

Purging the genetic load: Possible barriers to gene flow in outcrossing and selfing *Clarkia xantiana* subspecies

Rachel Edidin

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Abstract

Speciation events in plants are caused by a variety of biological, environmental, and genomic factors and can allow for formation of distinct species that remain in sympatry. Reproductive boundaries between sympatric species are often permeable; gene flow can occur between distinct species via backcrossing of hybrids with non-hybrid individuals. The extent of gene flow depends on reproductive barriers such as pollinator preference or fitness of hybrids. A common evolutionary change that can cause speciation while still allowing for gene flow between sympatric subspecies is a shift in mating system from outcrossing to selfing. When traditionally outcrossing populations self, plant species may experience inbreeding depression, or reduced fitness due to increased expression of harmful alleles. However, because individuals expressing harmful alleles have low reproductive success, over time these alleles get ‘purged’ from the population, causing selfing lineages to have lower frequencies of rare and harmful alleles than the outcrossing population they diverged from. These differences in harmful allele frequency could be a barrier to gene flow between selfing and outcrossing species. Here, we used phenotypic data to study the extent of purging in outcrossing and selfing subspecies of *Clarkia xantiana* wildflowers. F1s that each had one outcrossing and one selfing parent were both intercrossed and selfed, and phenotypic measures of fitness were quantified in their offspring. We hypothesized that offspring of selfed F1s would be less fit than their intercrossed counterparts because unpurged harmful alleles inherited from the outcrossing parent would be more likely to be expressed. We found weak signals for lower fitness in offspring of selfed F1s, suggesting that although harmful allele frequency may be a barrier to gene flow, it is not a strong one.

Introduction

The biological species concept defines a species as a group of organisms that can hybridize and exchange genes (Robson, 1928). Environmental, genetic, or behavioral changes can lead to speciation, where a group of individuals within the species diverges to become reproductively isolated from the rest of the species. When causes of speciation are genetic or behavioral in nature, such as a shift in flowering time, species can be reproductively isolated while still living in sympatry. In this case, reproductive isolation between species may not be complete as species continue to interact (Mallet et al., 2016; Osborne et al., 2020).

Incomplete reproductive isolation and permeable species boundaries can lead to the formation of hybrids: it's estimated that at least 25% of plant species form hybrids (Mallet, 2005). Gene flow thus occurs between sympatric subspecies by way of introgression: repeated backcrossing of hybrids with individuals from different populations leads to gene flow between them (Harrison and Larson, 2014). The extent to which gene flow occurs varies based on the factors keeping species distinct and can be uneven: that is, gene flow can occur more strongly in one direction (Martin et al., 2019).

Many factors affect the level of gene flow in any specific population. Barriers to gene flow can be both prezygotic, such as differences in geography or pollinator preference, or postzygotic, such as pollen incompatibility or unfit hybrids (Jiménez-López et al., 2023). Hybrid zone formation can be affected by the extent and parameters of these barriers, especially those relating to geography and fitness (Pickup et al., 2019). Such barriers can be uneven in their directionality, contributing to the strength of reproductive isolation in a population.

Mutations in hybrid zones can create barriers to gene flow through selection. Mutations can negatively impact hybrids in several ways. Incompatibility can arise through epistasis, where alleles are not harmful on their own but are harmful when expressed along with alleles from another species, as happens in hybrids (Orr, 1996). Hybrids can be in-

compatible with the environment: allele combinations can lead to lower fitness in the environment in which the hybrid lives (Chen et al., 2016). Lastly, mutations can be deleterious and cause lower hybrid fitness, but are not deleterious in non-hybrid species due to low effective population size, or persistence in heterozygosity, which would not have an effect on phenotypic fitness (Aeschbacher et al., 2017; Kim et al., 2018).

When selection acts on certain loci, such as when that loci contains a deleterious allele, the flow of any genes linked to said loci will be impeded because any hybrids expressing them will have lower reproductive success (Barton and Bengtsson, 1986). When many loci throughout the genome are selected against with small effect in hybrids, hybrid fitness and thus gene flow via introgression will be lower than if selection has large effects on few loci (Bierne et al., 2002).

One of the most common evolutionary changes is a change in mating system brought about by changes in morphology and environment, which not only increase variation within and between populations, but are also common catalysts for speciation (Goldberg and Igić, 2012; Karron et al., 2012). Organisms can outcross, where mating occurs with two unique plants, or self, where the maternal and paternal plants are the same. Although many species can reproduce either way, most species predominate toward one mating system (Igić and Kohn, 2006). Selfing may be advantageous, especially in the short term, because it provides reproductive assurance in the presence of reduced pollinators or mates (Wright et al., 2013). However, selfing can be detrimental in the short term due to inbreeding depression, and can cause species extinction in the long term: the method of which is not completely known but is likely associated with decreased genetic diversity (Igić and Busch, 2013; Wright et al., 2008).

One consequence of mating system variation in plants living in sympatry is a difference in allele frequency distribution—, specifically that of rare, recessive, and potentially harmful alleles (Huber et al., 2018). Plants with higher frequency of these alleles are said to have higher genetic load. Broadly, parental rare alleles are more likely to be pheno-

typically expressed when plants reproduce via selfing. Even if a rare allele exists at a frequency as low as 0.01% in a population, plants that are heterozygous for said allele have a 25% chance of their offspring from selfing expressing the rare phenotype. Outcrossed offspring are much less likely to be homozygous for harmful rare alleles because for that to happen, both parents must be heterozygous for that specific allele, which is unlikely when the allele occurs at a very low frequency. Rare, recessive alleles are often associated with lower fitness, and can cause offspring to fail to germinate, fail to grow to maturity, or produce few seeds. As a result, although selfing results in inbreeding depression by exposing rare, recessive alleles; selfing also ‘purges’ those alleles from the population as the plants expressing them have less reproductive success than individuals not expressing those alleles (Charlesworth and Willis, 2009; Khan et al., 2021; Szövényi et al., 2014). This does not happen in outcrossing lineages because harmful alleles are very unlikely to be phenotypically expressed, and thus persist in heterozygotes for generations. Therefore, over many generations, genetic load in selfing lineages is expected to be lower than the load in plants that outcross.

Because outcrossing plant lineages do not purge deleterious alleles the same way that selfing lineages do, outcrossers are predicted to have higher load, or higher frequency of harmful mutations. Outcrossers can mate with selfers, typically by way of outcrossing pollen fertilizing a selfer ovule, creating an F1 hybrid (Barrett, 2003). These hybrids can then either self or outcross. If the outcrossing parent of the F1 had a high load, F1 offspring following selfing are also likely to express high load, leading to lower reproductive success in the F2 hybrid generation, forming a barrier to introgression. Unpurged, harmful phenotypes require two copies of the recessive allele to be expressed, which is most likely to occur following selfing, because both parents of selfed offspring have a copy of the recessive allele that could be inherited: this is unlikely in outcrossing because both parents are unlikely to have the same recessive allele. Thus, F1 hybrids are more likely to have success outcrossing, as their offspring are less likely to express a high genetic load (Ruhsam et al.,

2011).

One such plant population that exhibits speciation following a shift in mating system is *Clarkia xantiana*, a wildflower native to the southern Sierra Nevada range in California. *C. xantiana* split into two distinct subspecies between 10,000 and 65,000 years ago (Pettengill and Moeller, 2012). Although both subspecies are self-compatible, *Clarkia xantiana ssp xantiana* primarily reproduces via outcrossing, whereas *Clarkia xantiana ssp parviflora* primarily self-fertilizes. Despite their partially overlapping ranges, and their ability to produce viable hybrids and exchange genetic information, *xantiana* and *parviflora* remain distinct subspecies (Briscoe Runquist et al., 2014; Pettengill and Moeller, 2012). However, the magnitude of gene flow has been observed to be larger from *C. x. parviflora* to *C. x. xantiana*, and not the other way around.

To study the effects of purging the genetic load on fitness in second-generation hybrid *Clarkia xantiana* plants, we created first generation hybrid plants that were each a cross between a *C. x. xantiana* and *C. x. parviflora* plant. Each F1 hybrid was both selfed and intercrossed (crossed with another F1 plant), and fitness was quantified with several phenotypic measurements. Our hypothesis, illustrated in Figure 1, is that F2s produced by selfing will be less fit than their intercrossed counterparts, because selfing is more likely to expose the high genetic load inherited from the outcrossing parent of the F1.

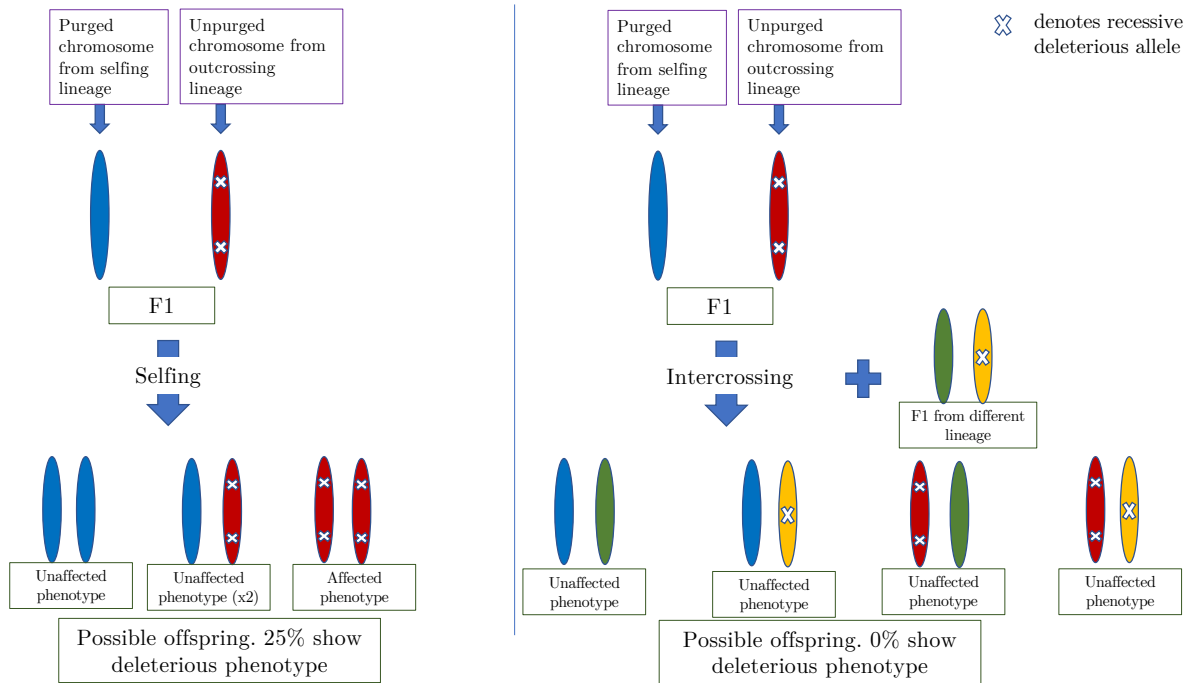


Figure 1: Hypothesis Schematic. Each F1 had a selfing and outcrossing parent. F1s were then selfed and outcrossed. We predict that F2s made via selfing will express more deleterious phenotypes inherited from the selfing F1 parent.

Methods

First generation (F1) plants were grown from a cross between an outcrossing lineage (*Clarkia xantiana ssp xantiana*) and a selfing lineage (*Clarkia xantiana ssp parviflora*). The F1 production scheme is shown in Figure 2. Following F1 maturity, F1 plants were both selfed (crossed with themselves) and intercrossed (crossed with another F1). Each plant was crossed both ways (acting as the maternal plant in both crosses).

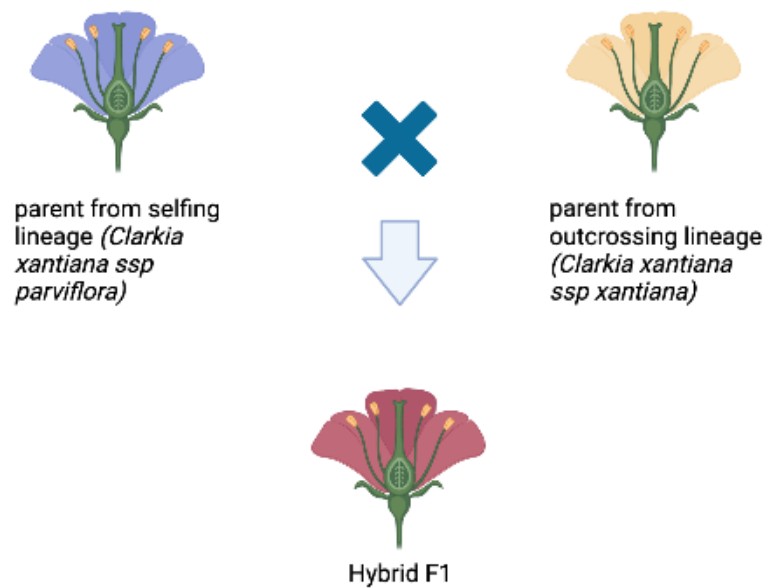


Figure 2: F1 cross scheme. F1s were made via a cross between a selfing lineage and an outcrossing lineage.

Two flowers from each cross were analyzed. In total, 42 unique maternal plants and 168 F2s were analyzed. Seeds from offspring (F2, second generation) were counted. Seeds were visually categorized into three groups: full, empty, and unfertilized. Full seeds were successfully fertilized, empty seeds had been fertilized but stopped growing due to nonviability, and unfertilized seeds had not been attempted to be fertilized. Seed fertilization

rate was calculated as the number of full seeds divided by the number of full seeds plus the number of empty seeds, as in Equation 1:

$$\frac{n_{fullseeds}}{n_{fullseeds} + n_{emptyseeds}}$$

Equation 1: Parental seed success rate.

Out of the seeds that were successfully fertilized, five per flower were planted in cone-shaped containers ('conetainers') that had been soaked in fertilizer for one day. Conetainers were placed in the cold room (30F) for seven days and misted daily. Following germination, conetainers were transferred to a growth chamber that had 12 hour days and was set to 13C for all 24 hours. Plants were watered as needed to keep soil moist (approximately every 2-3 days). Germination proportion was measured approximately three weeks after planting, quantified as the number of successfully germinated seeds per pot divided by five, the number of seeds planted. After, germinates were thinned to one plant per pot. The plant in the middle was kept. This planting scheme is shown in Figure 3.

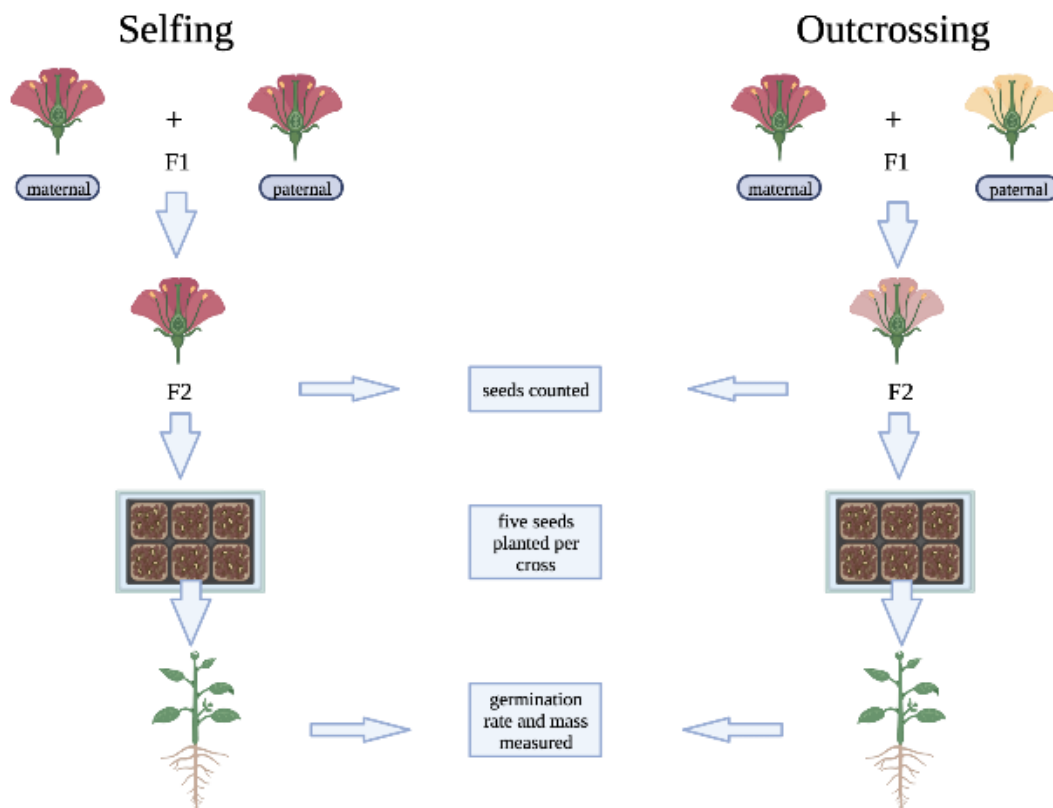


Figure 3: Planting scheme. F1s were both selfed and intercrossed (crossed with another F1). F2 seeds were counted and planted, after which germination proportion and mass were measured.

Approximately six weeks after the seeds were originally planted, leaf tissue was collected from leaves near the top of the plant. Further experiments could sequence this tissue and investigate genotypic association with phenotype.

Following leaf tissue collection, plants were cut with scissors at the base of the stem (as close to the dirt as possible), placed in coin envelopes, and dried in a 100 F oven for approximately three days. Following desiccation, the plant mass was measured.

To analyze the association between fitness-related phenotypic measures (seed fertilization, germination proportion, and mass) and cross type, generalized mixed effect models were built in R. A mixed effect model was built to predict cross type based on the three phenotypic predictors. A second model was built that used cross type to predict the proportion of seeds that were successfully fertilized. Because every maternal plant was both selfed and intercrossed, maternal plant was a random effect in every model, allowing for comparisons between both cross types for each plant.

Results

The average phenotypic measurements for each cross type are described in Table 1. Mass and germination proportion varied between cross type by only about 1%. Seed success rate was more variable across cross types, with intercrossed F1s producing approximately 4% more successfully fertilized seeds compared to selfed F1s.

Table 1: Phenotypic Measurements by Cross Type

Cross Type	Parental Seed Success Rate	Mass (g)	Germination Proportion
inter	0.934	0.189	0.94
self	0.899	0.194	0.93

Table 2 describes the results of the mixed effects model that used phenotypic elements to predict cross type. Parental seed success rate ('success_prop') was the only phenotypic variable that predicted cross type with a significant effect ($p < 0.05$).

Table 2: Mixed Effects Model with all Phenotypic Predictors

	Model 1
(Intercept)	5.58* (2.38)
germ_prop	-1.80 (1.42)
success_prop	-4.15* (1.92)
mass	0.08 (1.74)
Num. groups: momID	42
Var: momID (Intercept)	0.00

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 3 shows the results of the mixed effects model that used cross type to predict the proportion of F1 seeds that were successfully fertilized. Selfing was negatively associated with seed success; in other words, a plant crossed via selfing would be predicted to have a lower percentage of seeds successfully fertilized ($p < 0.001$).

Table 3: Seed Success and Cross Type Mixed Model

	Model 1
(Intercept)	2.88*** (0.15)
cross_type_self	-0.43*** (0.10)
Num. groups: momID	42
Var: momID (Intercept)	0.66

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

While the overall difference between intercrossing and selfing seed success was statistically significant, not all maternal plants expressed this: in fact, for some plants, selfing offspring had more viable seeds than intercrossing. Figure 4 shows the average seed viability for each maternal plant intercrossing (Y) and selfing (X). Each point corresponds to a maternal plant. Points above the line $y=x$ had higher intercrossing seed success vs selfing.

Most points are near the $y=x$ line (on either side), with only a few far above and below.

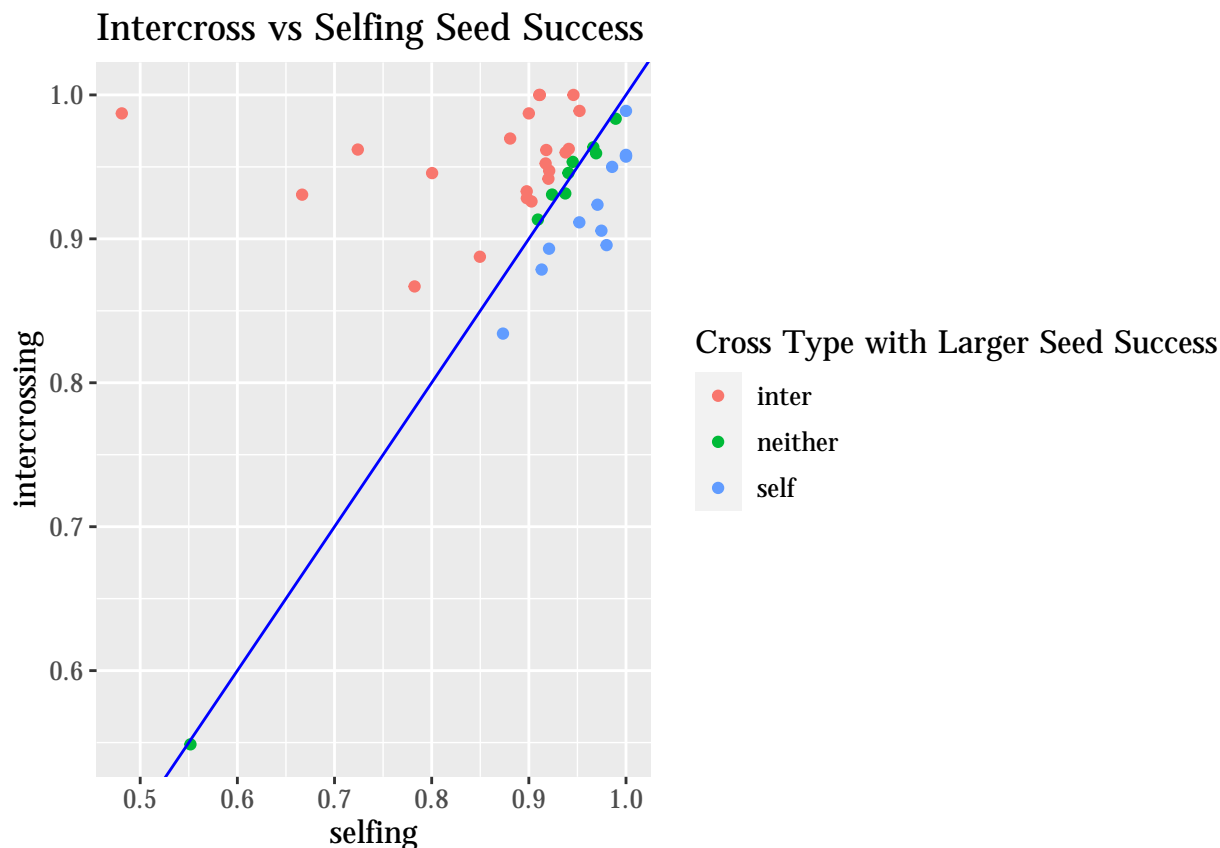


Figure 4: Intercrossing (y-axis) vs selfing (x-axis) seed success. Points correspond to maternal plants, plotted with respect to their average seed success when selfing and outcrossing. Point color corresponds to the cross type with a larger proportion of seeds germination (per maternal plant). Seed success proportion was considered to be significantly different when it was greater or less than 0.01. The blue line, $y=x$, shows what the association would look like if, for the same maternal plant, seed success did not vary by cross type.

To evaluate whether the average difference in seed success per maternal cross was significantly different from a null, a permuted null distribution was generated by flipping the cross type (inter/self) labels for each seed success rate per plant. The difference (inter-self) in seed success was taken for each pair. 50 null distributions were generated, shown in Figure 5 below. Pink lines correspond to null distributions, and the blue line is the true distribution for difference in seed success between intercross and selfing per maternal plant.

Distribution of Difference in Seed Success Between Intercrossing and Selfing Maternal Plants, Compared to Null Distribution

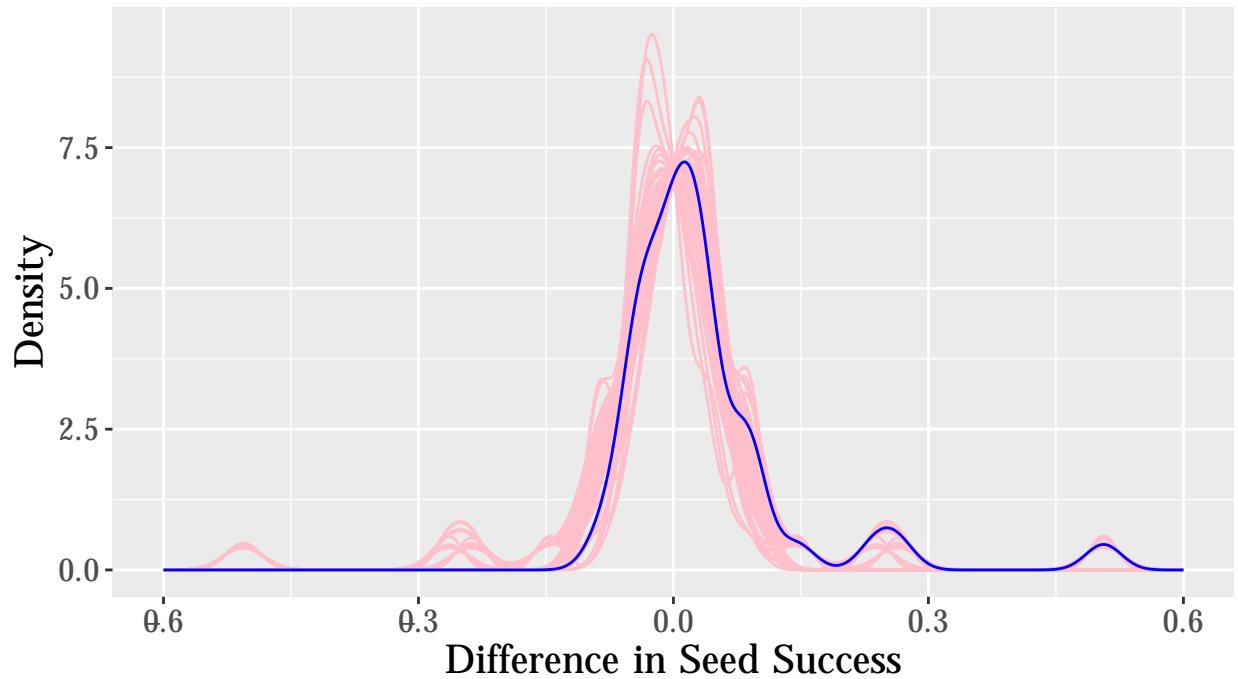


Figure 5: Distribution of difference in seed success between intercrossing and selfing maternal plants (blue) compared to the null distribution of difference in seed success (pink). The average seed success of maternal plants when selfing was subtracted from the average seed success of the same maternal plant when intercrossing. The pink lines are 50 different null distributions that were generated by randomly shuffling the 'cross type' label for seed success of each maternal parent, and represent the distribution of seed success if there was no difference in seed success between cross type for the same maternal plant.

The median of the actual distribution of seed success differences did not differ significantly from the median of the null distribution generated by permuting cross type labels within the same maternal plant ($p=0.0979$). Differences between the distributions, although they may exist, are likely slight and possibly driven by only a few plants.

Discussion

In this experiment, we tested the idea that selfing F1s containing genetic material from both selfing and outcrossing populations will expose unpurged recessive variants from the outcrossing population, which act as a barrier to hybrid success. Because outcrossing populations do not purge harmful alleles the same way that selfing populations do, an F1 plant that resulted from a cross between an outcrosser and a selfer will likely contain copies of harmful alleles that have persisted in outcrossing populations. When the F1 selfs, those alleles are much more likely to be expressed in its offspring, often in the form of lower reproductive fitness (Winn et al., 2011). We found one signal of lower fitness in selfed F1s: they tended to have fewer viable seeds compared to intercrossed F1s. However, this signal was subtle and varied by maternal line. Thus, although this barrier may exist it is not a major contributor to reproductive isolation.

Seed viability was the only phenotypic measurement that varied between selfed and intercrossed F1s. Because fertilization is the first step in plant growth, it is possible that successfully fertilized seeds were inherently more fit than non-viable seeds and went on to grow with less fitness variation regardless of cross type, explaining the lack of variation in germination proportion and mass between cross types. However, it is not clear from the data in this experiment if there is truly no difference in those metrics between cross types, or if it just was not possible to measure any difference because all the seeds that would have been significantly less fit did not reach maturity so were unable to be planted. Continued analysis could investigate these phenotypic parameters, possibly controlling for fertilization, to discern true differences in phenotype and make conclusions that could be

applied to other populations.

We assume that the decreased seed viability in selfed F1s reflects unpurged recessive variants in those maternal lineages (Crnokrak and Barrett, 2002). When an F1 has one selfing and one outcrossing parent, as in this experiment, they are likely to inherit some harmful alleles that have persisted recessively in outcrossing generations. When an F1 selfs, there is a nontrivial probability of at least one harmful recessive allele being expressed, possibly causing lower reproductive fitness in F2 offspring. This is unlikely to occur following F1 intercrossing: in order for a recessive genotype to be expressed, both parents must have the same rare allele (and offspring must inherit both copies) which is unlikely, and thus why we saw higher fitness in outcrossed F2s compared to selfed.

Because our experiment only looked at phenotypic data, it's not possible to completely conclude that our results are due to purging-related variations in frequency of certain genotypes. Although our results showed statistically significant variation in seed fertilization rates by cross type for F1s, these results were biologically subtle and, taken on their own, not sufficient evidence that purging occurred in selfing lineages of the plants we studied. Further research could explore genotypic evidence for purging in the same plants we studied, specifically comparing frequency of homozygosity in selfed vs intercrossed F2s. For the same hypothesized reasons that selfed F2s had lower fitness, they would also be expected to be homozygous for more recessive alleles that were inherited from the outcrossing (nonpurging) lineage of the F1 parent. These recessive alleles would have persisted heterozygously in outcrossing lineages. They would be predicted to appear more often homozygously in F2s produced by selfing due to both F1 parents having a recessive copy of the allele (García-Dorado, 2012).

Uneven genetic loads between selfing and outcrossing lineages due to purging can be a barrier to gene flow if they cause hybrids to have lower fitness, decreasing introgression in a population (Ruhsam et al., 2011). Although we found some evidence of fitness differences between selfed and intercrossed F1s, the differences were slight and don't indicate

that purging is a strong barrier to gene flow. Further research could continue investigating the relationship between purging, genetic load, and introgression by using genotypic data to quantify fitness differences between lineages with varied mating systems.

References

- Aeschbacher, S., Selby, J.P., Willis, J.H., and Coop, G. (2017). Population-genomic inference of the strength and timing of selection against gene flow. *Proc. Natl. Acad. Sci.* 114, 7061–7066.
- Barrett, S.C.H. (2003). Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 358, 991–1004.
- Barton, N., and Bengtsson, B.O. (1986). The barrier to genetic exchange between hybridising populations. *Heredity (Edinb.)*. 57, 357–376.
- Bierne, N., Lenormand, T., Bonhomme, F., and David, P. (2002). Deleterious mutations in a hybrid zone: Can mutational load decrease the barrier to gene flow? *Genet. Res.* 80, 197–204.
- Briscoe Runquist, R.D., Chu, E., Iverson, J.L., Kopp, J.C., and Moeller, D.A. (2014). Rapid evolution of reproductive isolation between incipient outcrossing and selfing *Clarkia* species. *Evolution (N. Y.)*. 68, 2885–2900.
- Charlesworth, D., and Willis, J.H. (2009). The genetics of inbreeding depression. *Nat. Rev. Genet.* 10, 783–796.
- Chen, C., E, Z., and Lin, H.-X. (2016). Evolution and Molecular Control of Hybrid Incompatibility in Plants. *Front. Plant Sci.* 7.
- Crnokrak, P., and Barrett, S.C.H. (2002). Perspective: Purging the genetic load: a review of the experimental evidence. *Evolution (N. Y.)*. 56, 2347–2358.
- García-Dorado, A. (2012). Understanding and predicting the fitness decline of shrunk populations: inbreeding, purging, mutation, and standard selection. *Genetics* 190, 1461–1476.
- Goldberg, E.E., and Igić, B. (2012). Tempo and mode in plant breeding system evolution. *Evolution (N. Y.)*. 66, 3701–3709.
- Harrison, R.G., and Larson, E.L. (2014). Hybridization, Introgression, and the Nature of Species Boundaries. *J. Hered.* 105, 795–809.
- Huber, C.D., Durvasula, A., Hancock, A.M., and Lohmueller, K.E. (2018). Gene

expression drives the evolution of dominance. *Nat. Commun.* 9, 2750.

Igic, B., and Busch, J.W. (2013). Is self-fertilization an evolutionary dead end? *New Phytol.* 198, 386–397.

Igic, B., and Kohn, J.R. (2006). The distribution of plant mating systems: Study bias against obligately outcrossing species. *Evolution* (N. Y). 60, 1098–1103.

Karron, J.D., Ivey, C.T., Mitchell, R.J., Whitehead, M.R., Peakall, R., and Case, A.L. (2012). New perspectives on the evolution of plant mating systems. *Ann. Bot.* 109, 493–503.

Khan, A., Patel, K., Shukla, H., Viswanathan, A., van der Valk, T., Borthakur, U., Nigam, P., Zachariah, A., Jhala, Y. V., Kardos, M., et al. (2021). Genomic evidence for inbreeding depression and purging of deleterious genetic variation in Indian tigers. *Proc. Natl. Acad. Sci.* 118.

Jiménez-López, F.J., Arista, M., Talavera, M., Cerdeira Morellato, L.P., Pannell, J.R., Viruel, J., and Ortiz Ballesteros, P.L. (2023). Multiple pre- and postzygotic components of reproductive isolation between two co-occurring *Lysimachia* species. *New Phytol.* 238, 874–887.

Kim, B.Y., Huber, C.D., and Lohmueller, K.E. (2018). Deleterious variation shapes the genomic landscape of introgression. *PLOS Genet.* 14, e1007741.

Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends Ecol. Evol.* 20, 229–237.

Mallet, J., Besansky, N., and Hahn, M.W. (2016). How reticulated are species? *BioEssays* 38, 140–149.

Martin, S.H., Davey, J.W., Salazar, C., and Jiggins, C.D. (2019). Recombination rate variation shapes barriers to introgression across butterfly genomes. *PLOS Biol.* 17, e2006288.

Orr, H.A. (1996). Dobzhansky, Bateson, and the genetics of speciation. *Genetics* 144, 1331–1335.

- Osborne, O.G., Kafle, T., Brewer, T., Dobрева, M.P., Hutton, I., and Savolainen, V. (2020). Sympatric speciation in mountain roses (*Metrosideros*) on an oceanic island. *Philos. Trans. R. Soc. B Biol. Sci.* 375, 20190542.
- Pettengill, J.B., and Moeller, D.A. (2012). Tempo and mode of mating system evolution between incipient *Clarkia* species. *Evolution* (N. Y). 66, 1210–1225.
- Pickup, M., Brandvain, Y., Fraïsse, C., Yakimowski, S., Barton, N.H., Dixit, T., Lexer, C., Cereghetti, E., and Field, D.L. (2019). Mating system variation in hybrid zones: facilitation, barriers and asymmetries to gene flow. *New Phytol.* 224, 1035–1047.
- Robson, G.C. (Guy C. (1928). The species problem: an introduction to the study of evolutionary divergence in natural populations. (Edinburgh [etc.]: Oliver and Boyd).
- Ruhsam, M., Hollingsworth, P.M., and Ennos, R.A. (2011). Early evolution in a hybrid swarm between outcrossing and selfing lineages in *Geum*. *Heredity* (Edinb). 107, 246–255.
- Szövényi, P., Devos, N., Weston, D.J., Yang, X., Hock, Z., Shaw, J.A., Shimizu, K.K., McDaniel, S.F., and Wagner, A. (2014). Efficient purging of deleterious mutations in plants with haploid selfing. *Genome Biol. Evol.* 6, 1238–1252.
- Winn, A.A., Elle, E., Kalisz, S., Cheptou, P.-O., Eckert, C.G., Goodwillie, C., Johnston, M.O., Moeller, D.A., Ree, R.H., Sargent, R.D., et al. (2011). Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interference and stable mixed mating. *Evolution* (N. Y). 65, 3339–3359.
- Wright, S.I., Ness, R.W., Foxe, J.P., and Barrett, S.C.H. (2008). Genomic Consequences of Outcrossing and Selfing in Plants. *Int. J. Plant Sci.* 169, 105–118.
- Wright, S.I., Kalisz, S., and Slotte, T. (2013). Evolutionary consequences of self-fertilization in plants. *Proceedings. Biol. Sci.* 280, 20130133.