Asymmetric gene flow causes cascading reproductive isolation

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Keywords: hybridization, reproductive barriers, speciation, *Ipomoea*, mating system

Abstract

The interplay between hybridization and reproductive isolation is critical to the origin and maintenance of species. Hybridization between species is known to affect reproductive barrier strength. An extension of this relationship is the expectation that asymmetric hybridization and gene flow will have asymmetric effects on reproductive barrier strength. We tested this hypothesis in a pair of morning glory species which exhibit asymmetric gene flow in sympatry from highly selfing *Ipomoea lacunosa* into mixed mating *I. cordatotriloba*. Because of the direction of hybridization, we predicted that reproductive barrier strength would change to a greater extent in *I. cordatotriloba* than *I. lacunosa.* We tested this prediction by measuring the strength of a barrier to seed set between these species across their ranges. Consistent with our prediction, we found that sympatric *I. cordatotriloba* was more compatible with range-wide *I. lacunosa* than allopatric *I. cordatotriloba,* whereas sympatric and allopatric *I. lacunosa* were equally compatible with *I. cordatotriloba.* This difference likely reflects an asymmetric decrease in reproductive barriers in sympatric *I. cordatotriloba*. We further demonstrated that changes to sympatric *I. cordatotriloba* have decreased compatibility with allopatric populations. Thus, in a manner analogous to cascade reinforcement, introgression associated with asymmetric hybridization not only influences between-species isolation but also contributes to isolation within a species.

Introduction

Reproductive barriers are fundamental to the evolution and maintenance of biological diversity. They maintain the integrity of species by preventing the homogenizing effect of gene flow. However, incomplete reproductive isolation is common, and consequently hybridization between species in sympatry is frequent (Mallet 2005, Whitney et al. 2010). When hybridization occurs, it can affect the reproductive barriers that separate species, which, in turn, affects the potential for future hybridization between those species. Species that initially hybridize in sympatry may collapse into a single species, complete speciation by undergoing reinforcement, or coexist in stable hybrid zones (Abbott et al. 2013, Todesco et al. 2016). Whether reproductive isolation increases, decreases, or remains unchanged during hybridization thus determines species boundaries in contact zones.

Hybridization affects reproductive barrier strength in two major ways: by homogenizing genotypes and traits and through interactions with natural selection. First, hybridization that results in introgression can cause traits that were previously differentiated between species to converge. If those traits underlie reproductive barriers, the strength of reproductive isolation between species will change. For example, when a trait that causes assortative mating on its own (e.g., self-referential mating preferences, selfing) spreads to a new species, reproductive isolation increases (Felsenstein 1981, Ortíz-Barrientos and Noor 2005). On the other hand, when a trait that produces reproductive isolation through differentiation (e.g., differences in flowering time) is homogenized, reproductive isolation decreases.

Second, hybridization that produces unfit offspring creates opportunities for selection to directly influence reproductive isolation. Selection can weed out unfit hybrids and consequently purge incompatible alleles and reduce postzygotic reproductive barrier strength (Clarke 1966, Endler 1977, Sanderson 1989, Gavrilets 1997). At the same time, in a process known as reinforcement, selection can favour earlier-acting reproductive barriers that increase total reproductive isolation and lessen resources wasted on unfit offspring (Dobzhansky 1940, Howard 1993, Servedio and Noor 2003). In some cases of reinforcement, new or strengthened reproductive barriers in sympatry cause incompatibility between sympatric and allopatric populations of the affected species (e.g., Hoskin et al. 2005, Jaenike et al. 2006, Kozak et al. 2015). This is known as “cascade reinforcement” or “cascade speciation” (Ortíz -Barrientos et al. 2009). However, any local change in reproductive barriers, including those caused by introgression, is likely to have cascading effects on reproductive isolation within and between species.

Asymmetric gene flow is known from a wide variety of organisms (Tiffin et al. 2001, Lowry et al. 2008, Todesco et al. 2016). It is common in selfing-outcrossing species pairs (Pickup et al. in press), and can result from asymmetric hybridization events, asymmetry in the fate of hybrids and introgressed alleles, or both processes. For example, asymmetries arise when one of the two hybridizing species is more often the maternal parent of hybrid offspring (Tiffin et al. 2001), is more common in regions of sympatry (Burgess et al. 2005), is more successful at backcrossing with hybrid offspring (Ippolito et al. 2004), or has a greater genetic load (Bierne et al. 2002, Kim et al. 2018, Pickup et al. in press). In systems with asymmetric hybridization and introgression, we expect that changes in reproductive barrier strength will also be asymmetric, regardless of whether reproductive barriers increase (Noor 1995, Jaenike et al. 2006, Yukilevich 2012) or decrease. In the most extreme cases, with unidirectional hybridization, we expect that only the species experiencing gene flow will experience homogenization, selection on introduced alleles, or reinforcing selection, and will thus have the potential to evolve in ways that change reproductive isolation. Here, we provide what we believe to be the first test of this hypothesis.

Rifkin et al. (2019a) identified extensive asymmetric gene flow between self-compatible morning glory species with different mating systems. In regions of sympatry (Fig. 1), there has been substantial introgression into the mixed-mater *I. cordatotriloba* while there has been essentially none into the highly selfing *I. lacunosa.* Although this direction of gene flow is counter to some expectations (Davis and Heywood 1963), a recent survey of gene flow between self-compatible species with different levels of selfing exclusively found more gene flow from selfing species into outcrossing species (Pickup et al. in press). This likely occurs because interspecific pollen that lands on the stigmas of outcrossing plants is more likely to fertilize ovules than interspecific pollen that arrives on the stigmas of selfing plants that have already self-fertilized (Lloyd 1979, Fishman and Wyatt 1999, Goodwillie and Weber 2018, Brys et al. 2016). This explanation is particularly likely in these *Ipomoea* species where anthers often dehisce before flowers open.

As a result of unidirectional hybridization and gene flow from the selfing to outcrossing species, we expect to see stronger effects of hybridization in the outcrossing species. Indeed, Rifkin et al. (2019a) observed phenotypic convergence between sympatric *I. cordatotriloba* and *I. lacunosa* caused by change in *I. cordatotriloba* but little change in *I. lacunosa.* Among the traits that showed this pattern were anther-stigma separation (herkogamy) and selfing rate. Because herkogamy is the main determinant of selfing rate in self-compatible *Ipomoea* (Duncan and Rausher 2013) and other plant species (e.g., Karron et al. 1997, Motten et al. 2000), the significantly reduced anther-stigma separation in sympatric *I. cordatotriloba* plants is consistent with the substantially higher selfing rates

of this species observed in sympatric regions. This increased selfing in *I. cordatotriloba* presumably constitutes a stronger barrier to gene flow from *I. lacunosa*.

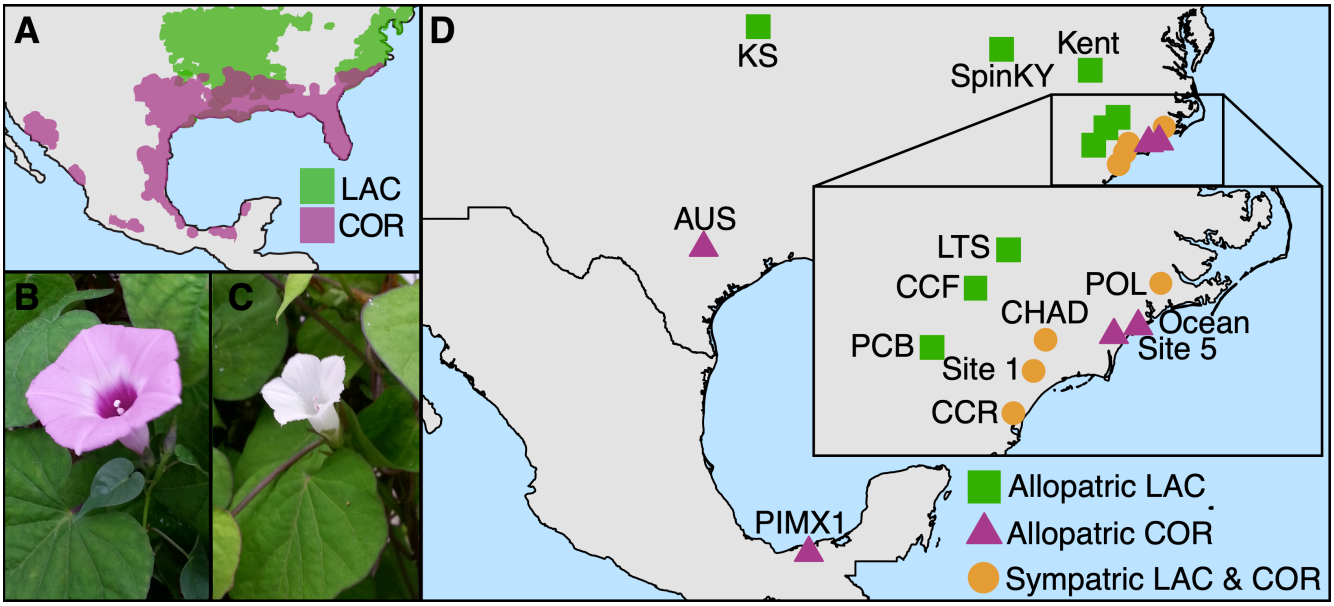

Figure 1 – A) Map showing the approximate distributions of *I. lacunosa* (green) and *I cordatotriloba* (purple) in North America. This map was redrawn based on Khoury et al. (2015) and generally matches collection records from the United States (USDA, NRCS 2019). B) A typical allopatric *I. cordatotriloba* flower. C) A typical *I. lacunosa* flower. D) Map showing the locations of populations used in this study.

In addition to mating system differences, *I. cordatotriloba* and *I. lacunosa* are partially reproductively isolated by a barrier to seed set (Martin 1970, Abel and Austin 1981, Diaz et al. 1996, Duncan and Rausher 2013b). This crossing barrier is potentially subject to all possible forces acting on reproductive barriers during hybridization. Although vigorous, hybrids between *I. cordatotriloba* and *I. lacunosa* tend to produce less pollen than the parental species (Rifkin 2017). The existence of less fertile hybrids means that selection could act to increase reproductive barriers that act before maternal provisioning is complete (e.g., a barrier to seed set, Hopkins 2013) or to lessen the postzygotic barrier where the species hybridize. Alternatively, the traits that underlie this crossing barrier could be homogenized by gene flow, possibly in conjunction with selection to eliminate incompatibility alleles in *I. cordatotriloba*. In the latter case, introgression of incompatibility alleles from *I. lacunosa* could then generate cross-incompatibility between allopatric and sympatric populations of *I. cordatotriloba.*

Here we experimentally assess seed set in crosses among sympatric and allopatric populations of the two morning glory species to determine whether gene flow in sympatry affects the strength of the crossing barrier. Given asymmetric hybridization and introgression from *I. lacunosa* into *I.*

cordatotriloba, we expect to find that, if changes to reproductive isolation occur, they will occur more strongly in sympatric populations of *I. cordatotriloba.* We also expect that local changes in reproductive barrier strength could cause reproductive barriers to arise within species, and therefore we examine reproductive isolation between allopatric and sympatric populations of *I. cordatotriloba.*

Results and Discussion

Controlled crosses between pairwise combinations of sympatric and allopatric *I. lacunosa* individuals and sympatric and allopatric *I. cordatotriloba* individuals from multiple populations (Fig. 1D, Fig. S1), revealed variation in the success of different cross types (Fig. 2, S2). First, our results confirm the existence of a strong reproductive barrier separating *I. cordatotriloba* and *I. lacunosa*. Overall, 42% of intraspecific crosses and only 5% of interspecific crosses set fruit and produced at least one seed (Fig. 2A). Interspecific crosses are significantly less likely to successfully produce seeds than both crosses within *I. cordatotriloba* ($t = 9.87$, $df = 310$, $p << 0.001$) and crosses within *I. lacunosa* ($t = 13.56$, $df = 310$, $p << 0.001$). However, we found no evidence that the success of interspecific crosses is affected by which species is used as the maternal parent (Fig. 2A; $t = 0.11$, $df = 310$, $p = 0.91$).

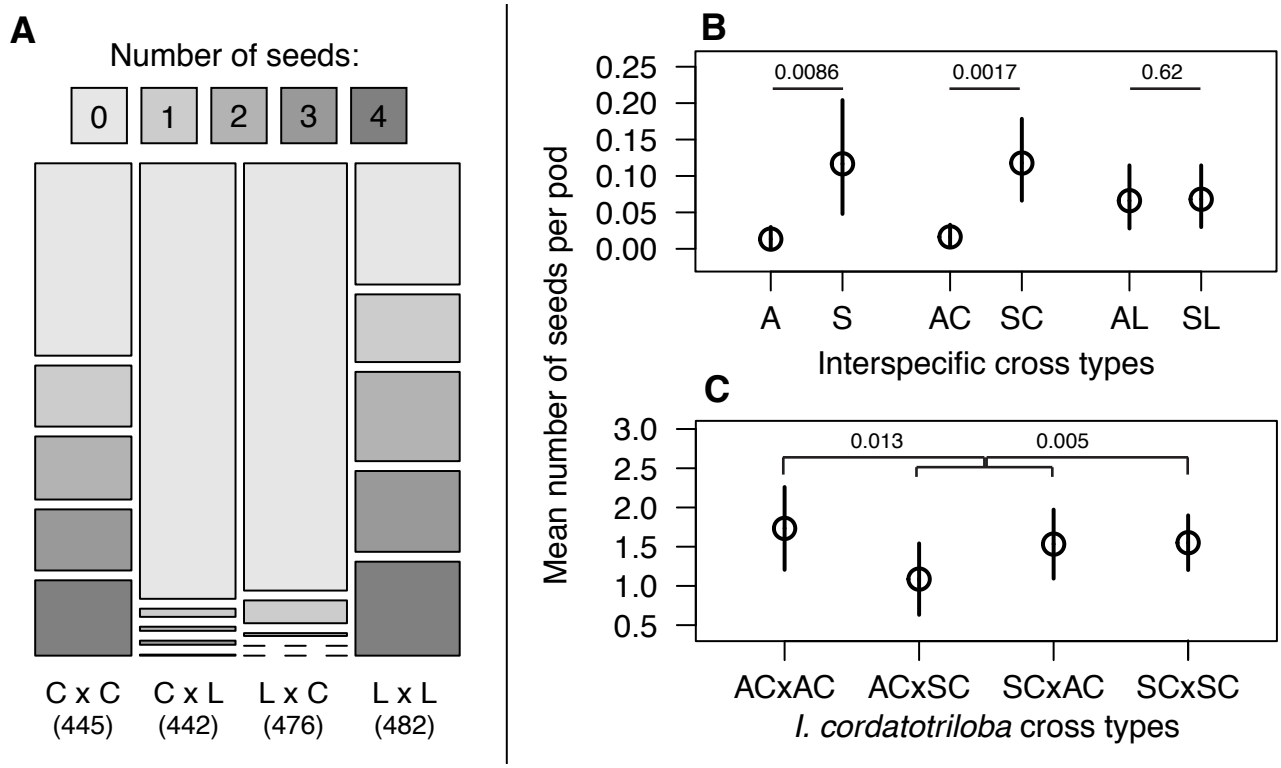

Figure 2 – A) The number of seeds produced by different types of crosses (ovule parent x pollen parent; C = *I. cordatotriloba*, L = *I. lacunosa*). Each box is proportional to the number of fruits that contained that many seeds after pollination. Dashed lines represent cases in which there were no fruits with a particular seed number. B) The mean and 95% confidence intervals (based on bootstrap percentiles) for the mean number of seeds

133 produced by different types of interspecific crosses: both species allopatric (A), both species sympatric (S),
134 allopatric *I. cordatotriloba* and any *I. lacunosa* (AC), sympatric *I. cordatotriloba* and any *I. lacunosa* (SC),
135 allopatric *I. lacunosa* and any *I. cordatotriloba* (AL), and sympatric *I. lacunosa* and all *I. cordatotriloba* (SL). C)
136 The mean and 95% confidence intervals for the mean number of seeds produced by different types of crosses
137 within *I. cordatotriloba*.
138
139 Second, we found that interspecific crosses with plants from sympatric sites set significantly more
140 seeds than those made with plants from allopatric sites (Fig. 2B: $t = 2.67$, $df = 146$, $p = 0.0085$). This
141 result could explain the wide variation in crossing success reported in previous studies (Martin 1970,
142 Abel and Austin 1981, Diaz et al. 1996, Duncan and Rausher 2013b). Further, it also suggests that the
143 crossing barrier has been degraded in sympatry. This result is significant because, although the
144 degradation of reproductive isolation in sympatry is supported by theory (Barton and Hewitt 1985,
145 Butlin 1987, Virdee and Hewitt 1994, Kelly and Noor 1996) and is implied by patterns of genetic
146 variation in many systems (e.g., Borge et al 2005, Sander et al. 2014, Zuellig and Sweigart 2019),
147 studies in which the strength of reproductive barriers have been explicitly quantified and shown to be
148 weaker in sympatry are rare (but see, Virdee and Hewitt 1994, Saetre et al. 1999, 2003,Carney et al.
149 2000, Collins and Rawlins 2013).
150
151 To determine whether changes in one or both species explains the pattern of higher seed set in
152 sympatric plants, we compared the success rates of interspecific crosses using sympatric and
153 allopatric plants within each species. We found that crosses between sympatric *I. cordatotriloba* and
154 range-wide *I. lacunosa* are more successful than between allopatric *I. cordatotriloba* and range-wide *I.*
155 *lacunosa* (Fig. 2B; $t = 3.19$, $df = 146$, $p = 0.0017$), whereas crosses between sympatric *I. lacunosa* and
156 range-wide *I. cordatotriloba* are no more likely to be successful than those between allopatric *I.*
157 *lacunosa* and range-wide *I. cordatotriloba* (Fig. 2B; $t = 0.49$, $df = 146$, $p = 0.62$). This result indicates
158 that only *I. cordatotriloba* experienced changes in characters that contribute to incompatibility and
159 supports the prediction that greater changes in the degree of reproductive isolation will occur in the
160 species experiencing greater gene flow. By itself, this type of asymmetric degradation of reproductive
161 barriers could have the potential to cause positive feedback in which asymmetric degradation of
162 reproductive barriers facilitates even more asymmetric gene flow.
163
164 Although one potential outcome of hybridization is reinforcement and increased reproductive
165 isolation in sympatry, we did not observe this outcome for cross compatibility. It is possible the
166 asymmetry itself was an important contributor to the lack of reinforcement in this system. Servedio
167 and Kirkpatrick (1997) showed theoretically that reinforcement is less likely in cases with one-way
168 migration relative to symmetric migration. Furthermore, in this system one barrier (high selfing rate;

169 Rifkin et al. 2019a) increased, while another (cross incompatibility) decreased, indicating that not all
170 barriers will necessarily respond to gene flow in the same way. This result is also consistent with
171 simulations by Castillo et al. (2016), which showed that increases in self-compatibility in sympatry
172 often preclude the evolution of assortative mating such as that represented by the crossing barrier in
173 this system. Finally, because we have not measured all possible reproductive barriers (e.g. flowering
174 time and pollinator isolation) and we observed both increases and decreases in barrier strength, we
175 cannot determine how total reproductive isolation between *I. cordatotriloba* from *I. lacunosa* has
176 changed.
177
178 The changes due to introgression into sympatric *I. cordatotriloba* have also affected compatibility
179 between sympatric and allopatric populations of this species. Specifically, crosses between sympatric
180 and allopatric individuals produce significantly fewer seeds than either crosses between sympatric
181 individuals (Fig. 2C; $t = 2.89$, $df = 75.5$, $p = 0.005$) or between allopatric individuals (Fig. 2C; $t = 2.54$, df
182 $= 75.7$, $p = 0.013$). This pattern is consistent with that expected if incompatibility alleles introgressed
183 into sympatric *I. cordatotriloba* from *I. lacunosa.* In particular, with complete introgression neither
184 allopatric nor sympatric *I. cordatotriloba* would be segregating for incompatibility, and should thus
185 exhibit similar, relatively high levels of compatibility in within-allopatry or within-sympatry crosses.
186 By contrast, because sympatric *I. cordatotriloba* has the alleles from *I. lacunosa* that originally caused
187 incompatibility, they should cause incompatibility with allopatric *I. cordatotriloba* in sympatric by
188 allopatric crosses. The approximately 25 percent reduction in compatibility between sympatric and
189 allopatric populations, which would reduce gene flow from sympatric *I. cordatotriloba* genotypes into
190 allopatric genotypes, coupled with the evolution of almost complete selfing of *I. cordatotriloba* in
191 sympatric populations (Rifkin et al. 2019a) that would likely impede gene flow in the opposite
192 direction, suggests the allopatric and sympatric populations are substantially isolated reproductively.
193 Because both of these effects are by-products of introgression in sympatry, our results provide an
194 example of how asymmetric introgression in sympatry has the potential to drive speciation between
195 allopatric and sympatric populations of the same species. This process is analogous to "cascade
196 reinforcement" or "cascade speciation" (Ortíz -Barrientos et al. 2009) except that erosion, rather than
197 reinforcement, of the between-species crossing barrier drives an increase in reproduction between
198 sympatric an allopatric populations. Our work thus demonstrates that any type of evolutionary
199 change in local reproductive barriers has the potential to affect within-species compatibility.

Methods

Species information

Ipomoea lacunosa and *I. cordatotriloba* are sister species (Muñoz-Rodríguez et al. 2018) in section *Batatas* of the morning glory genus (*Ipomoea*: Convolvulaceae). The two species have overlapping ranges in the southeastern United States, but only *I. lacunosa* occurs north of North Carolina into Canada and only *I. cordatotriloba* occurs south and west into Mexico (Fig. 1A). Both species produce many bisexual, self-compatible flowers that open for a single day (Fig. 1B-C). However, populations of *I. cordatotriloba* range from nearly complete outcrossing to nearly complete selfing, while all populations of *I. lacunosa* are highly selfing (all selfing rates ≥ 0.89, Duncan and Rausher 2013a). Accordingly, *I. lacunosa* exhibits many traits that are considered part of the "selfing syndrome" (Ornduff 1969, Sicard and Lenhard 2011), including small pale flowers, little nectar, and a low pollen:ovule ratio (Fig. 1C, McDonald et al. 2011, Duncan and Rausher 2013a, Rifkin et al. 2019a, Rifkin et al. 2019b).

Populations and crosses

To measure seed set between *I. lacunosa* and *I. cordatotriloba,* we grew 60 plants from 18 populations collected across 14 locations (Fig. 1D) under common greenhouse conditions. Seeds were scarified and germinated in potting mix in four-inch pots in a growth room under a long-day cycle (16:8 light:dark) and shifted to short-day cycle (13:11 light:dark) after approximately 7 weeks. When flower buds appeared (typically 2-3 weeks after the light change), we transferred plants to the Duke University Greenhouse Facility. Then, we selected 40 focal plants made up of 10 individuals from each of the following four population categories: allopatric *I. cordatotriloba* (AC), allopatric *I. lacunosa* (AL), sympatric *I. cordatotriloba* (SC), and sympatric *I. lacunosa* (SL). Allopatric populations were defined as populations growing outside the range of the other species or populations within the range of the other species where we observed only one species after a thorough search. Data from Rifkin et al. (2019a) suggests that these categorizations capture biologically relevant groups. In particular, STRUCTURE analysis revealed that *I. cordatotriloba* consists of two relatively homogeneous genetic groups corresponding to allopatric and sympatric populations (Rifkin et al. 2019a). By contrast, *I. lacunosa* populations consist of a single genetic group encompassing both allopatric and sympatric populations.

We made six reciprocal crosses between each focal plant and a representative of each population category, so that each focal plant was involved in 48 crosses (2x4x6), 24 as a pollen recipient and 24 as a pollen donor (Fig. S1). Within each cross type (i.e., all pairwise combinations of the population

233 categories), we made crosses between individuals from at least three populations within each
234 population category and we did not cross any combination of populations more than three times
235 (Table S1). To perform crosses, we emasculated flowers the day before they opened by dissecting the
236 corolla and removing the anthers. Between 8:00am and 12:00pm the next day, we pollinated the
237 emasculated flowers by dabbing a flower from the individual used as the male on the stigma of the
238 flower used as the female. This method transfers many more pollen grains than needed to fertilize
239 the 4 ovules present in each pistil. In these species, if a flower is left unpollinated or a cross fails, the
240 flower generally abscises and falls off the plant within 4 days. Therefore, we checked the crosses
241 every day until they abscised or until the seed capsule had dried and the sepals had reflexed,
242 indicating seed maturation. Finally, we counted and weighed the mature seeds and fruits.

243 Statistical Analyses

244 To determine whether cross type or location affected whether a cross was successful, we fit different
245 types of linear mixed effect models to the mean number of seeds produced by specific pairs of
246 individuals using R version 3.4.2 (R Core Team 2015) and the R packages *lme4* (Bates et al. 2015) and
247 *glmmTMB* (Brooks et al. 2017). We identified significant contrasts using least-squared means
248 implemented in the R package *emmeans* (Lenth 2016). Models fit to data that included interspecific
249 crosses included a term for zero-inflation and all models included maternal individual nested within
250 maternal population and paternal individual nested within paternal population as random effects.

251 Acknowledgements

252 We would like to thank Evangeline Mareki, Irene Liao, and Yuncheng Duan for help making crosses
253 and managing morning glories. We would also like to thank Jenn Coughlan, Kieran Samuk, Felix
254 Beaudry, Loren Rieseberg, Spencer Barrett, Stephen Wright, Elizabeth Lacey, and the Rausher lab for
255 helpful discussions and comments on earlier versions of this manuscript. This research was supported
256 by a National Science and Engineering Research Council of Canada Postdoctoral Fellowship (516658)
257 to KLO, a National Science Foundation Doctoral Dissertation Improvement Grant (DEB 1501954) to
258 JLR, and a National Science Foundation grant (DEB 1542387) to MDR.

259 Author Contributions

260 KLO, JLR, and MDR designed the study. KLO and HX collected the data. KLO and MDR analyzed the
261 data. KLO, JLR, and MDR drafted the manuscript. All authors read, edited, and approved the final
262 manuscript.

Data Accessibility

The data and scripts used in these analyses will be made available on an online data repository (Dryad) upon manuscript acceptance.

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398

Supplementary Figures

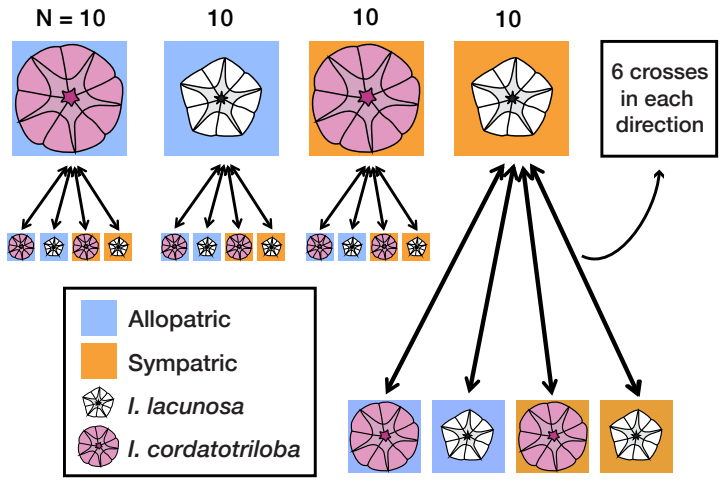

Figure S1 – Diagram of the full crossing design for this study.

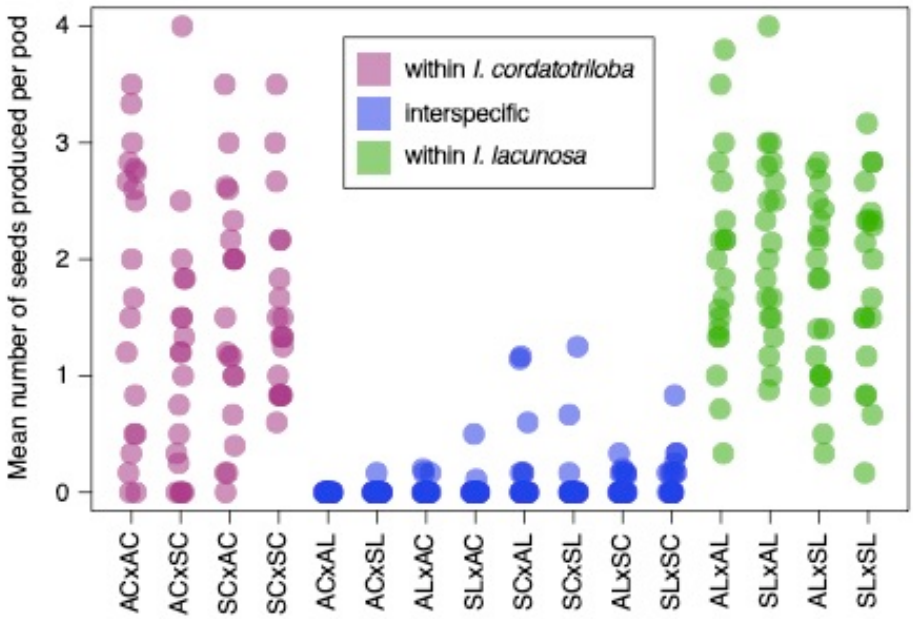

Figure S2 - The mean number of seeds produced by crosses between all pairwise combinations of population categories: allopatric *I. cordatotriloba* (AC), allopatric *I. lacunosa* (AL), sympatric *I. cordatotriloba* (SC), and sympatric *I. lacunosa* (SL). Each point represents 5-6 crosses.