The role of self-incompatibility systems in the prevention of bi-parental inbreeding

Tara N Furstenau [1,2], **Reed A Cartwright** [Corresp.] [1]

[1] School of Life Sciences and the Biodesign Institute, Arizona State University, Tempe, Arizona, United States of America

[2] The School of Informatics, Computing, and Cyber Systems, Northern Arizona University, Flagstaff, Arizona, United States

Corresponding Author: Reed A Cartwright
Email address: cartwright@asu.edu

Hermaphroditic plants experience inbreeding through both self-fertilization and bi-parental inbreeding. Therefore, many plant species have evolved either heteromorphic (morphology-based) or homomorphic (molecular-based) self-incompatibility (SI) systems. These SI systems limit extreme inbreeding through self-fertilization and, in the case of homomorphic SI systems, have the potential to limit bi-parental inbreeding, which is common when dispersal is restricted to a local region. Homomorphic SI species are prevalent across the angiosperms, and it is often assumed that the potential to reduce bi-parental inbreeding may be a factor in their success. To test this assumption, we developed a spatially-explicit, individual-based simulation of plant populations with either heteromorphic SI or one of three different types of homomorphic SI. In our simulations, we varied dispersal distance and the presence of inbreeding depression. We found that autozygosity in the homomorphic SI populations was significantly lower than in the heteromorphic SI populations and that this reduction was due to bi-parental inbreeding avoidance. As expected, the differences between the homomorphic and heteromorphic SI populations were more pronounced when seed and pollen dispersal was limited. However, levels of homozygosity and inbreeding depression between these plant populations were not different. At low dispersal, homomorphic SI populations also suffered reduced female fecundity and had smaller census population sizes. Our results suggest that bi-parental inbreeding avoidance was unlikely to be a major driver in the evolution of homomorphic SI systems.

The role of self-incompatibility systems in the prevention of bi-parental inbreeding

Tara N. Furstenau[1,2] and Reed A. Cartwright[2]

[1]The School of Informatics, Computing, and Cyber Systems, Northern Arizona University, Flagstaff, AZ 86011-0001, USA
[2]The Biodesign Institute and School of Life Sciences, Arizona State University, Tempe, AZ 85287-5301, USA

Corresponding author:
Reed A. Cartwright[2]

Email address: cartwright@asu.edu

ABSTRACT

Hermaphroditic plants experience inbreeding through both self-fertilization and bi-parental inbreeding. Therefore, many plant species have evolved either heteromorphic (morphology-based) or homomorphic (molecular-based) self-incompatibility (SI) systems. These SI systems limit extreme inbreeding through self-fertilization and, in the case of homomorphic SI systems, have the potential to limit bi-parental inbreeding, which is common when dispersal is restricted to a local region. Homomorphic SI species are prevalent across the angiosperms, and it is often assumed that the potential to reduce bi-parental inbreeding may be a factor in their success. To test this assumption, we developed a spatially-explicit, individual-based simulation of plant populations with either heteromorphic SI or one of three different types of homomorphic SI. In our simulations, we varied dispersal distance and the presence of inbreeding depression. We found that autozygosity in the homomorphic SI populations was significantly lower than in the heteromorphic SI populations and that this reduction was due to bi-parental inbreeding avoidance. As expected, the differences between the homomorphic and heteromorphic SI populations were more pronounced when seed and pollen dispersal was limited. However, levels of homozygosity and inbreeding depression between these plant populations were not different. At low dispersal, homomorphic SI populations also suffered reduced female fecundity and had smaller census population sizes. Our results suggest that bi-parental inbreeding avoidance was unlikely to be a major driver in the evolution of homomorphic SI systems.

INTRODUCTION

A large portion of angiosperm species ($\sim$95%) are hermaphroditic (Renner, 2014) meaning that a single plant is capable of self-fertilizing and reproducing without a mating partner. There are several selective advantages to self-fertilization including reproductive assurance when mates are limited (Darwin, 1876; Baker, 1955; Stebbins, 1957; Busch, 2005; Herlihy and Eckert, 2005) and the ability to transmit a higher proportion of genes to offspring (Fisher, 1941). In many cases however, the negative effects of inbreeding depression associated with self-fertilization out weigh these advantages. Consequently, outcrossing remains the dominant reproductive strategy in angiosperms (Igic and Kohn, 2006) and many plant species have evolved a wide variety of morphologically- or molecularly-enforced self-incompatiblity (SI) systems to avoid self-fertilization.

In morphology-enforced or heteromorphic SI systems, self-fertilization is reduced through spatial or temporal separation of the male and female reproductive organs (anther and stigma, respectively). For example, Darwin (1862) first described the heterostyly SI system in *Priumula* (*P. vulgaris* and *veris*), in which each plant expresses one of two flower morphologies that differ in the relative heights of the anther and stigma. The different arrangements ensure that pollenating insects that visit the anther of one morph will only deposit pollen on stigmas with the opposite morph.

Molecularly-enforced or homomorphic SI systems are more common and are found in species spanning at least 100 angiosperm families (Igic et al., 2008). In homomorphic plants, the stigma is

able to recognize and reject self-generated pollen using various molecular mechanisms. In order for self-recognition to be successful, the genes controlling the molecular phenotypes of the pollen and the stigma must be inherited together. Typically, these phenotypes are controlled by two genes at the *S* locus that are tightly linked due to repressed recombination (Casselman et al., 2000; Castric et al., 2010; Charlesworth and Awadalla, 1998; Kamau et al., 2007; Kawabe et al., 2006; Vieira et al., 2003) and highly polymorphic to allow for successful outcrossing. Because a plant passes one of its two *S* haploypes to its pollen, all self-generated pollen will be recognized and rejected. Additionally, pollen from closely related plants that express the same haplotype will also be rejected. As a result, homomorphic SI systems not only reduce inbreeding by preventing self-fertilization, they also reduce mating between close relatives (bi-parental inbreeding) (Charlesworth and Charlesworth, 1987).

It is often assumed that the success of homomorphic SI systems across the angiosperms is due to this two-fold inbreeding avoidance strategy. Unfortunately, because the genetic outcomes of bi-parental inbreeding and self-fertilization are similar, it is difficult to distinguish between these two types of inbreeding in natural populations without a controlled experimental setup (Griffin and Eckert, 2003). This makes it difficult to draw strong conclusions about the role of bi-parental inbreeding avoidance in the evolution of homomorphic SI systems.

Mixed-mating models and genetic markers are often used to estimate levels of bi-parental inbreeding (Ennos and Clegg, 1982), but these estimates can be inaccurate even when a large number of loci are used (Ritland, 2002). These estimates are complicated by the fact that mixed-mating models assume that a certain proportion of progeny are a product of self-fertilization while the rest are a product of outcrossing with random unrelated individuals. In natural plant populations, however, outcrossing is more likely to occur with related individuals. Due to the sessile nature of angiosperms, offspring dispersal occurs through the movement of pollen and seed, and in many plant species, pollen and seed dispersal distances rarely exceed a few meters from the parent (Fenster, 1991; Levin, 1981). Plants are therefore more likely to become established near their parents and be surrounded by related individuals. Under these conditions, populations become spatially structured due to isolation-by-distance, and if pollen dispersal is also limited, these related individuals will interbreed. Under isolation-by-distance, bi-parental inbreeding potentially reduces the genetic cost of outcrossing by increasing parent-offspring genetic relatedness (Uyenoyama, 1986). On the other hand, it may also increase homozygosity and the expression of deleterious recessive alleles.

The extent to which inbreeding is detrimental depends on the history of inbreeding in the population. Both bi-parental inbreeding and self-fertilization can increase homozygosity within a genome and the resulting offspring may be more likely to express recessive deleterious alleles and suffer reduced viability and fecundity (Charlesworth and Charlesworth, 1987; Charlesworth et al., 1990). While, self-fertilizing species have more opportunities to purge highly deleterious alleles, they tend to maintain a large number of only slightly deleterious alleles (Charlesworth et al., 1990; Wang et al., 1999). Outcrossing species tend to maintain recessive deleterious alleles in a heterozygous state, which can lead to inbreeding depression. However, when bi-parental inbreeding is common, some of the segregating deleterious alleles can be purged in outcrossing populations. However, in many plant species, crosses between close neighbors have been shown to produce less fit offspring, and because the reduction in fitness is associated with spatial proximity, this is likely evidence of inbreeding depression resulting from isolation-by-distance (Heywood, 1991).

In this current study, we test whether bi-parental inbreeding avoidance is a driving force behind the evolution of homomorphic SI systems in angiosperms. We use a spatially-explicit, individual-based simulation to model continuous populations of plants with various mating systems: three different homomorphic SI systems, a heteromorphic SI system, and a self-compatible system. To differentiate between inbreeding due to self-fertilization and bi-parental inbreeding we compare the difference in the amount of inbreeding observed in heteromorphic populations, which only prevent self-fertilization, and the inbreeding observed in homomorphic populations, which prevent self-fertilization and reduce bi-parental inbreeding. If there is a large decrease in total inbreeding and inbreeding depression in homomorphic SI populations we can conclude that bi-parental inbreeding avoidance may have provided a selective advantage in the evolution of homomorphic SI systems. Simulations with different seed and pollen dispersal distances are used to determine if homomorphic SI provides a greater advantage when dispersal is local and bi-parental inbreeding is more prevalent.

The three homomorphic SI systems we model vary in the way they discriminate against pollen

from plants with a matching *S* allele and they are described in the Fig. 1 diagram. The first system is modeled after the gametophytic SI system (GSI) which is the most widespread SI system and is found in Solanaceae, Rosaceae and Scrophulariaceae (Franklin-Tong and Franklin, 2003). In GSI systems, the pollen phenotype is solely determined by the *S* haplotype that it inherits. From a single diploid plant, roughly 50% of the pollen will carry one *S* haplotype and 50% will carry the other *S* haplotype. If two plants have one *S* allele in common, half of the pollen from each plant — those that do not carry the common haplotype — will be able to fertilize the other plant.

The second system is modeled after the sporophytic SI system that is common in Brassicaceae (BSI). One often studied example is *Arabidopsis lyrata*, a self-incompatible relative of the self-compatible model angiosperm, *Arabidopsis thaliana* (Kusaba et al., 2001; Charlesworth et al., 2003; Mable et al., 2003; Kawabe et al., 2006; Kamau et al., 2007; Schierup et al., 2008). In the BSI system, the phenotype of the pollen is determined by the diploid *S* genotype of the parent plant. Dominance relationships exist between the *S* alleles and the pollen will therefore express the phenotype of the dominant allele. If two plants share the same dominant *S* allele, they will be unable to interbreed; however, if they share the same recessive *S* allele, all of the pollen will be compatible between the two plants. Consequently, this is the only system that potentially allows a plant to become homozygous for recessive *S* alleles (Hiscock and Tabah, 2003).

Finally, we model a sporophytic SI (SSI) system that is similar to BSI except all *S* alleles are codominant. There is no known biological equivalent of this SI system, and a situation where all *S* alleles are equally codominant is highly unlikely. Nevertheless, the SSI system serves to model an extreme case of discrimination where pollen is prevented from fertilizing any plant that shares either *S* allele. We predict that this more stringent SI system will show the greatest reduction in bi-parental inbreeding. In each of the homomorphic SI systems, we treat the *S* alleles that control the stigma phenotype as codominant.

In the heteromorphic or physical SI (PSI) system, individuals are obligate out-crossers but no genetic mating system is in place to prevent bi-parental inbreeding. Our synthetic PSI system is 100% efficient at preventing self-fertilization. In the self-compatible system (NSI for not self-incompatible), plants are able to self-fertilize and outcross. Self-fertilization increases relative to outcrossing when pollen dispersal distance is limited.

Previous studies have provided evidence that bi-parental inbreeding is reduced in regions of the genome that are linked to the *S* locus. The forced heterozygosity at the *S* locus extends to other linked loci and can reduce the expression of recessive deleterious alleles at those loci. Deleterious alleles can accumulate in this region because they are sheltered from selection (Llaurens et al., 2009). It remains unclear, however, whether homomorphic SI systems reduce bi-parental inbreeding at loci that are not linked to the *S* locus. Cartwright (2009) presented results from a simulation study which compared the amount of inbreeding in populations with heteromorphic or homomorphic SI systems. There was a large decrease in bi-parental inbreeding in homomorphic SI simulations near the *S* locus compared to heteromorphic SI systems, but at unlinked loci, the reduction in bi-parental inbreeding was relatively small and the amount of inbreeding was similar to heteromorphic populations. This suggests that at unlinked loci, homomorphic SI systems only have a small impact on the amount of bi-parental inbreeding; however, this study did not model inbreeding depression which may provide a selective advantage to avoid inbreeding. In this study, we focus on inbreeding at loci that are not linked to the *S* locus. We also incorporate inbreeding depression by simulating the segregation of recessive deleterious alleles in the population, which penalizes homozygous individuals with sterility.

Self-compatible plants have a strong advantage over self-incompatible plants when mates are limited because they are able to self-fertilize and reproduce in isolation. Homomorphic SI plants are at an even greater disadvantage if pollen dispersal is limited and local mates are incompatible. In these situations female fecundity can suffer (Larson and Barrett, 2000). The *S* locus is under negative, frequency-dependent selection and pollen with a rare *S* phenotype will be favored. For this reason, a large number of *S* alleles need to be maintained in the population for mating to be successful. When isolation-by-distance is strong, the pollen pool is reduced and individuals may struggle to find mates. There is evidence that suggests that effective dispersal at the *S* locus increases in SI populations (Cartwright, 2009; Leducq et al., 2011) but mate limitation will still likely reduce total seed production. We monitored the number of viable seeds produced in our simulations to determine how female fecundity is affected by the different mating systems.

METHODS

Simulation

We developed a spatially-explicit, individual-based simulation to model discrete generations of self-incompatible plant populations. In the simulation, populations inhabit a toroidal lattice where each cell is occupied by a single, hermaphroditic individual. The plants are diploid and have several independently assorting genetic loci.

Although in plants the *S* locus has multiple, tightly linked genes, in our simulations we treated it as a single gene, with multiple haplotypes. Typically, the formation of novel functional *S* haplotypes through mutation is rare because it requires coordination between the genes controlling both the pollen and the stigma; a mutation in just one component will result in the breakdown of self-incompatibility (Charlesworth and Charlesworth, 1979; Uyenoyama et al., 2001; Igic et al., 2008). Therefore, in the simulation, we keep the mutation rate at the *S* locus low ($\mu_s = 10^{-5}$) and each mutation results in a completely new *S* haplotype according to the infinite alleles mutation model. We did not allow mutations that would result in the breakdown of SI.

The marker locus, *M*, is used to measure the amount of inbreeding in the population. The alleles at the *M* locus are all selectively neutral and mutate at rate $\mu_m = 10^{-4}$ under the infinite alleles model. The higher mutation rate at the *M* locus maintains polymorphism which aids in the estimation of inbreeding. In the initial population, each *S* and *M* allele is unique and the simulation must run for a burn-in period to reach a drift-mutation equilibrium.

Each individual also carries a total of 10 independent deleterious loci ($D_1, D_2, \ldots, D_{10}$) that are not linked to each other or to any other locus. Each *D* locus carries either a wild-type allele or a recessive deleterious allele. In the initial population, all individuals carry wild-type alleles that will permanently mutate into a deleterious allele at rate $\mu_d = 0.1$; this results in a genome-wide recessive mutation rate that is close to 1. Each homozygous recessive genotype at a *D* locus increases the probability that an individual will be sterile by 0.005. Individually, these alleles are only slightly deleterious and thus are more likely to be maintained in the population; in combination, they should produce an appreciable number of sterile individuals. Affected individuals are viable but are unable to produce pollen or seed. Typically, the probability that a deleterious mutation occurs at a single locus is rare, but the probability of a deleterious mutation occurring across the whole genome is high. Therefore, to maintain a large enough penalty for inbreeding, we used a high mutation rate at each *D* locus so that, on average, there would be one new deleterious mutation per haplotype.

At the beginning of each generation, fertile parent plants produce gametes — 10 pollen grains and 5 ovules — through the independent assortment of loci. Pollen grains are dispersed from the parent's location according to a normal distribution along each axis with standard deviation σ. Incoming pollen is checked for compatibility with the plant in the new location based on the rules of the designated SI system. If compatible, the pollen is randomly assigned to an ovule; otherwise it is discarded. When pollen dispersal is complete, some ovules will remain unfertilized while other ovules will have a pool of pollen from which one pollen grain will be randomly chosen. Unfertilized ovules will be aborted and fertilized ovules will form seeds. Seeds are then dispersed from the parent's location in the same way as the pollen. When seed dispersal is complete, a single seed from each cell will be randomly selected to become a parent in the next generation. Mutations occur in the germ line of the parents before they produce gametes so all of their offspring will carry the mutation.

In each simulation, pollen compatibility is determined by one of the five different mating systems: NSI, PSI, GSI, BSI, and SSI. A serial dominance scheme, similar to that described in Vekemans et al. (1998), is used to model the dominance relationships between the *S* alleles in the BSI system. The *S* alleles are sorted into a dominance hierarchy such that each allele is dominant to all alleles below it and recessive to all alleles above it in the hierarchy; new alleles, introduced through mutations, are randomly inserted into the hierarchy. For the self-compatible NSI system, outcrossing occurs when pollen disperses outside of the parent cell; otherwise, self-fertilization occurs.

Analysis

Simulations were run on a 100×100 landscape with the pollen and seed dispersal parameters both set to either $\sigma = 1$, 2, 4, or 6. After a 10,000 generation burn-in period, a random sample of 500 individuals was collected from the population every 10,000 generations for a total of 500 nearly independent samples.

To measure inbreeding in each sample, we calculated the proportion of sampled individuals that were

209 autozygous and the proportion that were homozygous at the *M* locus. An individual's *M* alleles were
210 considered to be autozygous (identical-by-descent) if they both descended from the same allele in a
211 grandparent, regardless of mutation. Autozygosity is therefore a measurement of recent inbreeding events.
212 Under the infinite alleles mutation model, homozygosity also implies identity-by-descent; however, the
213 inbreeding event may have occurred in the distant past.

214 We also recorded the average number of alleles at the *S* and *M* locus, and the average squared parent-
215 offspring dispersal distance (s^2). In plants, $s^2 = \sigma_s^2 + \sigma_p^2/2$, where σ_s represents seed movement and
216 σ_p represents pollen movement (Crawford, 1984). In this formula, seed dispersal contributes more than
217 pollen dispersal because seeds carry gametes from both parents whereas pollen only carries gametes
218 from the father. From the whole population, we recorded the total number of adults, the number of seeds
219 produced, and the number of sterile individuals.

220 To analyze the results, we used the Anderson-Darling two-sample test (Scholz and Stephens, 1987)
221 implemented in the kSamples R package (R Core Team, 2015; Scholz and Zhu, 2016). The test statistic
222 was $T_a = (AD - (k-1))/\sigma$, and the p-value estimation method was set to simulate the default 10,000
223 random rank permutations using the average rank score for ties. The distribution of values for each
224 measurement was compared between the different simulations under the null hypothesis that the values
225 came from the same underlying distribution. The p-values from the pairwise comparisons were adjusted
226 for multiple tests using the Holm correction (Holm, 1979), and the significance criterion was set at 0.05
227 for all tests.

RESULTS

Effect of Inbreeding Depression

230 Introducing inbreeding depression in the homomorphic SI simulations resulted in a significant difference
231 in the level of homozygosity. Figure 2 compares the level of homozygosity and autozygosity in simulations
232 with and without a penalty for inbreeding. Median homozygosity was lower in each mating system when
233 inbreeding depression occurred. Comparing across the different mating systems, the PSI, GSI, BSI, and
234 SSI mating systems were not significantly different from each other in simulations with and without
235 inbreeding depression. Under the NSI mating system, there was not a significant difference between
236 homozygosity in simulations with and without inbreeding depression. Homozygosity was significantly
237 higher in the NSI simulations compared to the SI systems.

238 Autozygosity, which measures very recent inbreeding, was not significantly different in simulations
239 with and without inbreeding depression within the same mating system, except in the PSI system. The
240 median autozygosity increased with inbreeding depression for the PSI and GSI systems. In simulations
241 with and without inbreeding depression, the highest level of autozygosity was observed under the
242 NSI system and the lowest level was observed under the SSI system; in both cases, autozygosity was
243 significantly different from all of the other mating systems.

Reduction in Bi-Parental Inbreeding

245 The amount of inbreeding in the PSI system was used as a baseline value to determine how much bi-
246 parental inbreeding avoidance occurred in the homomorphic SI simulations. Because the PSI system only
247 prevented self-fertilization, any reduction in inbreeding below the level observed in the PSI simulations
248 represented a reduction in bi-parental inbreeding. We found that compared to the large drop in inbreeding
249 between the NSI and PSI systems, the decrease in inbreeding between PSI and the homomorphic SI
250 systems was relatively small. Figure 3 shows the empirical density plot of measures of autozygosity in
251 each of the simulations and, while it is clear that autozygosity was significantly lower in the homomorphic
252 SI systems compared to the PSI system (inset), the difference was about an order of magnitude smaller
253 than the difference between PSI and NSI. Autozygosity was not significantly different between the GSI
254 and BSI systems, but it was significantly lower in the more stringent SSI system. The results in Fig. 3
255 are from simulations with a population size of 10,000 individuals. We repeated these simulations with
256 a range of population sizes (2,500, 40,000, and 160,000) and verified that the pattern we observed was
257 consistent (results not shown).

Isolation-by-Distance

259 Bi-parental inbreeding is more common under isolation-by-distance and the bi-parental inbreeding
260 avoidance strategy provided by homomorphic SI systems may provide a greater advantage in this situation.

5/15

To test this, we compared the amount of inbreeding in simulations with various dispersal distance parameters for pollen and seed. Figure 4 shows pairwise comparisons of the level of homozygosity and autozygosity in each SI system with a range of dispersal distance parameters. Homozygosity was significantly higher when isolation-by-distance was strongest but there was no difference between the different SI systems. When isolation-by-distance was strongest ($\sigma = 1$), autozygosity was significantly different between all of the SI systems except BSI and GSI. The median autozygosity was highest for PSI, lowest for SSI, and BSI and GSI were tied in the middle. When isolation-by-distance was weak ($\sigma = 6$), autozygosity was not significantly different between BSI, GSI, and SSI but each of these were still significantly different from PSI. The median autozygosity at $\sigma = 6$ was 0 for each SI system and the average autozygosity was 0.0011 for PSI, 0.0007 for GSI, 0.0008 for BSI, and 0.0006 for SSI. Overall, we observed a greater decrease in the median autozygosity levels, in both absolute and relative differences, between PSI and the homomorphic SI simulations when isolation-by-distance was stronger.

Population Demographics and Allele Diversity

Table 1 provides a summary of median per-generation population demographic values for each of the simulations including: census population size, the number of sterile individuals, seed set, dispersal distance, and the number of alleles at the M locus and the S locus. In the simulations, the maximum allowable population size was 10,000 individuals; however, in many cases the population size was smaller because seeds would fail to disperse into some locations and therefore no plants would grow in those locations for a generation. The PSI simulations had the largest median population size and the population size did not seem to be affected by different dispersal distance parameters. In the homomorphic SI simulations, the number of individuals increased with dispersal distance. When $\sigma = 1$, the homomorphic SI simulations all had significantly reduced population size with the greatest reduction observed under the SSI system.

Inbreeding in the population increased the probability that sterile individuals were produced. The average number of sterile individuals per generation across all simulations was 484.5 which represents approximately 5% of the population. For most of the dispersal levels, the PSI simulations had the highest number of sterile individuals; although, none of the differences were statistically significant.

A maximum of 50,000 seeds can be produced in one generation, assuming all five ovules were fertilized in 10,000 individuals. Seed set was highest in the PSI simulations and lowest in the SSI simulations. In each of the SI systems, seed set increased as dispersal distance increased.

The expected mean-squared parent-offspring dispersal distances were 1.5, 6, 24, and 54 for dispersal parameters 1, 2, 4, and 6, respectively. The observed s^2 values were slightly higher across all simulations but the relative difference was much greater when isolation-by-distance was strong. The s^2 values were not significantly different between the different SI systems when $\sigma = 2$, 4, and 6, but when $\sigma = 1$, the SSI simulation had significantly higher effective dispersal than GSI and PSI.

In the homomorphic SI systems, high diversity is maintained at the S locus. The SSI system maintained the largest number of S alleles followed by the GSI system then the BSI system. Few alleles were maintained at the S locus in the PSI system because the S allele was not active, essentially behaving as a selectively neutral marker. The number of alleles maintained at both the S locus and the M locus decreased as the average dispersal distance increased in all of the SI systems.

DISCUSSION

Introducing a penalty for inbreeding had a significant impact on the homozygosity in each mating system. It did not, however, affect very recent autozygosity except in the case of the PSI population. In the PSI population there was a significant increase in autozygosity when the inbreeding penalty was in effect. This unexpected increase may have been a consequence of the type of inbreeding penalty that we introduced. Seeds that were impacted by the deleterious effects of inbreeding were viable but they were not fertile so they effectively took up space and reduced the number of potential mates for neighboring plants. The reduced mating pool near these individuals may have increased the potential for bi-parental inbreeding particularly in the PSI system where there was no genetic mechanism to avoid it. Due to the finite number of ovules, plants that produced sterile seeds through inbreeding produced fewer fertile seeds that then had to compete with with the sterile seeds for space. The average number of sterile individuals that resulted from inbreeding was just under 5% of the population, and it was not significantly different for any of the

SI systems. The fact that homozygosity decreases but very recent autozygosity does not change suggests that several generations are required before the selection has an effect.

Measuring the amount of inbreeding in the PSI simulations was important because it allowed us to tease apart inbreeding due to self-fertilization and inbreeding between related individuals in the homomorphic SI simulations. The significant decrease in autozygosity in the homomorphic SI systems compared to the PSI system supports the assumption that homomorphic SI reduces inbreeding through bi-parental inbreeding avoidance. These results also support our hypothesis that bi-parental inbreeding avoidance is more prominent when seed and pollen dispersal distance is limited. Nevertheless, the effect of bi-parental inbreeding avoidance on total inbreeding was small compared to selfing avoidance, even when isolation-by-distance was strongest. Therefore, it is unlikely that bi-parental avoidance was a major factor contributing to the evolution of homomorphic SI multiple times in the angiosperm lineage.

Among the different homomorphic SI systems, the BSI and GSI outcomes were not significantly different in most cases. The simple linear dominance scheme that we used to model the relationships between the S alleles in the BSI system is likely responsible for the similarities between the two systems. Under the GSI model, if we consider three related plants with S genotypes S_1S_2, S_2S_3, and S_1S_3, the first plant would be able to accept approximately 50% of the pollen produced by both plants two and three — the pollen with the S_3 haplotype in both cases. Under the BSI system with linear dominance ($S_1 > S_2 > S_3$), the first plant can accept 100% of the pollen from the second plant but it would not be compatible with the third plant. In both systems, the first plant receives the same total amount of pollen from the related plants, the only difference is that the number of compatible mates is higher under the GSI system. In many cases in Brassicaceae, the dominance between S alleles is not linear; S alleles in self-incompatible field mustard (*Brassica campestis*) and marrow-stem kale (*Brassica oleracea*) fall into general classes that are dominant and recessive to each other while alleles within the same group are codominant (Bateman, 1955; Thompson, 1957; Thompson and Taylor, 1966; Hatakeyama et al., 1998). Under these more complicated dominance patterns, the S allele frequency dynamics may cause very different behavior in the BSI system.

In homomorphic SI systems, the S-locus experiences negative frequency-dependent selection which favors low frequency alleles (Wright, 1939). This type of selection allows a large number of S alleles to be maintained in the population which is necessary to keep the number of available mates high (Byers and Meagher, 1992). This is especially true under isolation-by-distance because the number of potential mates is already restricted to a local region. Here we found that, at equilibrium, the SSI simulations maintained the highest number of S alleles, especially when dispersal was restricted. This was expected because, under the SSI system, plants are only compatible when they do not share any S alleles and therefore a larger number of alleles are required to ensure reproductive success. The BSI populations had the lowest number of S alleles and this is likely because recessive S alleles are masked by dominant alleles and therefore fewer total S alleles need to be maintained because more crosses are compatible (Hiscock and Tabah, 2003). Although fewer S alleles are required for reproductive success in BSI, the number of available mates is limited compared to GSI (Vekemans et al., 1998). This is supported by the significantly higher effective dispersal distances observed in the BSI and SSI populations when isolation-by-distance was strongest.

Homomorphic SI systems have a negative effect on population size and female fecundity (Vekemans et al., 1998). Fecundity selection in the simulation was modeled by limiting the number of pollen grains produced by each plant. After pollen dispersal, each plant had a finite pollen pool that was further reduced when a high proportion of the pollen grains are incompatible. If the number of compatible pollen grains was less than the number of ovules, there was a reduction in seed set. The lowest seed set was observed in the SSI simulations because it had the strictest rules for compatibility. Seed set was lowest when dispersal was limited because the pollen pool consisted of a higher proportion of close neighbors which were more likely to be related and thus incompatible. The reduction in seed set also translated into a reduction in the census population size which then further limited the number of available mates in the next generation. Seed set and population size was significantly higher for the PSI populations at each dispersal level, which suggests that reduced fecundity and population size was unique to the homomorphic mating systems. Smaller populations are not able to maintain high levels of S diversity which reduces the number of compatible mates and ultimately reduces seed set. As a result, population size continues to decline and the population is likely to go extinct. This raises concerns for endangered SI species suffering from habitat fragmentation and population bottlenecks such as *Arnica montana*, a grassland perennial in

Europe (Luijten et al., 2000); *Aster furcatus* (Forked aster) of the midwestern United States (Les et al., 1991); three cliff dwelling species, *Sonchus pustulatus*, *S. fragilis*, and *S. masguindalii*, of the western Mediterranean Basin (Silva et al., 2016); and *Hymenoxys acaulis var. glabra* (grassland daisy) of the Great Lakes region (Demauro, 1993).

Homomorphic SI systems seem to provide only a small bi-parental inbreeding avoidance advantage at the expense of reduced fecundity. Despite that, homomorphic SI systems have evolved a number of times in angiosperms so there is likely beneficial features outside of inbreeding avoidance that may explain why these systems evolved in place of heteromorphic SI in many cases. The heteromorphic system that we simulated was designed to simply provide a baseline level of inbreeding that occurred when self-fertilization is prevented and it was not intended to be a realistic representation of a natural heteromorphic SI system. Therefore, we cannot make any meaningful comparisons between our simulated heteromorphic and homomorphic SI systems for characteristics other than inbreeding. Nonetheless, we can propose several reasons why homomorphic SI may be more beneficial in certain situations. One of the most obvious situations is when a species depends on abiotic factors for pollen dispersal rather than pollinators. The differences in flower morphology in heteromorphic systems like distyly are a result of the mechanics of insect pollination. When a species has some other mechanism of pollen dispersal, such as wind dispersal in the case of SI *Beta vulgaris* (beet) (Laporte et al., 2001), changes to flower morphology would not be a valid strategy for preventing selfing. Another potential advantage is that, in some cases, homomorphic SI species allow delayed self-fertilization. In such cases, SI weakens in older flowers allowing ovules that were not fertilized during outcrossing to be self-fertilized. This scenario is the best of both worlds because outcrossing dominates when possible but the plant has reproductive assurance when mates are limited (Lloyd, 1979; Kalisz et al., 2004; Busch and Schoen, 2008).

Further studies should be carried out to better understand the evolutionary dynamics of homomorphic SI systems compared to heteromorphic SI systems to better understand the selective advantages of each mating system. Competition or invasion simulations with heteromorphic SI plants and homomorphic SI plants with delayed self-fertilization may provide a better understanding of the adaptive dynamics. Furthermore, seed and pollen dispersal parameters that mimic a particular species can be used in competition simulations to predict which SI system should be more successful under such conditions.

ACKNOWLEDGMENTS

REFERENCES

Baker, H. G. (1955). Self-compatibility and establishment after "long-distance" dispersal. *Evolution*, 9(3):347–349.

Bateman, A. (1955). Self-incompatibility systems in angiosperms: III. Cruciferae. *Heredity*, 9(1):53–68.

Busch, J. W. (2005). The evolution of self-compatibility in geographically peripheral populations of *Leavenworthia alabamica* (Brassicaceae). *American Journal of Botany*, 92(9):1503–1512.

Busch, J. W. and Schoen, D. J. (2008). The evolution of self-incompatibility when mates are limiting. *Trends in Plant Science*, 13(3):128 – 136.

Byers, D. L. and Meagher, T. R. (1992). Mate availability in small populations of plant species with homomorphic sporophytic self-incompatiblity. *Heredity*, 68:353–359.

Cartwright, R. A. (2009). Antagonism between local dispersal and self-incompatibility systems in a continuous plant population. *Molecular Ecology*, 18(11):2327–2336.

Casselman, A. L., Vrebalov, J., Conner, J. A., Singhal, A., Giovannoni, J., Nasrallah, M. E., and Nasrallah, J. B. (2000). Determining the physical limits of the Brassica S locus by recombinational analysis. *The Plant Cell*, 12:23–33.

Castric, V., Bechsgaard, J. S., Grenier, S., Noureddine, R., Schierup, M. H., and Vekemans, X. (2010). Molecular evolution within and between self-incompatibility specificities. *Molecular Biology and Evolution*, 27(1):11–20.

Charlesworth, D. and Awadalla, P. (1998). Flowering plant self-incompatibility: The molecular population genetics of Brassica S-loci. *Heredity*, 81(1):1–9.

Charlesworth, D., Bartolomé, C., Schierup, M. H., and Mable, B. K. (2003). Haplotype structure of the stigmatic self-incompatibility gene in natural populations of *Arabidopsis lyrata*. *Molecular Biology and Evolution*, 20(11):1741.

Charlesworth, D. and Charlesworth, B. (1979). The evolution and breakdown of S-allele systems. *Heredity*, 43(1):41–55.

Charlesworth, D. and Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, 18:237–268.

Charlesworth, D., Morgan, M. T., and Charlesworth, B. (1990). Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution*, 44(6):1469–1489.

Crawford, T. J. (1984). The estimation of neighbourhood parameters for plant populations. *Heredity*, 52:273–283.

Darwin, C. (1862). On the two forms, or dimorphic condition, in the species of *Primula*, and on their remarkable sexual relations. *Journal of the Proceedings of the Linnean Society of London. Botany*, 6(22):77–96.

Darwin, C. R. (1876). *The Effects of Cross and Self-Fertilization in the Vegetable Kingdom*. John Murray.

Demauro, M. M. (1993). Relationship of breeding system to rarity in the Lakeside Daisy (*Hymenoxys acaulis* var. *glabra*). *Conservation Biology*, 7(3):542–550.

Ennos, R. A. and Clegg, M. T. (1982). Effect of population substructuring on estimates of outcrossing rate in plant populations. *Heredity*, 48:283–292.

Fenster, C. B. (1991). Gene flow in *Chamaecrista fasciculata* (Leguminosae) I. Gene dispersal. *Evolution*, 45(2):398–409.

Fisher, R. A. (1941). Average excess and average effect of a gene substitution. *Annals of Eugenics*, 11(1):53–63.

Franklin-Tong, V. E. and Franklin, F. C. H. (2003). The different mechanisms of gametophytic self-incompatibility. *Philosophical Transactions Royal Society London B Biological Sciences*, 358(1434):1025–1032.

Griffin, C. A. M. and Eckert, C. G. (2003). Experimental analysis of biparental inbreeding in a self-fertilizing plant. *Evolution*, 57(7):1513–1519.

Hatakeyama, K., Watanabe, M., Takasaki, T., Ojima, K., and Hinata, K. (1998). Dominance relationships between S-alleles in self-incompatible *Brassica campestris* L. *Heredity*, 80(2):241–247.

Herlihy, C. R. and Eckert, C. G. (2005). Evolution of self-fertilization at geographical range margins? A comparison of demographic, floral, and mating system variables in central vs. peripheral populations of *Aquilegia canadensis* (Ranunculaceae). *American Journal of Botany*, 92(4):744–751.

Heywood, J. S. (1991). Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology, Evolution, and Systematics*, 22:335–355.

Hiscock, S. J. and Tabah, D. A. (2003). The different mechanisms of sporophytic self-incompatiblity. *Philosophical Transactions of the Royal Society B*, 358(1434):1037–1045.

Holm, D. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 6(2):65–70.

Igic, B. and Kohn, J. R. (2006). The distribution of plant mating systems: Study bias against obligately outcrossing species. *Evolution*, 60(5):1098–1103.

Igic, B., Lande, R., and Kohn, J. (2008). Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences*, 169(1):93–104.

Kalisz, S., Vogler, D. W., and Hanley, K. M. (2004). Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature*, 430(7002):884–887.

Kamau, E., Charlesworth, B., and Charlesworth, D. (2007). Linkage disequilibrium and recombination rate estimates in the self-incompatibility region of *Arabidopsis lyrata*. *Genetics*, 176(4):2357–2369.

Kawabe, A., Hansson, B., Forrest, A., Hagenblad, J., and Charlesworth, D. (2006). Comparative gene mapping in *Arabidopsis lyrata* chromosomes 6 and 7 and *A. thaliana* chromosome IV: Evolutionary history, rearrangements and local recombination rates. *Genetical Research*, 88(1):45–56.

Kusaba, M., Dwyer, K., Hendershot, J., Vrebalov, J., Nasrallah, J. B., and Nasrallah, M. E. (2001). Self-incompatibility in the genus *Arabidopsis*: Characterization of the S locus in the outcrossing *A. lyrata* and its autogamous relative *A. thaliana*. *The Plant Cell*, 13(3):627–643.

Laporte, V., Viard, F., Bena, G., Valero, M., and Cuguen, J. (2001). The spatial structure of sexual and cytonuclear polymorphism in the gynodioecious *Beta vulgaris* ssp. *maritima*: I. At a local scale.

Genetics, 157(4):1699–1710.

Larson, B. M. H. and Barrett, S. C. H. (2000). A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, 69:503–520.

Leducq, J. B., Llaurens, V., Castric, V., Saumitou-Laprade, P., Hardy, O. J., and Vekemans, X. (2011). Effect of balancing selection on spatial genetic structure within populations: Theoretical investigations on the self-incompatibility locus and empirical studies in *Arabidopsis halleri*. *Heredity*, 106(2):319–329.

Les, D. H., Reinartz, J. A., and Esselman, E. J. (1991). Genetic consequences of rarity in *Aster furcatus* (Asteraceae), a threatened, self-incompatible plant. *Evolution*, 45(7):1641–1650.

Levin, D. A. (1981). Dispersal versus gene flow in plants. *Annals of the Missouri Botanical Garden*, 68(2):233–253.

Llaurens, V., Gonthier, L., and Billiard, S. (2009). The sheltered genetic load linked to the *S* locus in plants: New insights from theoretical and empirical approaches in sporophytic self-incompatibility. *Genetics*, 183(3):1105–1118.

Lloyd, D. G. (1979). Some reproductive factors affecting the selection of self-fertilization in plants. *The American Naturalist*, 113(1):67–79.

Luijten, S. H., Dierick, A., Gerard, J., Oostermeijer, B., Raijmann, L. E. L., and Den Nijs, H. C. M. (2000). Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in the Netherlands. *Conservation Biology*, 14(6):1776–1787.

Mable, B. K., Schierup, M. H., and Charlesworth, D. (2003). Estimating the number, frequency, and dominance of S-alleles in a natural population of *Arabidopsis lyrata* (Brassicaceae) with sporophytic control of self-incompatibility. *Heredity*, 90:422–431.

R Core Team (2015). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Renner, S. S. (2014). The relative and absolute frequencies of angiosperm sexual systems: Dioecy, monoecy, gynodioecy, and an updated online database. *American Journal of Botany*, 101(10):1588–1596.

Ritland, K. (2002). Extensions of models for the estimation of mating systems using *n* independent loci. *Heredity*, 88:221–228.

Schierup, M. H., Bechsgaard, J. S., and Christiansen, F. B. (2008). Selection at work in self-incompatible *Arabidopsis lyrata*. II. Spatial distribution of S haplotypes in Iceland. *Genetics*, 180(2):1051–1059.

Scholz, F. and Zhu, A. (2016). *kSamples: K-Sample Rank Tests and their Combinations*. R package version 1.2-3.

Scholz, F. W. and Stephens, M. A. (1987). K-sample Anderson-Darling tests. *Journal of the American Statistical Association*, 82(399):918–924.

Silva, J. L., Brennan, A. C., and Mejías, J. A. (2016). Population genetics of self-incompatibility in a clade of relict cliff-dwelling plant species. *AoB PLANTS*, 8:plw029.

Stebbins, G. L. (1957). Self fertilization and population variability in the higher plants. *The American Naturalist*, 91(861):337–354.

Thompson, K. F. (1957). Self-incompatibility in marrow-stem kale, *Brassica oleracea* var. *acephala* I. Demonstration of a sporophytic system. *Journal of Genetics*, 55(1):45–60.

Thompson, K. F. and Taylor, J. P. (1966). Non-linear dominance relationships between S alleles. *Heredity*, 21:345–362.

Uyenoyama, M. K. (1986). Inbreeding and the cost of meiosis: The evolution of selfing in populations practicing partial biparental inbreeding. *Evolution*, 40(2):388–404.

Uyenoyama, M. K., Zhang, Y., and Newbigin, E. (2001). On the origin of self-incompatibility haplotypes: Transition through self-compatible intermediates. *Genetics*, 157(4):1805–1817.

Vekemans, X., Schierup, M. H., and Christiansen, F. B. (1998). Mate availability and fecundity selection in multi-allelic self-incompatibility systems in plants. *Evolution*, 52(1):19–29.

Vieira, C. P., Charlesworth, D., and Vieira, J. (2003). Evidence for rare recombination at the gametophytic self-incompatibility locus. *Heredity*, 91(3):262–267.

Wang, J., Hill, W. G., Charlesworth, D., and Charlesworth, B. (1999). Dynamics of inbreeding depression due to deleterious mutations in small populations: Mutation parameters and inbreeding rate. *Genetical Research*, 74(2):165–178.

Wright, S. (1939). The distribution of self-sterility alleles in populations. *Genetics*, 24(4):538–552.

Table 1. Seed set and population size is reduced when the SI system is more stringent. The table provides the medians for the number of individuals (N), the number of sterile individuals, the seed set, the mean squared parent-offspring dispersal distance (s^2), the number of unique M alleles, and the number of unique S alleles for simulations with different SI systems and different dispersal parameters (σ). The maximum possible number of individuals in the population is 10,000 and the maximum number of seeds is 50,000.

|	σ	N	Sterile	Seed Set	s^2	M alleles	S alleles
PSI	1	9907	486	45679	1.72	24	4
	2	9905	485	46351	6.20	23	4
	4	9905	486	46458	24.24	22	4
	6	9905	485	46481	54.11	22	4
GSI	1	9886	484	44127	1.72	23	75
	2	9902	486	45963	6.21	22	73
	4	9903	482	46296	24.27	22	73
	6	9903	484	46340	54.06	22	73
BSI	1	9879	484	43547	1.73	24	50
	2	9900	485	45755	6.21	22	38
	4	9902	484	46107	24.22	22	35
	6	9901	484	46166	54.16	22	34
SSI	1	9847	482	41555	1.73	24	81
	2	9897	484	45447	6.24	23	76
	4	9901	485	46072	24.18	22	75
	6	9901	484	46174	54.10	22	75
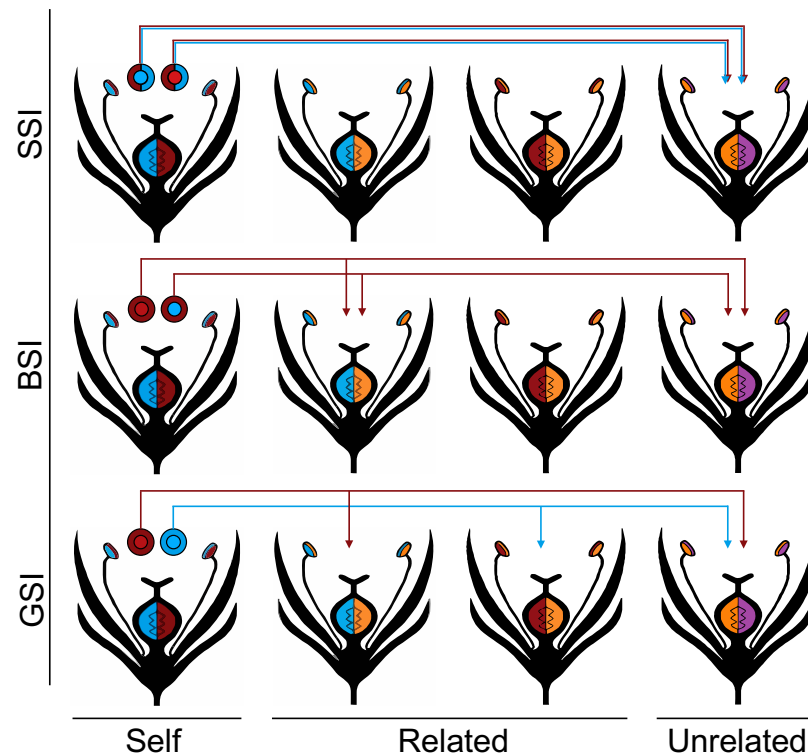
Figure 1. Homomorphic self-incompatibility systems. Four different *S* haplotypes are represented by the colors red, blue, orange, and purple. The plants in the first column produce the pollen represented by the circles above each plant. The color of the inner circle indicates the pollen's haplotype and the outer circle indicates the pollen's phenotype in each of the three SI systems: GSI, BSI, and SSI. Under the GSI system, the pollen phenotype is the same as the pollen haplotype; under the BSI system, the red allele is dominant to the blue allele so all of the pollen are phenotypically red; and under the SSI system, both of the parental alleles are codominant so both are expressed in the pollen phenotype. The *S* alleles in the stigma are all codominant. In every SI system, none of the pollen is compatible with the plant that produced it (self) and all of the pollen is compatible with unrelated individuals that do not share any of the same *S* alleles with the parent plant. The arrows indicate which related plants (those that share one *S* allele with the parent plant) are compatible with each of the pollen types.
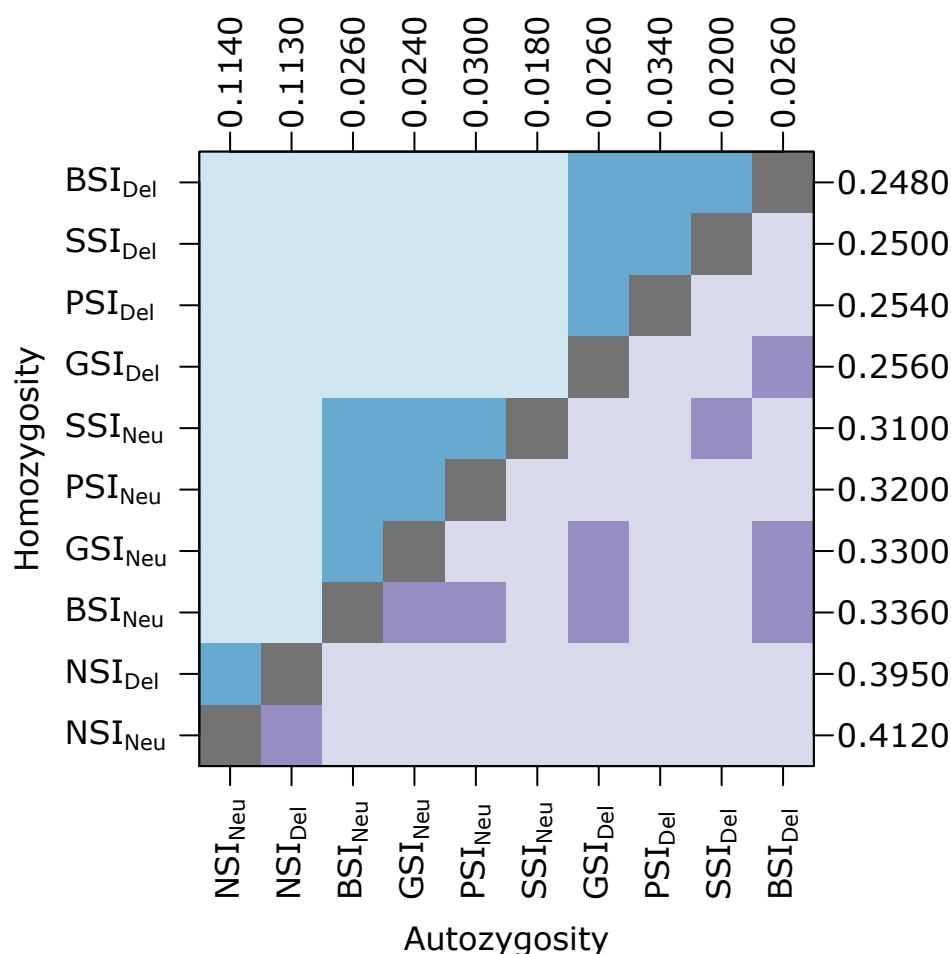
Figure 2. In the homomorphic SI simulations, inbreeding depression had a significant effect on homozygosity. This plot shows pairwise comparisons for each SI system with inbreeding depression (Del) or without inbreeding depression (Neu). The upper (blue) and lower (purple) triangles compare the distribution of proportions of homozygotes and autozygotes, respectively, in 500 samples from each simulation. The color of each square indicates whether the two distributions were significantly different (light) or not (dark). The values along the right and top axes are the median homozygosity and autozygosity for each simulation, respectively. The simulations are sorted on both axes by median homozygosity. The simulations were run on a 100×100 landscape with pollen and seed dispersal parameter $\sigma = 1$.
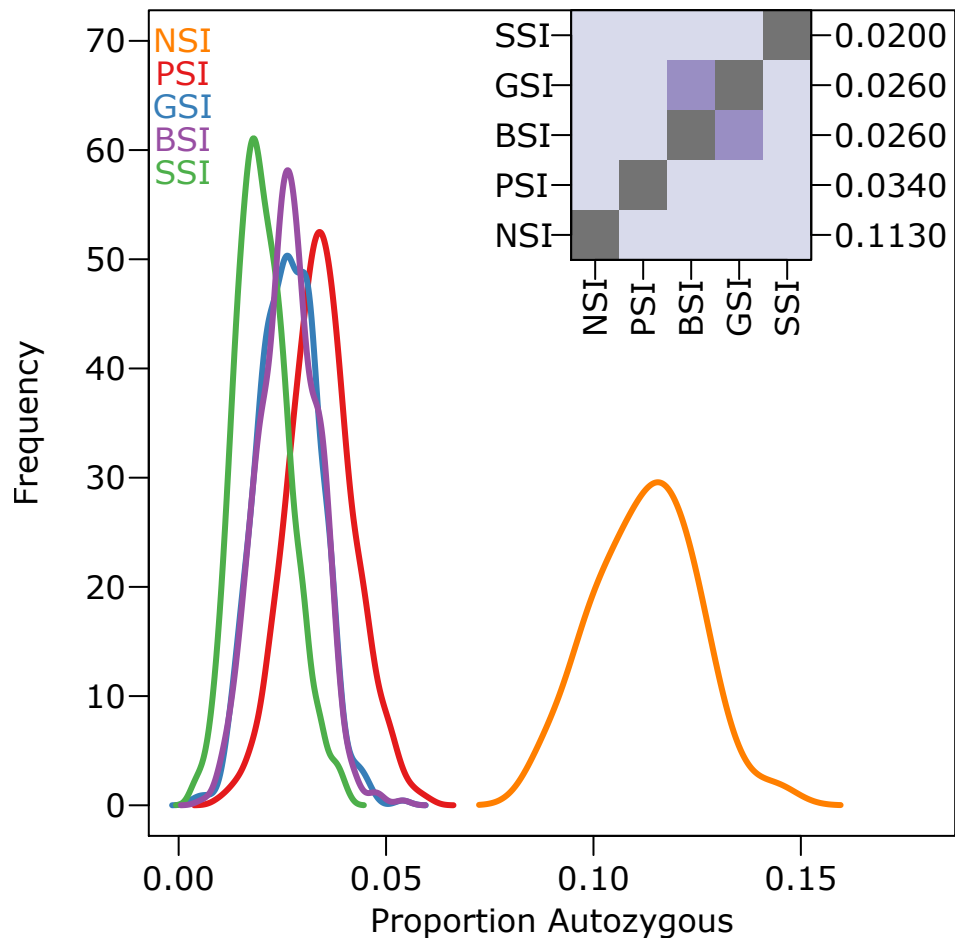
Figure 3. **The reduction in bi-parental inbreeding in homomorphic SI systems was small compared to the reduction in self-fertilization** The empirical density plot (main) shows the distribution of the proportion of autozygotes in 500 samples from simulations of each mating system. The inset shows the pairwise comparisons of each distribution where the color of each square indicates whether the pair is significantly different (light) or not (dark). The values along the right axis of the inset are the medians in increasing order. The simulations included inbreeding depression and were run on a 100×100 landscape with pollen and seed dispersal parameter $\sigma = 1$.
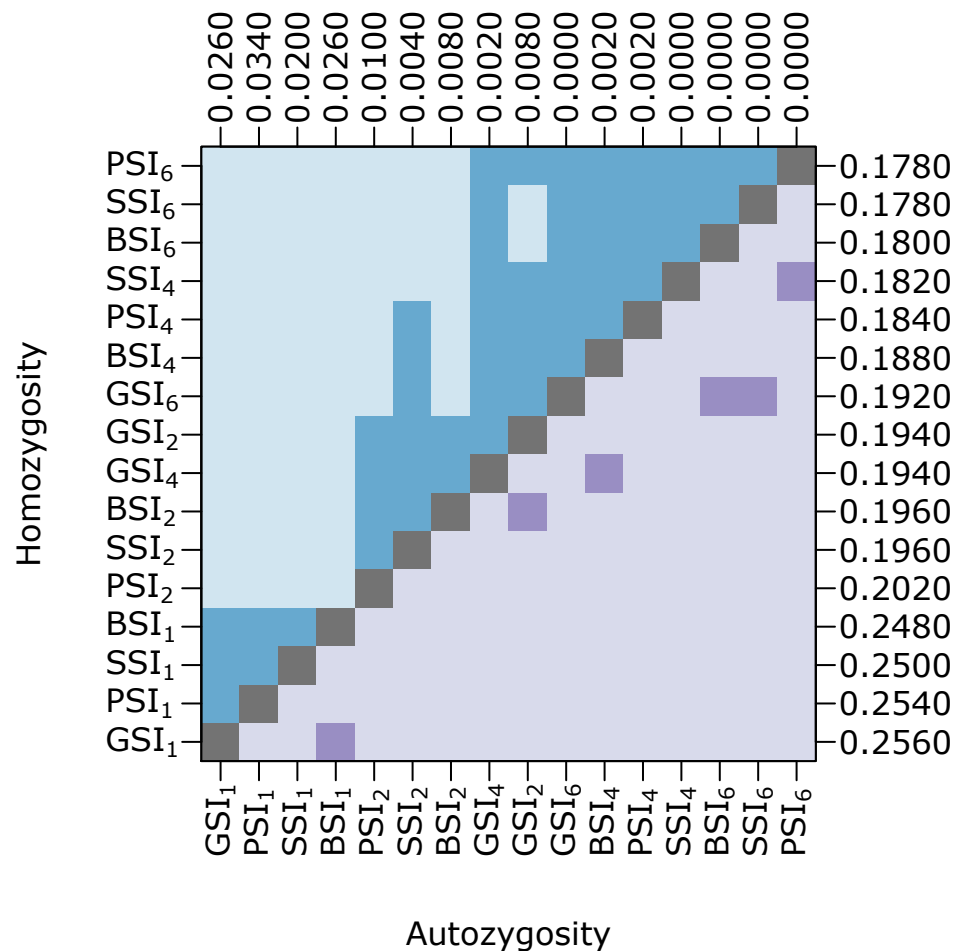
Figure 4. Bi-parental inbreeding avoidance in homomorphic SI systems was greater when isolation-by-distance was stronger This plot shows pairwise comparisons for simulations of each SI system at different dispersal levels. The upper (blue) and lower (purple) triangles compare the proportion of homozygotes and autozygotes, respectively, in 500 samples from each simulation. The color of each square indicates whether the two distributions are significantly different (light) or not (dark). The values along the right and top axes are the medians for homozygosity and autozygosity, respectively. The simulations are sorted on both axes by median homozygosity. The simulations included inbreeding depression and were run on a 100×100 landscape with pollen and seed dispersal parameters $\sigma = 1, 2, 4,$ and 6, indicated in the subscript.