Asymmetric gene flow causes cascading reproductive isolation

Kate L Ostevik[1], Joanna L Rifkin[2], Hanhan Xia[3], and Mark D Rausher[1]

1. Department of Biology, Duke University, Durham, NC, USA, 27708
2. Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada, M5S 3B2
3. College of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou, 510225 China

Correspondence to: kate.ostevik@gmail.com

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, introgression associated with asymmetric hybridization not only influences between-species isolation but also contributes to isolation within a species in a manner analogous to cascade reinforcement.

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 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., selfing) spreads to a new species, reproductive isolation increases. 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). 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, Butlin 1987, 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” (Ortiz-Barrientos 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 reinforcement, 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 morning glory species. 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.* As observed in other plant species, this asymmetry is likely a consequence of the divergent mating system 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). As a result of this unidirectional hybridization and gene flow, 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*.

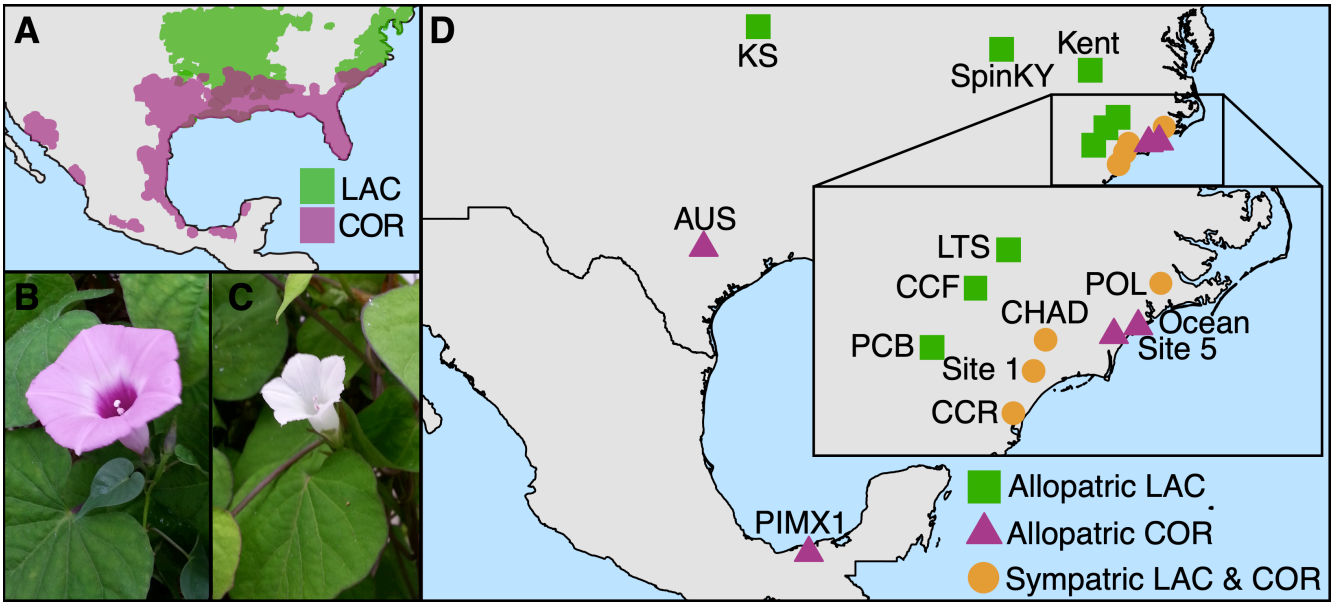

Figure 1 – A) Map showing the approximate distributions of *I. lacunosa* (green) and *I cordatotriloba* (purple) in North America. This map was redrawn based on Khoury et al. (2015) and generally matches collection records from the United States (USDA, NRCS 2019). B) A typical allopatric *I. cordatotriloba* 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 examine whether this gene flow affects 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; $t = 0.11$, $df = 310$, $p = 0.91$).

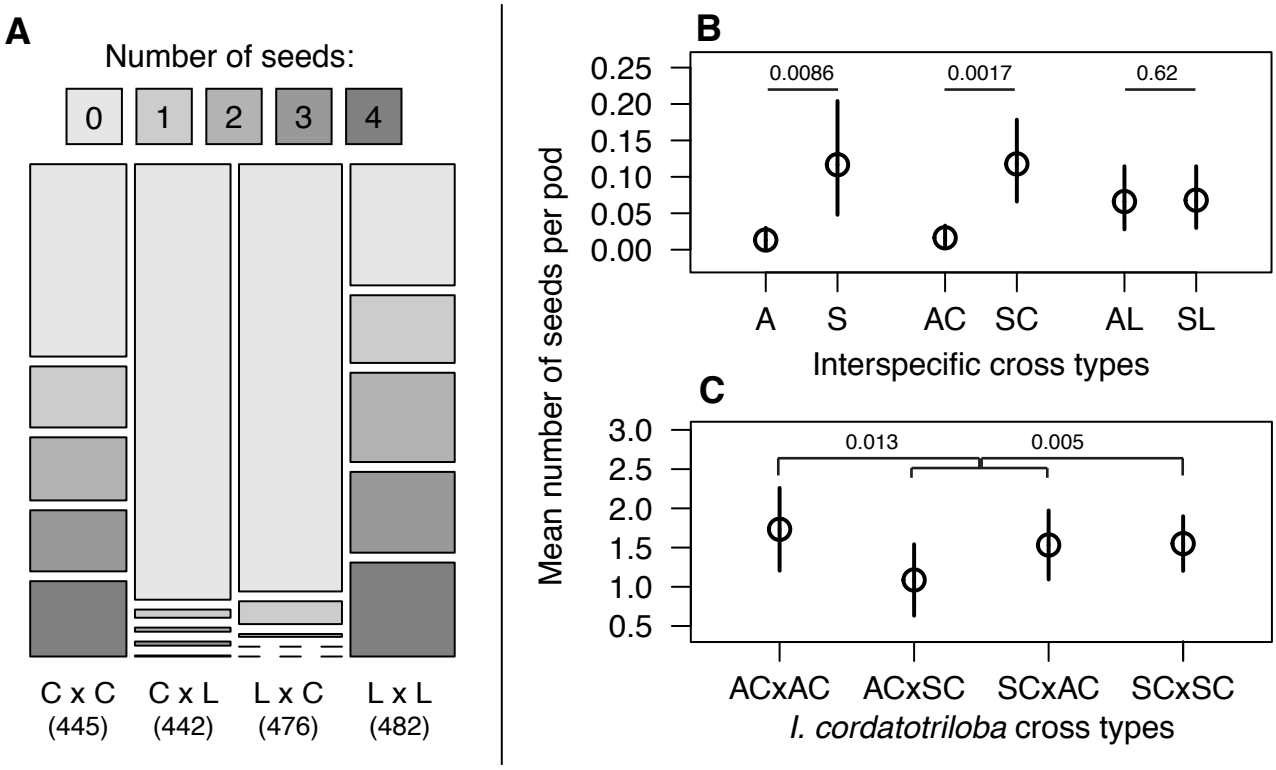

Figure 2 – A) The number of seeds produced by different types of crosses (ovule parent x pollen parent; C = *I. cordatotriloba*, L = *I. lacunosa*). Each box is proportional to the number of fruits that contained that many seeds after pollination. Dashed lines represent cases in which there were no fruits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

Second, we found that interspecific crosses with plants from sympatric sites set significantly more seeds than those made with plants from allopatric sites (Fig. 2B: $t = 2.67$, $df = 146$, $p = 0.0085$). This result could explain the wide variation in crossing success reported in previous studies (Martin 1970, Abel and Austin 1981, Diaz et al. 1996, Duncan and Rausher 2013b). Further, it also suggests that the crossing barrier has been degraded in sympatry. This result is significant because, although the degradation of reproductive isolation in sympatry is supported by theory (Barton and Hewitt 1985, Butlin 1987, Virdee and Hewitt 1994, Kelly and Noor 1996) and is implied by patterns of genetic variation in many systems (e.g., Borge et al 2005, Sander et al. 2014), studies in which the strength of reproductive barriers have been explicitly quantified and shown to be degraded in sympatry are rare (but see Carney et al. 2000, Collins and Rawlins 2013, Virdee and Hewitt 1994, Saetre et al. 1999, 2003, Zuellig and Sweigart 2019).

To determine whether changes in one or both species explains the pattern of higher seed set in sympatric plants, we compared the success rates of interspecific crosses using sympatric and allopatric plants within each species. We found that interspecific crosses using sympatric *I. cordatotriloba* tend to be more successful than interspecific crosses using allopatric *I. cordatotriloba* (Fig. 2B; $t = 3.19$, $df = 146$, $p = 0.0017$), while crosses using sympatric *I. lacunosa* are no more likely to be successful than those using allopatric *I. lacunosa* (Fig. 2B; $t = 0.49$, $df = 146$, $p = 0.62$). This result indicates that only *I. cordatotriloba* experienced changes in characters that contribute to incompatibility and supports the prediction that greater changes in the degree of reproductive isolation will occur in the species experiencing greater gene flow. By itself, this type of asymmetric degradation of reproductive barriers could have the potential to cause positive feedback in which asymmetric degradation of reproductive barriers facilitates even more asymmetric gene flow.

Although one potential outcome of hybridization is reinforcement and increased reproductive isolation in sympatry, we did not observe this outcome for cross compatibility. It is possible the asymmetry itself was an important contributor to the lack of reinforcement in this system. Servedio and Kirkpatrick (1997) showed theoretically that reinforcement is less likely in cases with one-way migration relative to symmetric migration. Furthermore, in this system one barrier (high selfing rate; Rifkin et al. 2019a) increased, while another (cross incompatibility) decreased, indicating that not all barriers will necessarily respond to gene flow in the same way. This result is also consistent with simulations by Castillo et al. (2016), which showed that increases in self-compatibility in sympatry often preclude the evolution of assortative mating such as that represented by the crossing barrier in this system. Finally, because we have not measured all possible reproductive barriers (e.g. flowering

time and pollinator isolation) and we observed both increases and decreases in barrier strength, we cannot determine how total reproductive isolation between *I. cordatotriloba* from *I. lacunosa* has changed.

The changes due to introgression into sympatric *I. cordatotriloba* have also affected compatibility between sympatric and allopatric populations of this species. Specifically, crosses between sympatric and allopatric individuals produce significantly fewer seeds than either crosses between sympatric individuals (Fig. 2C; $t = 2.89$, $df = 75.5$, $p = 0.005$) or between allopatric individuals (Fig. 2C; $t = 2.54$, $df = 75.7$, $p = 0.013$). This pattern is consistent with that expected if incompatibility alleles introgressed into sympatric *I. cordatotriloba* from *I. lacunosa.* In particular, with complete introgression neither allopatric nor sympatric *I. cordatotriloba* would be segregating for incompatibility, and should thus exhibit similar, relatively high levels of compatibility in within-allopatry or within-sympatry crosses. By contrast, because sympatric *I. cordatotriloba* has the alleles from *I. lacunosa* that originally caused incompatibility, they should cause incompatibility with allopatric *I. cordatotriloba* in sympatric by allopatric crosses. The approximately 25 percent reduction in compatibility between sympatric and allopatric populations, which would reduce gene flow from sympatric *I. cordatotriloba* genotypes into allopatric genotypes, coupled with the evolution of almost complete selfing of *I. cordatotriloba* in sympatric populations (Rifkin et al. 2019a) that would likely impede gene flow in the opposite direction, suggests the allopatric and sympatric populations are substantially isolated reproductively. Because both of these effects are by-products of introgression in sympatry, our results provide an example of how asymmetric introgression in sympatry has the potential to drive speciation between allopatric and sympatric populations of the same species. This process is analogous to "cascade reinforcement" or "cascade speciation" (Ortiz-Barrientos et al. 2009) except that erosion, rather than reinforcement, of the between-species crossing barrier drives an increase in reproduction between sympatric an allopatric populations. Our work thus demonstrates that any type of evolutionary 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

197 *I. cordatotriloba* range from nearly complete outcrossing to nearly complete selfing, while all
198 populations of *I. lacunosa* are highly selfing (Duncan and Rausher 2013a). Accordingly, *I. lacunosa*
199 exhibits many traits that are considered part of the "selfing syndrome" (Ornduff 1969, Sicard and
200 Lenhard 2011), including small pale flowers, little nectar, and a low pollen:ovule ratio (Fig. 1C,
201 McDonald et al. 2011, Duncan and Rausher 2013a, Rifkin et al. 2019a, Rifkin et al. 2019b).

202 Populations and crosses

203 To measure seed set between *I. lacunosa* and *I. cordatotriloba,* we grew 60 plants from 18
204 populations collected across 14 locations (Fig. 1D) under common greenhouse conditions. Seeds were
205 scarified and germinated in potting mix in four-inch pots in a growth room under a long-day cycle
206 (16:8 light:dark) and shifted to short-day cycle (13:11 light:dark) after approximately 7 weeks. When
207 flower buds appeared (typically 2-3 weeks after the light change), we transferred plants to the Duke
208 University Greenhouse Facility. Then, we selected 40 focal plants made up of 10 individuals from each
209 of the following four population categories: allopatric *I. cordatotriloba* (AC), allopatric *I. lacunosa* (AL),
210 sympatric *I. cordatotriloba* (SC), and sympatric *I. lacunosa* (SL). Allopatric populations were defined as
211 populations growing outside the range of the other species or populations within the range of the
212 other species where we observed only one species after a thorough search. Data from Rifkin et al.
213 (2019a) suggests that these categorizations capture biologically relevant groups. In particular,
214 STRUCTURE analysis revealed that *I. cordatotriloba* consists of two relatively homogeneous genetic
215 groups corresponding to allopatric and sympatric populations (Rifkin et al. 2019a). By contrast, *I.*
216 *lacunosa* populations consist of a single genetic group encompassing both allopatric and sympatric
217 populations.
218
219 We made six reciprocal crosses between each focal plant and a representative of each population
220 category, so that each focal plant was involved in 48 crosses (2x4x6), 24 as a pollen recipient and 24
221 as a pollen donor (Fig. S1). Within each cross type (i.e., all pairwise combinations of the population
222 categories), we made crosses between individuals from at least three populations within each
223 population category and we did not cross any combination of populations more than three times
224 (Table S1). To perform crosses, we emasculated flowers the day before they opened by dissecting the
225 corolla and removing the anthers. Between 8:00am and 12:00pm the next day, we pollinated the
226 emasculated flowers by dabbing a flower from the individual used as the male on the stigma of the
227 flower used as the female. This method transfers many more pollen grains than needed to fertilize
228 the 4 ovules present in each pistil. In these species, if a flower is left unpollinated or a cross fails, the
229 flower generally abscises and falls off the plant within 4 days. Therefore, we checked the crosses

every day until they abscised or until the seed capsule had dried and the sepals had reflexed, indicating seed maturation. Finally, we counted and weighed the mature seeds and fruits.

Statistical Analyses

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

Acknowledgements

We would like to thank Evangeline Mareki, Irene Liao, and Yuncheng Duan for help making crosses and managing morning glories. We would also like to thank Jenn Coughlan, Kieran Samuk, Felix Beaudry, Loren Rieseberg, Spencer Barrett, Stephen Wright, and the Rausher lab for helpful discussions and comments on earlier versions of this manuscript. This research was supported by an NSERC PDF fellowship 516658 to KLO, and an NSF DDIG grant DEB 1501954 to JLR, and an NSF grant DEB 1542387 to MDR.

Author Contributions

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

Data Accessibility

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

References

Abbott R., Albach D., Ansell S., Arntzen J. W., Baird S. J. E., Bierne N., Boughman J., Brelsford A., Buerkle C. A., Buggs R., Butlin R. K., Dieckmann U., Eroukhmanoff F., Grill A., Cahan S. H., Hermansen J. S., Hewitt G., Hudson A. G., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Martinez-Rodriguez P., Möst M., Mullen S., Nichols R., Nolte A. W., Parisod C., Pfennig K., Rice A. M., Ritchie M. G., Seifert B., Smadja C. M., Stelkens R., Szymura J. M., Väinölä R., Wolf J. B. W., Zinner D., 2013 Hybridization and speciation. Journal of Evolutionary Biology **26**: 229–246.

Abel W. E., Austin D. F., 1981 Introgressive hybridization between *Ipomoea trichocarpa* and *Ipomoea lacunosa* (Convolvulaceae). Bulletin of the Torrey Botanical Club **108**: 231–239.

Ortiz-Barrientos D., Grealy A., Nosil P., 2009 The genetics and ecology of reinforcement: implications for the evolution of prezygotic isolation in sympatry and beyond. Annals of the New York Academy of Sciences **1168**: 156–182.

Barton N. H., Hewitt G. M., 1985 Analysis of hybrid zones. Annual Review of Ecology and Systematics **16**: 113–148.

Bates D., Mächler M., Bolker B., Walker S., 2015 Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**: 1–48.

Bierne N., Lenormand T., Bonhomme F., David P., 2003 Deleterious mutations in a hybrid zone: can mutational load decrease the barrier to gene flow? Genetics Research **80**: 197–204.

Borge T., Lindroos K., Nádvokník P., Syvänen A. C., Saetre G. P., 2005 Amount of introgression in flycatcher hybrid zones reflects regional differences in pre and post-zygotic barriers to gene exchange. Journal of Evolutionary Biology **18**: 1416–1424.

Brooks M. E., Kristensen K., van Benthem K. J., Magnusson A., Berg C. W., Nielsen A., Skaug H. J., Mächler M., Bolker B. M., 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal **9**: 378–400.

Brys R., van Cauwenberghe J., Jacquemyn H., 2016 The importance of autonomous selfing in preventing hybridization in three closely related plant species. Journal of Ecology **104**: 601–610.

Burgess K. S., Morgan M., Deverno L., Husband B. C., 2005 Asymmetrical introgression between two *Morus* species (*M. alba*, *M. rubra*) that differ in abundance. Molecular Ecology **14**: 3471–3483.

Butlin R., 1987 Speciation by reinforcement. Trends in Ecology & Evolution **2**: 8-13.

Carney S. E., Gardner K. A., Rieseberg L. H., 2000 Evolutionary changes over the fifty-year history of a hybrid population of sunflowers (*Helianthus*). Evolution **54**: 462–474.

Castillo D. M., Gibson A. K., Moyle L. C., 2016 Assortative mating and self-fertilization differ in their contributions to reinforcement, cascade speciation, and diversification. Current Zoology **62**: 169–181.

288 Clarke B., 1966 The evolution of morph-ratio clines. The American Naturalist **100**: 389–402.

289 Collins M. M., Rawlins J. E., 2013 A transect for reproductive compatibility and evidence for a "hybrid
290 sink" in a hybrid zone of *Hyalophora* (Insecta: Lepidoptera: Saturniidae). Annals of Carnegie
291 Museum **82**: 193–223.

292 Diaz J., Schmiediche P., Austin D. F., 1996 Polygon of crossability between eleven species of *Ipomoea*:
293 section Batatas (Convolvulaceae). Euphytica **88**: 189–200.

294 Dobzhansky T., 1940 Speciation as a stage in evolutionary divergence. The American Naturalist **74**:
295 312–321.

296 Duncan T. M., Rausher M. D., 2013a Evolution of the selfing syndrome in *Ipomoea*. Frontiers in Plant
297 Science **4**: 301.

298 Duncan T. M., Rausher M. D., 2013b Morphological and genetic differentiation and reproductive
299 isolation among closely related taxa in the *Ipomoea* series Batatas. American Journal of Botany
300 **100**: 2183–2193.

301 Endler J. A., 1977 *Geographic variation, speciation, and clines*. Princeton University Press, Princeton,
302 NJ.

303 Fishman L., Wyatt R., 1999 Pollinator-mediated competition, reproductive character displacement,
304 and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution **53**: 1723–1733.

305 Goodwillie C., Weber J. J., 2018 The best of both worlds? A review of delayed selfing in flowering
306 plants. American Journal of Botany **105**: 641–655.

307 Hoskin C. J., Higgie M., McDonald K. R., Moritz C., 2005 Reinforcement drives rapid allopatric
308 speciation. Nature **437**: 1353–1356.

309 Hopkins R., 2013 Reinforcement in plants. New Phytologist **197**: 1095–1103.

310 Howard D. J., 1993 Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis. In:
311 Hybrid zones and the evolutionary process (R.G. Harrison eds.), pp. 46-69. Oxford University
312 Press, New York.

313 Jaenike J., Dyer K. A., Cornish C., Minhas M. S., 2006 Asymmetrical reinforcement and *Wolbachia*
314 infection in *Drosophila*. PLoS Biology **4**: 1852–1862.

315 Karron J. D., Jackson R. T., Thumser N. N., Schlicht S. L., 1997 Outcrossing rates of individual *Mimulus*
316 *ringens* genets are correlated with anther-stigma separation. Heredity **79**: 365–370.

317 Kelly J. K., Noor M., 1996 Speciation by reinforcement: A model derived from studies of *Drosophila*.
318 Genetics **143**: 1485–1497.

319 Kim B. Y., Huber C. D., Lohmueller K. E., 2018 Deleterious variation shapes the genomic landscape of
320 introgression. PLoS Genetics **14**: e1007741–30.

321 Kozak G. M., Roland G., Rankhorn C., Falater A., Berdan E. L., Fuller R. C., 2015 Behavioral Isolation
322 due to Cascade Reinforcement in *Lucania* Killifish. The American Naturalist **185:** 491–506.

323 Lenth R. V., 2016 Least-Squares Means: The R package lsmeans. Journal of Statistical Software **69**: 1–
324 33.

325 Lloyd D. G., 1979 Some reproductive factors affecting the selection of self-fertilization in plants. The
326 American Naturalist **113**: 67–79.

327 Lowry D. B., Modliszewski J. L., Wright K. M., Wu C. A., Willis J. H., 2008 The strength and genetic basis
328 of reproductive isolating barriers in flowering plants. Philosophical Transactions of the Royal
329 Society B: Biological Sciences **363**: 3009–3021.

330 Mallet J., 2005 Hybridization as an invasion of the genome. Trends in Ecology & Evolution **20**: 229–
331 237.

332 Martin F. W., 1970 Self- and Interspecific Incompatibility in the Convolvulaceae. Botanical Gazette
333 **131**: 139–144.

334 McDonald J. A., Hansen D. R., McDill J. R., Simpson B., 2011 A phylogenetic assessment of breeding
335 systems and floral morphology of North American *Ipomoea* (Convolvulaceae). Journal of the
336 Botanical Research Institute of Texas **5**: 159–177.

337 Motten A. F., Stone J. L., 2000 Heritability of stigma position and the effect of stigma-anther
338 separation on outcrossing in a predominantly self-fertilizing weed, *Datura stramonium*
339 (Solanaceae). American Journal of Botany **87**: 339–347.

340 Muñoz-Rodríguez P., Carruthers T., Wood J. R. I., Williams B. R. M., Weitemier K., Kronmiller B., Ellis
341 D., Anglin N. L., Longway L., Harris S. A., Rausher M. D., Kelly S., Liston A., Scotland R. W., 2018
342 Reconciling conflicting phylogenies in the origin of sweet potato and dispersal to Polynesia.
343 Current Biology **28**: 1246–1256.e12.

344 Noor M. A., 1995 Speciation driven by natural selection in *Drosophila*. Nature **375**: 674–675.

345 Ornduff R., 1969 Reproductive biology in relation to systematics. Taxon **18**: 121–133.

346 Pickup M., Branvain Y., Fraisse C., Yakimowski S., Barton N., Dixit T., Lexer C., Cereghetti E., Field D.,
347 2019 Mating system variation in hybrid zones: facilitation, barrier and asymmetries to gene flow.
348 New Phytologist accepted online.

349 Rifkin J. L., 2017. Population genetics, natural selection and genetic architecture of the selfing
350 syndrome in the morning glory *Ipomoea lacunosa*. Retrieved from ProQuest Dissertations &
351 Theses Global. (1935570052). https://doi.org/10.1534/genetics.106.064469

352 Rifkin J. L., Castillo A. S., Liao I. T., Rausher M. D., 2019a Gene flow, divergent selection and resistance
353 to introgression in two species of morning glories (*Ipomoea*). Molecular Ecology **28**: 1709–1729.

354 Rifkin J. L., Castillo A. S., Liao I. T., Rausher M. D., 2019b Multiple aspects of the selfing syndrome of

355 the morning glory *Ipomoea lacunosa* evolved in response to selection: A Qst-Fst comparison.
356 Ecology and Evolution **9**(13): 7712-7725.

357 Sanders K. L., Rasmussen A. R., Guinea M. L., 2014 High rates of hybridisation reveal fragile
358 reproductive barriers between endangered Australian sea snakes. Biological Conservation **171**:
359 200–208.

360 Sanderson N., 1989 Can gene flow prevent reinforcement? Evolution **43**: 1223–1235.

361 Servedio M. R., Noor M. A. F., 2003 The role of reinforcement in speciation: Theory and data. Annual
362 Reveiw of Ecology, Evolution and Systematics **34**: 339–364.

363 Sicard A., Lenhard M., 2011 The selfing syndrome: a model for studying the genetic and evolutionary
364 basis of morphological adaptation in plants. Annals of Botany **107**: 1433–1443.

365 Tiffin P., Olson S., Moyle L. C., 2001 Asymmetrical crossing barriers in angiosperms. Proceedings of the
366 Royal Society B: Biological Sciences **268**: 861–867.

367 Todesco M., Pascual M. A., Owens G. L., Ostevik K. L., Moyers B. T., Hubner S., Heredia S. M., Hahn M.
368 A., Caseys C., Bock D. G., Rieseberg L. H., 2016 Hybridization and extinction. Evolutionary
369 Applications **9**: 892–908.

370 USDA, NRCS (2019). The PLANTS Database. Greensboro, NC: National Plant Data Team. Retrieved
371 from http://plants.usda.gov (accessed 16 January 2019).

372 Virdee S. R., Hewitt G. M., 1994 Clines for hybrid dysfunction in a grasshopper hybrid zone. Evolution
373 **48**: 392.

374 Whitney K. D., Ahern J. R., Campbell L. G., Albert L. P., King M. S., 2010 Patterns of hybridization in
375 plants. Perspectives in Plant Ecology, Evolution and Systematics 12: 175–182.

376 Yukilevich R., 2012 Asymmetrical patterns of speciation uniquely support reinforcement in
377 Drosophila. Evolution **66**: 1430–1446.

378 Zuellig, M.P. and Sweigart, A.L., 2019. A two-locus hybrid incompatibility is widespread, polymorphic,
379 and active in natural populations of *Mimulus*. Evolution, **72**: 2394-2405.

380

Supplementary Figures

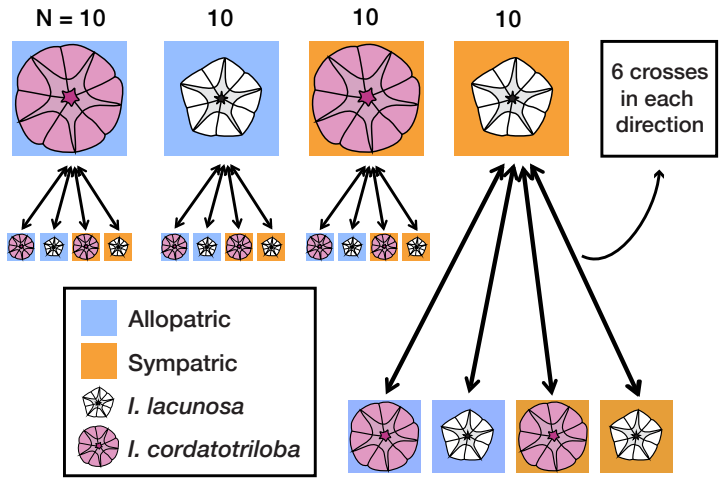

Figure S1 – Diagram of the full crossing design for this study.

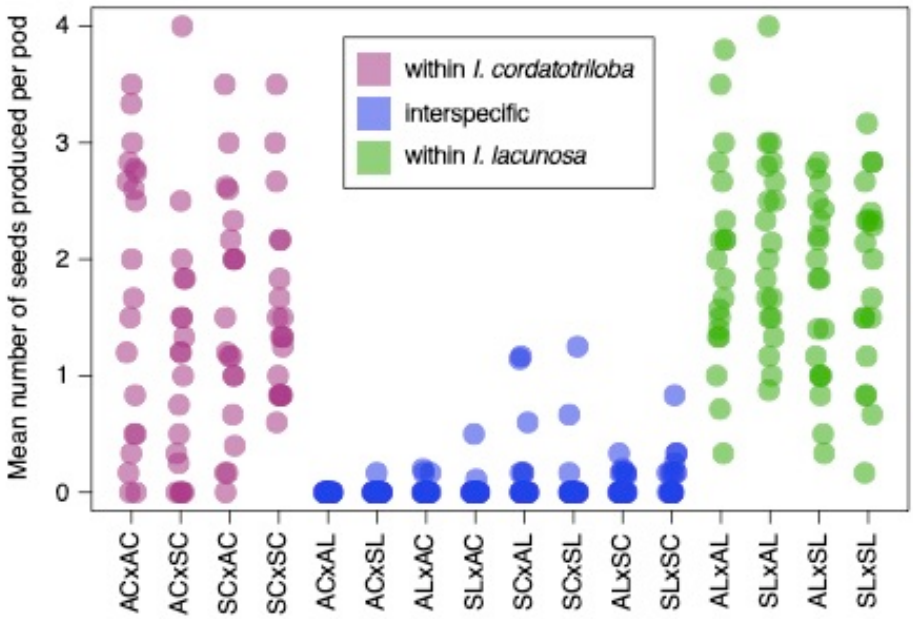

Figure S2 - The mean number of seeds produced by crosses between all pairwise combinations of population categories: allopatric *I. cordatotriloba* (AC), allopatric *I. lacunosa* (AL), sympatric *I. cordatotriloba* (SC), and sympatric *I. lacunosa* (SL). Each point represents 5-6 crosses.