studies (49-52), we found little evidence for additive

221   genetic effects on seed size. Indeed, in both datasets, hybrid relative seed size was not

222   detectably affected by the average seed size in the paternal population relative to the maternal

223   population (crosses between the nine populations: $\beta = 10.37 \pm 13.38$, diallel crosses: $\beta = 3.34$

224   ± 4.12; Table S7). This observation further supports our main result that seed size is affected

225   by a more complex mechanism of inheritance.

226   Inbreeding depression in seed size, measured as the difference in size between seeds

227   produced by selfing versus outcrossing within populations, tended to increase with

228   outcrossing rate in the four diallel populations. Although this result is consistent with the

229   purging of deleterious alleles in the most inbred populations (Fig. S1; 53), these differences

230   were limited and statistically not significant (Table S8). Nevertheless, an increase in the size

231   of hybrid seeds relative to the seeds produced by within-population crosses could have

232   resulted from heterosis (i.e. hybrid vigour due to the restoration of heterozygosity in crosses

233   between inbred populations: 53, 54, 55). Although heterosis effects cannot explain the

234   decrease in seed size observed in some hybrid crosses as predicted by the tug-of-war

235   mechanism, it may explain the small upward shift in the intercept of the relationship between

236   hybrid relative seed size and relative outcrossing rate in the diallel data (Fig. 3c).

237   The present study supports the idea that uniparentally expressed genes with

238   antagonistic effects on seed growth have coevolved within natural populations of

239   *Dalechampia scandens* as an outcome of intersexual conflict over maternal investment. It

240   further suggests that subtle differences in mating system have resulted in the rapid evolution

241   of genes involved in this conflict. The importance of imprinted genes influencing offspring

242   growth has been well documented in mammals (18), some crops (56, 57), and in the model

243   organism *Arabidopsis thaliana* (17, 58-60). However, knowledge derived from artificially

244   selected or highly inbred species is of limited relevance for understanding the evolution of

245   intersexual conflict in a natural context. To our knowledge, only one prior study, that of Willi

246   (19), has performed the kinds of inter-population crosses between natural populations needed

247   to determine which of the two mechanisms, tug-of-war versus recognition-avoidance,

248   mediates intersexual conflict over maternal resources. Our study differs from that of Willi

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249   (19) in several aspects. First, Willi (19) used populations that were either predominantly

250   selfing or predominantly outcrossing (with the exception of one mixed-mating population),

251   while all our populations were mixed-mating with relatively small differences in outcrossing

252   rates, as inferred from the limited variation in herkogamy. Second, instead of comparing

253   hybrid seed size with the mid-parent average (which assumes an additive genetic effect as the

254   null hypothesis), we compared the hybrid seed size with the average seed size in the maternal

255   population, therefore accounting for the strong maternal effects generally observed on seed

256   size (e.g. 44, 45-47). Finally, based on the mating systems of the populations involved (22),

257   we made clear predictions regarding the direction of the deviation in hybrid seed size

258   compared to the average seed size in the maternal population. In contrast to Willi (19), we did

259   not observe a consistent increase in hybrid relative seed size when the pollen donor came

260   from another outcrossing population, suggesting that the recognition-avoidance mechanism is

261   of limited importance in our study system. The upward shift in the intercept of the

262   relationship between hybrid relative seed size and relative outcrossing rate could be explained

263   by the fact that maternal plants in our system partly fail to control some of the effects of

264   foreign paternal growth-enhancing alleles, but as previously mentioned, this could also be

265   explained by heterosis effects. However, the general pattern in both datasets suggests that a

266   tug-of-war mechanism is the most prevalent in these populations.

267   To conclude, our study on a mixed-mating, perennial plant shows that crosses between

268   populations with subtle differences in mating systems yield hybrid seeds that deviate from the

269   within-population equilibrium seed size in the manner predicted by the tug-of-war hypothesis,

270   but not by the recognition-avoidance hypothesis. These results support the idea that

271   maternally and paternally derived alleles with antagonistic effects on seed growth have

272   coevolved within populations, and that antagonistic forces are stronger in more outcrossed

273   populations that have histories of more intense intersexual conflict over maternal investment.

274   This may have important consequences for the maintenance of local adaptation in the

275   presence of gene flow. Indeed, if seed size is locally adapted, depending on environmental

276   conditions and size-number trade-off (4), any gene flow from populations with different

277   outcrossing rate should negatively affect maternal fitness by affecting the size of the seeds

278   produced. However, the fitness of individual seeds (e.g. germination and establishment

279   success) is predicted to increase when the paternal population is more outcrossed but decrease

280   in the opposite case. Although neither additive genetic effects nor heterosis seemed to explain

281   much of the variation in hybrid seed size, it would still be interesting to investigate

282 systematically how genomic imprinting due to intersexual conflict interacts with local

283 selection pressures on maternal and paternal genetic components of seed size.

284 Methods

285

286 STUDY SPECIES AND POPULATIONS

287 Crosses were made within and among populations of *Dalechampia scandens* L.

288 (Euphorbiaceae), a mixed-mating, perennial vine with a distribution ranging from Mexico to

289 Argentina (61). The bisexual, pseudanthial inflorescences (blossoms) comprise ten male

290 flowers clustered above three female flowers. Each female flower contains three ovules, so

291 that a blossom can produce up to nine seeds (62). A gland situated above the male flowers

292 secretes a terpenoid resin, which functions as pollinator reward, attracting apid and

293 megachilid bees that use resin for nest building (61, 63). Two petaloid bracts subtend the

294 flowers and function as an advertisement to attract pollinators (64).

295   Blossoms are functionally protogynous with a female phase preceding a bisexual

296 phase during which autogamous selfing can occur (65). Pollinators visit both female-phase

297 and bisexual-phase inflorescences and they commonly visit multiple plants per foraging bout

298 (40). The rate of autofertility (seed set in the absence of pollinators) declines with increasing

299 distance between anthers and stigmas (herkogamy), a highly evolvable trait that varies among

300 populations (41, 66, 67). In plants in general, populations with high autofertility rates tend to

301 have low outcrossing rates, and thus herkogamy offers a reliable proxy for variation in mating

302 system (i.e. outcrossing rate) (43). This was confirmed for *D. scandens* where variation in

303 outcrossing rates among natural populations was positively correlated with population-mean

304 herkogamy ($r = 0.93$, $n = 4$ populations) (41).

305   The first dataset comprised measurements of seeds produced as part of a larger study

306 of population differentiation in *D. scandens*, where crosses were performed among 9

307 populations originating from Mexico (Tables S1, S2). Because of the unbalanced sampling in

308 this first dataset (missing data for many cross-combinations and few crosses per combination,

309 $n = 635$ seeds from 86 inter-population crosses) and the absence of information on covariates

310 that could potentially influence seed size (see below), we obtained a second dataset by

311 crossing four populations originating from Veracruz and the Yucatán peninsula in Mexico in

312 a full-diallel design, also including self-pollination (Tables S3, S4). All populations differed

313 in average seed size and herkogamy (Table S1; 68). The populations are inter-fertile but

314 geographically separated by at least 225 km, so natural gene flow should be extremely rare

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315   (40). Population differences in seed size and herkogamy were observed on individuals grown

316   in the greenhouse and therefore represent genetic differences. Note that the range of

317   herkogamy among the four populations included in the diallel is similar to the range observed

318   among the populations analysed by Opedal *et al.* (41), for which the rate of outcrossing

319   ranged from 0.16 to 0.49.

320

321   EXPERIMENTAL DESIGN AND MEASUREMENTS

322   Crosses for the first dataset were performed between 2007 and 2009 between individuals

323   grown in the greenhouse from field-collected seeds. Between May and July 2016, we used

324   seeds obtained from random crosses within four of the nine populations to grow the plants

325   that we used in the diallel experiment. Hence, the experimental individuals were second

326   greenhouse-generation plants. We used ten plants per population and distributed them evenly

327   across two tables in a single room in the greenhouse with 13/11 light/dark regime and 25°C

328   during the day and 23°C at night. We watered the plants every day by flooding the tables with

329   ca. 5 cm of water. Plants were moved weekly to avoid positional effects.

330   All four populations were crossed in a complete diallel design, with each population

331   used both as paternal and maternal population (Table S4). This resulted in four sets of within-

332   population crosses and twelve sets of between-population crosses. Within populations, each

333   plant was crossed with two different plants from the same population (outcrossing) and once

334   with itself (geitonogamous selfing). For the between-population crosses, each population was

335   crossed both as maternal and paternal population with each of the three other populations. For

336   each combination of two populations, ten plants from the maternal population were crossed

337   with three different plants from the paternal population. Hence, each individual was

338   represented three times as maternal and three times as paternal plant in the crossing design.

339   Crosses (total $n = 460$) were made from August to December 2016 by AR. Blossoms were

340   emasculated and hand-pollinated during the female phase with an ample amount of pollen

341   from a freshly dehisced male flower. Crosses that failed were recorded, and repeated. Crosses

342   were performed in a random order to avoid possible confounding factors associated with the

343   timing of pollination and uncontrolled variation in the greenhouse environment. To control

344   for the effect of blossom size on seed size, the peduncle diameter, which correlates with

345   blossom size (50), was measured with digital callipers (0.01 mm precision). Hand-pollinated

346   blossoms were enclosed in empty tea bags to collect seeds after explosive dehiscence.

347   We counted the number of seeds produced per blossom (*seed set*), and measured the

348   diameter of each individual seed (*seed size*) with digital callipers (0.01 mm precision; all

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349   measurements were made by AR). Repeatability of seed measurements estimated by repeated

350   measurements of one seed per seed set was high ($r^2 = 0.99$, $n = 447$). Seeds were measured in

351   random order. We used seed diameter as a measure of seed size because seed diameter is less

352   prone than seed mass to vary with time due to water loss. Still, we weighed the seed sets to

353   estimate the allometric relationship between seed diameter and average seed mass. Seed

354   diameter and mass were strongly correlated ($r^2 = 0.90$, $n = 428$ seed sets) and the allometric

355   exponent was very close to three (3.04 ± 0.07) as expected for an allometry between a length

356   and a mass. This allometric relationship indicates that percent differences in seed mass can be

357   accurately estimated by simply multiplying the percent difference in diameter by three.

358

359   STATISTICAL ANALYSES

360   To assess whether the mating system of the parental populations affect seed size in inter-

361   population crosses, and which of the two genetic mechanisms, tug-of-war or recognition-

362   avoidance occurs, we compared hybrid seed size with the size of the seeds normally produced

363   in the maternal populations. Maternal effects are expected to strongly influence seed size even

364   in hybrid crosses. Therefore, we expressed hybrid seed size as a percent deviation from the

365   average seed size produced by within-population crosses (excluding selfing) in the maternal

366   population: $Hybrid\ relative\ seed\ size = 100 \times \left(\frac{Hybrid\ seed\ diameter}{Mean\ seed\ diameter\ in\ the\ maternal\ population} \right)$. For the data

367   including nine populations, we calculated the mean seed diameter within each maternal

368   population from the raw data, while for the four populations in the diallel we estimated this

369   for each maternal populations from linear mixed-effect models where paternal population

370   identity was set as predictor variable with five levels: pollen from another individual in the

371   same population (within-population outcross), pollen from the same plant (selfing), and

372   pollen from each of the three other populations (between-population cross). Effects of

373   peduncle diameter and number of seeds per blossom on seed size were population-specific

374   (Table S8). Therefore, both variables were centred on the mean of the maternal population

375   and included as covariates. Number of seeds per blossom was also allowed to interact with

376   paternal population identity to account for a potential cross-specific size-number trade-off.

377   Paternal plant identity, and blossom identity nested within maternal plant identity were set as

378   random factors.

379   To test the tug-of-war model (Fig. 1a), we quantified the relative outcrossing rate of

380   the two parental populations inferred from their mean herkogamy (41) as:

381     $Relative\ outcrossing\ rate = log_e \left(\frac{Paternal\ population\ herkogamy}{Maternal\ population\ herkogamy} \right)$. This index is symmetrical around

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zero and will take positive values when the paternal population is more outcrossed than the maternal population, and negative values in the opposite case. We fitted a linear mixed-effects model with hybrid relative seed size as the response variable and relative outcrossing rate as the predictor variable. We also included blossom identity nested within maternal plant identity, nested within maternal population identity, and paternal plant identity nested within paternal population identity as random factors. For the diallel data, we also included mean-centred peduncle diameter in interaction with maternal population identity, and mean-centred seed number in interaction with maternal and paternal population identity as covariates.

To test the recognition-avoidance model (Fig. 1b), we fitted two different linear mixed-effects models testing two distinct predictions. If hybridisation leads to failed maternal recognition of foreign paternal allele products, seeds from inter-population crosses are expected to be larger than seeds produced by within-population crosses. We tested this prediction by fitting a linear mixed-effects model with hybrid relative seed size as response variable and cross type (between- vs. within-population) as predictor variable. In addition, hybrid relative seed size should increase with the outcrossing rate of the paternal population, because growth-promoting alleles from more outcrossed populations are expected to have stronger effects. To test this second prediction, we fitted a linear mixed-effects model using hybrid relative seed size as the response variable and the mean herkogamy (as a proxy for outcrossing rate) of the paternal population as the predictor variable. In both models, random effects and covariates were specified as above.

Finally, we tested for a model where seed size determination follows a simple additive genetic inheritance pattern. In this case, hybrid relative seed size should depend on the relative seed size in the two paternal populations. We quantified relative average seed size of the two parental populations (excluding selfed seeds) as:

$Relative\ seed\ size = log_e \left(\frac{Paternal\ population\ seed\ diameter}{Maternal\ population\ seed\ diameter} \right)$. We then fitted a linear mixed-effects model with hybrid relative seed size as the response variable and relative seed size as the predictor variable. Random effects and covariates were specified as above.

All statistical analyses were conducted in *R* version 3.3.3 (69), and linear mixed-effects models were fitted using the *lme4*-package (70).

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Figure Legends

Figure 1. Predicted outcomes for hybrid offspring size (solid lines) in crosses between populations relative to the average normal offspring size in the maternal population (horizontal dashed lines) under two different genetic mechanisms. With the tug-of-war mechanism (a) hybrid offspring are predicted to become larger than the offspring normally produced in the maternal population (horizontal dashed line) if the paternal population has experienced more intense intersexual conflict than the maternal population, and smaller in the opposite cross direction. The vertical dashed line represents the point where the intensity of intersexual conflict is similar in both parental populations, and therefore where genes originating from each population have exact opposite effects on seed size. With the recognition-avoidance mechanism (b) hybrid offspring should become consistently larger than the offspring normally produced in the maternal populations (dashed line), and the magnitude of the difference may increase with the intensity of intersexual conflict in the paternal population.

Figure 2. Blossom inflorescence of *Dalechampia scandens* with the first (terminal) male flower open above the three female flowers. In this species, the shortest distance between anthers and the stigmas (ASD) affects the outcrossing rate. (Photo C. Pélabon).

Figure 3. Results of the experimental tests of the tug-of-war and recognition-avoidance mechanisms. The hybrid relative seed size of crosses among populations of *Dalechampia scandens* is regressed against the relative outcrossing rate of parental populations (a, c) to test the tug-of-war hypothesis, and against the outcrossing rate of the paternal population (b, d) to test the recognition-avoidance mechanism. Data in panels a and b are from the crosses among nine populations covering a wide range of outcrossing rates and data in panels c and d are from the diallel among four populations. The vertical dashed lines on panel a and b mark the range in relative outcrossing rate and paternal population outcrossing rate covered by the populations in the diallel experiment. The hybrid relative seed size is the hybrid seed diameter (dm) expressed as a percent deviation from the average seed diameter within the maternal population. Shaded areas represent 95% confidence intervals for the regression models. Regression lines are estimated using individual seed data in mixed-effect models. Each point represents the mean ($\pm$ SE) relative seed diameter for each cross combination. The relative outcrossing rate of the parental populations is estimated as: $log_e \left(\frac{Paternal\ population\ herkogamy}{Maternal\ population\ herkogamy} \right)$ and the outcrossing rate of the paternal population is estimated as the mean herkogamy (mm).