NORTHWESTERN UNIVERSITY

Plant Breeding System and Primary Pollinator as a Proxy to Estimate Inbreeding and Inbreeding Depression

A DISSERTATION

SUBMITTED TO THE GRADUATE SCHOOL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

for the degree

DOCTOR OF PHILOSOPHY

Field of Plant Biology and Conservation

By

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EVANSTON, ILLINOIS

March 2021

ABSTRACT

Genetic factors such as loss of genetic diversity, mutations, and inbreeding depression can increase a populations chances of extinction, therefore it is important to understand how these factors can contribute to populations decline. Among the genetic factors that can contribute to populations extinction, inbreeding depression is the only one that has direct consequences on the fitness of the individuals and therefore can contribute to fitness changes in the next generations. Inbreeding depression refers to the fitness decline of inbreed individuals compared to outcross individuals. Increased inbreeding can lead to inbreeding depression, however, this relationship is not always straightforward and multiple factors can contribute to differences in inbreeding and its impact on the expression of inbreeding depression. Species reproductive system and pollinators, can influence on the rates of inbreeding and outcrossing in a population and therefore should play an important role on the expression of inbreeding depression.

In this dissertation I tested the influence that reproductive system and different pollinators have on inbreeding and inbreeding depression. In chapter 1, I performed two meta-analyses to test the role that plant breeding system and type of pollinators have on inbreeding levels (F_{IS}) and inbreeding depression (F) across taxa. The results indicated that self-compatible taxa have an overall higher level of inbreeding within a population and reduced inbreeding depression compared to self-incompatible taxa, while the role of pollinators does not show a clear or significant pattern. Next, I focus on testing the relation between rates of inbreeding and inbreeding depression, using populations of three different species that differ on their reproductive system or in their pollinators to estimate inbreeding and inbreeding depression. In chapter 2 and 3, I focus on populations of *Oenothera primiveris*, which have variation on their reproductive system across their range of distribution. In chapter 2, I tested differences on the

species reproductive system and how these differences associate with floral traits and with population genetic parameters. The results indicate a transition towards the a selfing syndrome in *O. primiveris* moving from west to east across its geographic range. This shift includes variation in the breeding system, reduction of floral traits (flower diameter, herkogamy, and scent production), and reduced genetic diversity with increased inbreeding. While, in chapter 3, I tested how variation on the breeding system and the inbreeding coefficient in *Oenothera primiveris* can impact the expression of inbreeding depression. The results of this chapter do not support the hypothesis that populations with self-incompatible individuals will have higher inbreeding depression than self-compatible populations, suggesting that not only the reproductive system can influence inbreeding depression and that knowing more about the history of the populations is necessary.

Finally, in chapter 4, I tested differences on inbreeding depression in two sister species with contrasting pollinators. *Clarkia breweri* is mainly pollinated by hawkmoths, which are known to migrate long distances and be sporadic foragers, while *Clarkia concinna* subsp. *concinna* is mainly pollinated by bees and beeflies, which tend to be local pollinators. The results do not support the hypothesis that hawkmoth-pollinated population will lead to higher inbreeding depression under inbreeding compared to bee-pollinated populations. The results suggest that pollinators influence on the populations mating system might be more variable than expected based on the pollinators size and behavior.

Overall my results show that inbreeding depression is population-specific and more variable than anticipated based on the populations reproductive system or their main pollinator. My results also suggest that knowing more about the populations history and demography is important to contextualize inbreeding depression in the populations.

AKNOWLEDGMENTS

First, infinite thanks to my family who have been my main support through out the years. I would like to specially recognize my father, Manuel Cisternas, my mother Yolanda Fuentes (R.I.P) and my sister, Isabel Cisternas who have been there every step of the way and have helped me and accompany me despite the distance. I would also like to thanks my little niece, Sofia Fica, who was born after I started grad school but she has been my daily encouragement to keep going and who has give me happiness and endless conversations who have kept me going and cheer my days, specially during the pandemic. Thanks for the endless video calls and laughs, sweetie.

My sincerest and grateful recognition to my advisor Dr. Jeremie Fant, who helped to grow as a scientist and as mentor by providing encouragement, ideas and feedback in every step of the process. I also want to thanks the members of my committee Drs. Krissa Skogen, Michele Dudash and Stuart Wagenius. Thanks to challenge me to do better and make me grow as a scientist. I would also like to thank the undergraduate students that help me to collect data for my project but also help me discover my passion and interest in teaching and mentoring, Raudel Cabral, Angelica Muñoz, Luis Anzures, Iris Moore, Connor Grant, Anna Od, Juan Gonzalez, Beatriz Torres and Megan Crapo.

I am so grateful for the PBC program and the Chicago Botanic Garden for allowing me to be part of their community and allowing me to use the facilities to carry on my research. Thanks to Dr. Nyree Zerega and Amanda Bartosiak for helping me navigate the PhD requirements and all the paperwork to maintain my visa status. Thanks to the Skogen-Fant lab members all these years for the feedback, discussions, wine tastings and hall conversations, particularly to Katie

Wenzell, Alicia Foxx, and Hilary Noble. Thanks to the greenhouse people who did a great job keeping my plants alive.

Finally, I would like to thank my friend Dr. Jessa Finch, for her kind words, encouragement, unnumbered drinks and conversations. Also thanks to H. Azizi for all his unconditional support, hugs, encouragement and kind words. I couldn't have done it without having you by my side.

DEDICATION

This dissertation is dedicated to my wonderful mother (1952 – 2016) to my family, and H.A. who have unconditionally supported me along the way.

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CHAPTER 1

INFLUENCE OF POLLINATORS AND BREEDING SYSTEM ON INBREEDING AND INBREEDING DEPRESSION: A META-ANALYIS

Abstract

Genetic factors such as loss of genetic diversity, mutations, and inbreeding depression can increase the chances of extinction, therefore it is important to understand how this can happen. Inbreeding depression has direct consequences on the fitness of inbred individuals compared to outcross individuals, influencing the overall fitness of the next generation. Increased inbreeding can lead to inbreeding depression, however, this relationship is not straightforward and multiple factors can contribute to differences in inbreeding and its impact on the expression of inbreeding depression. Along with these factors we have species breeding systems and type of pollinator. In this chapter, I performed two meta-analyses to test the role that plant breeding system and type of pollinators have on inbreeding levels (F_{IS}) and inbreeding depression (F). The datasets consisted of 206 studies that reported inbreeding coefficient across taxa and 194 studies that reported inbreeding depression. The results indicated that self-compatible taxa have an overall higher level of inbreeding within a population and reduced inbreeding depression compared to self-incompatible taxa, while the role of pollinators does not show a clear or significant pattern. The results obtained in the meta-analysis of inbreeding coefficient and inbreeding depression indicate that the factors analyzed here, pollinators and breeding system, might not be enough to explain the heterogeneity observed on the data and that other factors that can influence within-population mating might be important such as, mating system, generational times, population size, etc. These results agree with previous meta-analyses that focus on F_{IS} and

F, although the datasets created here use more specific classifications for breeding system and pollinator functional groups. The variability of inbreeding coefficient and inbreeding depression across taxa and for each evaluated category of pollinator suggest that both estimates depend on the specific species and therefore should be evaluated together more often than currently.

Introduction

To slow the unprecedented loss of species worldwide (Ellis et al., 2012; Pimm et al., 2014) will require an understanding of the factors that make a species vulnerable to extinction. The elements that increase the risk of extinction are a combination of deterministic (habitat loss, overexploitation, introduced species, and pollution) and stochastic factors (demographic, catastrophes, environment, and genetics) (Shaffer, 1981; Frankham et al., 2002; Frankham, 2005). These factors work together to accelerate the species decline, spiraling populations downward in a trajectory known as the 'extinction vortex' (Gilpin and Soulé, 1986). One important component of the extinction vortex is the genetic changes that occur when populations become small. Frankham (2005), identified three genetic factors which will increase the chances of extinction: loss of genetic diversity, mutation accumulation, and inbreeding depression. The genetic factors are all major issue of conservation concern because they can affect all aspects of reproduction and survival and can lead to an increase in mortality of the next generation (Frankham, 2005; Charlesworth and Willis, 2009). Although these factors are recognized as major factors in species extinction, we still do not have a clear understanding on how these different factors can increase the risk of extinction (Frankham, 2005; Ouborg et al., 2006; Paige, 2010; Frankham et al., 2017).

Identifying traits and factors that increase a species or population's susceptibility to extinction can help prioritize efforts on those groups most at risk. Of the three genetic factors associated with extinction, inbreeding and inbreeding depression have been shown to vary with life-history and population parameters (Leimu et al., 2006; Duminil et al., 2009; Angeloni et al., 2011a). Inbreeding, although often considered as the mating between relatives, is more accurately described as the increased likelihood that an allele that is identical by descent, comes together in the same individual (Charlesworth and Charlesworth, 1987, 1999). The factors that can influence the levels of inbreeding in a population include a degree of isolation, the species breeding and mating system (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987) and in the case of plant species, pollinator behavior or limitation (Mitchell et al., 2009). All of these components separately and/or combined can influence the rate of gene exchange between populations and the likelihood of inbreeding. Although the traits or factors that increase rates of inbreeding are usually thought to be undesirable, they can also have a selective advantage when providing reproductive assurance (Lloyd, 1992; Herlihy and Eckert, 2002; Fenster and Martén-Rodríguez, 2007; Busch and Delph, 2012). Hence inbreeding per se is not an issue unless it leads to a decline in fitness, known as inbreeding depression.

The expression of inbreeding depression often occurs when there is a change in the rate of inbreeding (Charlesworth and Charlesworth, 1999; Keller and Waller, 2002), often associated with a reduction in the effective population size, as a consequence of a population bottleneck or through a founder effect (Frankham et al., 2002). There are two main hypotheses as to how increasing rates of inbreeding can reduce fitness: overdominance and dominance hypothesis (Charlesworth and Charlesworth, 1987, 1999; Roff, 2002; Charlesworth and Willis, 2009). The overdominance hypothesis suggests that there is overall a fitness advantage in higher

heterozygosity across the genome, often termed heterozygous advantage (Charlesworth and Willis, 2009). Hence the loss of heterozygosity over time will lead to a decrease in the mean fitness value of the population (Lynch and O'Hely, 2001; Schou et al., 2017). By contrast, the dominance (or partial dominance) hypothesis, refers to the increased expression of the genetic load (Keller and Waller, 2002), which are recessive deleterious mutations that have little or no negative effects in heterozygous individuals (Haliburton, 2004; Wright et al., 2008). Higher rates of inbreeding will increase the likelihood that these traits are expressed. Whenever this genetic load is expressed, selection will act to eliminate these alleles from the population. If frequent enough this can result in the purging of the genetic load from the population, which is why the negative effects of inbreeding depression are not always found in all populations even under higher inbreeding scenarios (Dudash and Carr, 1998; Crnokrak and Barrett, 2002).

Given the variation in expression of inbreeding and inbreeding depression seen across taxa, several meta-analyses investigated how the surrounding environment and other life-history traits will impact the expression of inbreeding and inbreeding depression (Byers and Waller, 1999; Crnokrak and Roff, 1999; Keller and Waller, 2002; O'Grady et al., 2006; Duminil et al., 2007, 2009; Angeloni et al., 2011a; Fox and Reed, 2011). These studies have found that inbreeding depression and purging are not consistent across populations. However, some consistent trends show that inbreeding rates were related to generational time and mating system (Duminil et al., 2009). While the expression of inbreeding depression was highest in larger population sizes compared to small populations (Angeloni et al., 2011a). This latter result was contrary to the expected, which might suggest purging of the genetic load is more frequent in small populations (Angeloni et al., 2011a). However, two important traits that were not included in these meta-analyses are breeding system and pollinator functional group.

Both breeding system and pollinator functional group will directly influence the amount of inbreeding a population experiences. Plant breeding systems shape the mating patterns within a population (Neal and Anderson, 2005), by determining the extent to which selfing can occur (Charlesworth, 2006; Raduski et al., 2012). Hence self-compatible families could express less inbreeding depression as they had more opportunities to express their genetic load, purging it in each subsequent generation. Several studies looking at contrasting breeding system (SI compared to SC) or contrasting mating system (outcrossing compared to selfing) have supported this response (Dudash and Carr, 1998; Fishman, 2001; Ishida, 2008; Voillemot and Pannell, 2017a), while others have not (Guillaume and Jacquemart, 1999; Busch, 2005a; Ruhsam et al., 2010). Similarly, for animal pollinated plants, the amount of inbreeding in a population would also depend on the species main pollinator and characteristics such as size and behavior. Species pollinated by local foragers with a short flight distance might result in greater geitonogamy, higher biparental inbreeding and greater isolation (Mitchell et al., 2009). Genetic patterns such as differentiation and diversity for plant species with different pollinators has supported this hypothesis (Jabis et al., 2011; Kramer et al., 2011; Howell and Jesson, 2013; Wessinger et al., 2019), while other did not see this relationship (Collevatti et al., 2010; Torres-Vanegas et al., 2019). Given the wide variety of pollinators that can be found in nature (Ollerton, 2017), it seems relevant to understand the impact they have on inbreeding and therefore the expression of inbreeding depression. To my knowledge, no attempt has been done to evaluate the relationship between type of pollinator and level of inbreeding across taxa.

In this chapter, I performed two meta-analyses focusing on the influence that different breeding systems and different pollinator functional groups have on inbreeding coefficient (F_{IS}) and inbreeding depression (F). Molecular data can be used to estimate the inbreeding coefficient

(Wright, 1951; Weir and Cockerham, 1984). F_{IS} is an indirect estimate of the likelihood of inbreeding of an individual with respect to the subpopulation (Wright, 1951). It represents the degree of inbreeding over the lifetime of the population, as molecular changes can take time to accumulate and be reflected in the estimate. Populations with a high F_{IS} (~ >0.25) value indicate that mating between relatives is common or has happened in the past. By contrast, estimations of inbreeding depression (hereafter refer to as F) are obtained through the comparison of fitness traits between inbred and outcrossed individuals. Since the measurement of fitness traits can be difficult under natural conditions, the most common way to estimate inbreeding depression is through experiments under controlled environmental conditions. Using two separate meta-analyses, the following hypotheses were tested: (1) inbreeding coefficient (F_{IS}) would be higher in self-compatible than self-incompatible taxa, while inbreeding depression will lower in self-compatible compare to self-incompatible taxa and (2) taxa pollinated by large pollinators with large foraging distances will have a lower estimate of F_{IS} and under inbreeding express higher inbreeding depression than species pollinated by small insects.

Methods

Compiling the inbreeding coefficient (F_{IS}) dataset

The search for data looking at inbreeding coefficients was done by performing an extensive search in the Web of Science using the keywords, f-statistics and plants (Search was done in December 2018). I reviewed the abstracts following Preferred Reporting Items for Systematic Reviews and Meta-analyses or PRISMA recommendations for data selection (Figure 1.1, Moher et al., 2009). Only articles that included molecular data in angiosperms were included. Based on the preliminary screening of the articles, two data sets were created. One

dataset included population-level data while the second data set only included information at the species level. Both were later collapsed into a single data file for species-level comparison (Supplemental material Table S1.1), by calculating average of F_{IS} across populations. Further information about the species and details of each study where recorded when available, including family, mating system, growth form and type of reproduction. The mating system was defined as selfing, mixed-mating or outcrossing, and was only included if the authors mentioned the specific mating system or reported outcrossing or selfing rates for the evaluated populations. If outcrossing rate was reported, a value below 0.2 was categorized as selfing, a value above 0.8 was categorized as outcrossing and values between 0.2 and 0.8 as mixed mating. Growth form was categorized into three groups, as annual, short-live perennial and long-live perennial, as well as if the species was able to clonally reproduce. Annual refers to herbaceous species that have a short generational time (either one or two years), short-lived perennials refer to forbs, or herbaceous perennial while perennial referred to long-lived species (usually referring to long-lived taxa such as tress, members of the Cactaceae family and others). Type of reproduction was defined as sexual or asexual, since this was rarely provided in the paper, a search for the species was done on Google to find out if asexual reproduction was possible or not. Specific information also recorded included number of populations studied, mean sample size used, the mean number of alleles per locus and the type of genetic marker. I only considered studies that included a minimum of 10 individuals on average to evaluate inbreeding, given that the resolution of genetic markers might be reduced with small sample sizes. If an article reported a clear distinction in breeding system, type of pollinator or mating system for the taxa evaluated (or between populations of the same taxa), this was incorporated into separate entries in the dataset reflecting on these differences. Furthermore, if a species was evaluated in multiple studies, the

information provided from each study was entered separately into the dataset because of the uncertainty of material coming from the same populations and/or differences in molecular markers used to obtain the data or by the authors of the article.

Compiling inbreeding depression dataset (F)

Similar guidelines were used to create the inbreeding depression dataset. Papers were identified on Web of Science using the keywords inbreeding depression and plants (Search was done in December 2018). Only articles that explicitly mentioned measuring inbreeding depression or evaluating fitness changes under inbreeding in the abstract were included. Based on the preliminary screening, I focused on changes in cumulative fitness after one generation of inbreeding, hence if multiple generations were measured, only the first was used. In the case where a mean value of fitness was available (either by traits measured or a cumulative estimate was provided) for self and outcross pollination, I calculated inbreeding depression following the recommendations of Agren and Schemske (1993) using the following equations, where W_S is the fitness of the self cross and W_O represent fitness of the outcross:

Equation 1: Inbreeding depression when $W_O > W_S$

$$ID = 1 - \frac{W_S}{W_O}$$

Equation 2: Inbreeding depression when $W_O < W_S$

$$ID = \frac{W_O}{W_S} - 1$$

If the authors provided only the mean fitness value, I calculated cumulative fitness by multiplying all means and entering the resulting value into the Agren and Schemske (1993) equation to obtain inbreeding depression. If an article reported multiple species or different populations, these were incorporated as independent values into the dataset. From each article

incorporated into the dataset, I also recorded species breeding system and/or pollinator, family, growth form and mating system and the number of traits used in the article to calculate inbreeding depression. The full inbreeding depression dataset used can be found in the Appendix (Appendix Table S1.2).

Category definitions

Recognizing the variability of data reporting and information provided across papers, the breeding system and type of major pollinators were categorized into broad categories to increase statistical power. Plant breeding systems were categorized for this study into self-incompatible and self-compatible according to the amount of expected outcrossing. For example, dioecious species were classified as self-incompatible since they promote outcrossing, likewise for heterostylous species. Similarly, main pollination strategy of the taxa were categorized according to broad functional groups (Fenster et al., 2004). If a study reported that a species main pollinator belonged to different functional groups, this was classified as a generalist. Pollinators were also classified according to their body size, which was used as an estimation of foraging behavior, to address the hypothesis that foraging distance can influence the inbreeding coefficient and inbreeding depression. This included extra small (Thrips), Small (Bees, beetles, wasp, flies); Medium (Noctuid moths and butterflies); Large (Bumblebees and hawkmoths), while extra-large included (Birds and bats).

Meta-analysis for inbreeding coefficient (F_{IS})

To test the influence that different pollinator functional groups and the breeding system have on the inbreeding coefficient, I performed a meta-regression on the effect size of F_{IS} and variance to determine which category varied from zero. The meta-analysis (including a meta-regression approach) provides a powerful, informative and unbiased tool for summarizing results

of studies (Koricheva et al., 2013). In general, this type of analysis uses effect size and their respective variance to equalize precision of the magnitude for each study to their main effect. This is done by weighting each effect size by the inverse of variance, therefore, an estimate with a higher variance would indicate lower precision compared to an estimate with lower variance. Inbreeding coefficient (F_{IS}), represents an unconventional statistic in the field of molecular ecology because the measurement of F_{IS} can vary with population size and the variance of the effect size is not usually reported. To rectify this, I calculated the variance of each study in our data set. This was done separately for the population dataset (Equation 3) and the species (Equation 4) data set. The calculation of variance was done as follows:

Equation 3: used for studies with multiple populations

$$\sigma^2 = \frac{\Sigma(x-\bar{x})^2}{n-1}$$

Equation 4: used for studies where only one value of FIS is provided

$$\sigma^2 = \frac{(x)^2}{n-1}$$

Where σ^2 represents variance, Σ represents the sum across populations, x represents the value of F_{IS} for any population or species, $\bar{x}$ represent the average F_{IS} across populations and n represents the average population size used for the study. The variance was calculated for each study. With population-level datasets, the parameters were averaged to create a specie level mean and merged with the species dataset, to obtain the final data for the analysis.

For the analysis, I constructed random effect models and mixed effect meta-regression models to compare the influence of pollinator functional group and breeding system on F_{IS} values. The models were compared using restricted maximum likelihood. To test whether pollinator functional group and breeding system influence F, I created a random effect model

without any moderators and a full model that incorporated the interaction between the different combinations of pollinator functional group and breeding system. For comparing the statistics of both models, I calculated how much of the heterogeneity can be explained by adding the moderators (Pollinator functional groups*Breeding system) using the following equation

Equation 5:

$$\%\text{Heterogeneity} = \frac{{\tau^2}_A - {\tau^2}_B}{{\tau^2}_A} \text{ x } 100$$

where ${\tau^2}_A$ represents the τ^2 statistics for the most basic model while ${\tau^2}_B$ represents the τ^2 statistics for the model with moderators.

Models were also created to analyze the amount of heterogeneity explained by the moderators separately. The reduced models were then compared to the simpler model to determine which of the moderators best explained most of the variation observed in F_{IS} values. This analysis was done using the package metafor (Wolfgang, 2010) in R version 3.3.3 (R Core Team, 2017). The model results can be compared by the following parameters obtained from each model, Q_E and their P value represents significance between-study variance; τ^2 measures the between-study variance; I^2 measures the variance explained by heterogeneity between studies. Considering that not all studies in the dataset had information on pollinator functional group and breeding system, I also evaluated pollinator functional group and breeding system separately, to account for all the variation and categories captured in the dataset. This was done using the same approach mentioned above.

As a complementary approach, I also performed an analysis of variance to test differences between the evaluated categories. I did this using a two-way ANOVA to evaluate if pollinators and breeding system interact to influence the levels of F_{IS} seen. I used a one-way ANOVA to test for differences in the mean F_{IS} across the different pollinator functional group

categories or t-test to evaluate differences in mean values of F_{IS} between breeding systems. Using the same approach, I evaluated how other life-history categories (growth form, mating system and type of reproduction) influenced the inbreeding coefficient. If more than three categories were evaluated and significant differences were obtained, I used a Tukey's posthoc test to identify specific differences between the categories.

Meta-analysis for inbreeding depression (F)

Inbreeding depression represents a more conventional measurement of effect size across studies as inbreeding depression is calculated consistently. The variance could not always be calculated due to the differences in the way sample size was reported. While some studies reported the initial sample size used, others reported the number of families or the sample size for each evaluated category. These differences made it difficult to generate a consistent measure of variance for each study, therefore this dataset was analyzed using analysis of variance only. This was done by testing the influence of the pollinators and breeding system and their interaction and also by pollinator functional groups and breeding systems on their own

Results

Influence of pollinators and breeding system on inbreeding coefficient (F_{IS})

For the inbreeding coefficient dataset, the search resulted in 473 relevant studies (Figure 1.1). Some articles were rejected as they focused on theoretical models and did not provide species or population data. The remaining 425 articles recovered were downloaded based on the information provided in the abstract. For this chapter, I reviewed 210 articles and 140 had the appropriate data which were incorporated in our dataset. From this data, a total of 11 pollinator categories and 2 breeding system were captured in the inbreeding coefficient dataset (Table 1.1).

The inbreeding coefficient dataset consisted of 206 studies representing 185 species distributed among 68 families of Angiosperms. The most common families represented in the dataset were Orchidaceae, Asteraceae, Fabaceae, Ericaceae, Brassicaceae, Phrymaceae and Plantaginaceae, together representing nearly one-third of the studies in the dataset. Estimations of the inbreeding coefficient varied from -0.61 to 0.98. The model without moderators showed a significant positive effect (0.169 with a 95% confidence intervals of 0.13 to 0.20) which was significantly different from zero (P <0.0001) with a τ^2 of 0.31. A mixed effect model including the interaction between pollinators and breeding system as moderators indicated a significant residual heterogeneity (Q_E= 15,020.7, P <0.0001; Table 1.2) possible indicating that other moderators not included in the model are influencing the F_{IS} estimates, and a τ^2 of 0.11. Using τ^2 values of both models, I calculated how much of the total amount of heterogeneity can be accounted for by including the interaction between the breeding system and pollinators and found that only 13.6% of the total amount of heterogeneity could be explained by the interaction between moderators. Using the same dataset but only including studies with information on both breeding system and pollinator functional group, the model that can best explained the observed variation was the one with the sum of factors (15%; Table 2) followed by the interaction term (13.6%). It is also relevant that the interaction or sum of moderators explained more variation than either of the factors separated. This indicates that pollinator functional group and breeding system can explain the variation on F_{IS} but other factors not considered in this work may also be important.

Considering that of the 206 studies in the data, only 151 included information about both pollinator and the breeding system, I also evaluated the influence that pollinators and breeding system separately. The dataset with only pollinators included 192 studies, while 141 could be

categorized by body size once wind-pollinated species and generalists were omitted. The dataset where breeding system was known included only 160 studies. Pollinators showed a significant amount of heterogeneity ($Q_{E\,(176)} = 46{,}736.1$, $P < 0.0001$) between studies. A test for moderators indicated that type of pollinators overall does not have a significant influence on the average effect of F_{IS} ($Q_{M\,(10)} = 15.78$, $P = 0.10$). However, some of the pollinators categories such as, bats, bees, birds, generalists, moths, and abiotic-pollinated species do have a significant effect on F_{IS} that is different from 0 (Figure 1.2A). When data were analyzed based on pollinators categorized according to body size, the model shows that body size has a significant amount of heterogeneity ($Q_{E\,(136)} = 51209.8$, $P < 0.0001$) between studies. A test for moderators indicated that pollinator body size overall does not have a significant influence on the average effect of F_{IS} ($F_{1,136} = 0.88$, $P = 0.47$). However, some of the body size categories such as, small, medium, large, extra-large do have a significant effect on F_{IS} (Figure 1.3A). To corroborate the results obtained in the meta-regression I also used analysis of variances to test differences between the evaluated categories. The effect of pollinators on the inbreeding coefficient indicates that F_{IS} across pollinators show a significant difference from the null ($F_{10,181} = 2.09$, $P = 0.027$; Figure 1.2B), while pollinators classified according to their body size does not ($F_{4,136} = 1.12$, $P = 0.35$; Figure 1.3B).

In the meta-analysis regression, the breeding system model also shows a significant amount of heterogeneity ($Q_{E\,(136)} = 47{,}0098.01$, $P < 0.0001$) between studies. While a test for moderators indicated that breeding system does have a significant effect on the average effect of F_{IS} ($F_{1,163} = 19.4$, $P < 0.0001$; Figure 1.4A), where self-compatible taxa had a higher F_{IS} than self-incompatible taxa. When using the analysis of variance, there was a significant difference between the mean value of F_{IS} according to their breeding system ($t_{155.7} = 4.69$, $P < 0.0001$; Figure

1.4B), where similar to the meta-analysis regression, the self-compatible taxa show higher F_{IS} than self-incompatible.

Considering that other life-history traits such as mating system and growth form can influence the amount of inbreeding within a population, I also tested if there were any significant differences between the different mating systems, growth forms, type of reproduction and genetic markers used on the studies and their influence on inbreeding coefficient. Selfing taxa had higher F_{IS} than taxa with mixed-mating and outcrossing mating systems ($F_{2,91}$= 49.2, P <0.0001; Figure 1.5A), while annual taxa had a higher F_{IS} on average than short-lived and long-lived perennials ($F_{2,203}$ = 14.27, P <0.0001; Figure 1.5B). Species with asexual reproductive strategy had a slightly lower F_{IS} ($F_{1,204}$= 4.64, P= 0.03; Figure 1.5C). Finally, the type of molecular marker used had no impact on F_{IS} ($F_{4,201}$= 0.2, P= 0.94; Figure 1.5D).

Influence of pollinators and breeding system on inbreeding depression (F)

For the inbreeding depression dataset, the search results indicated 2,602 articles, although most of them did not directly measure inbreeding depression therefore they were excluded from the study (Figure 1.1). Of these 233 articles were downloaded based on the information in their abstract. Of the 150 reviewed for this chapter, information from 94 papers were incorporated in our dataset. A total of 10 pollinator categories and 4 types of the breeding system were captured (15 studies were Dioecious and 4 were Heterostylous, all of them classified as SI in this chapter; Table 1.1).

The inbreeding depression dataset consisted of 194 values of inbreeding depression representing 105 species distributed among 40 families of Angiosperms. The most common families represented in the dataset were Onagraceae, Caryophyllaceae, Plantaginaceae, Boraginaceae, Ericaceae, Orchidaceae, and Passifloraceae, together representing nearly half of

the studies present in the dataset. Estimations of inbreeding depression captured in the dataset generated the expected range of inbreeding depression varying from -0.89 to 0.99, with a mean cumulative value of 0.38 ± 0.39.

The interaction between the breeding system and pollinators did not show a significant influence on inbreeding depression ($F_{17,169}$= 1.10, P= 0.36; Table 1.3). The model with breeding system was significant, indicating that there is a significant difference between self-compatible and self-incompatible taxa in inbreeding depression, with SI species showing a higher mean inbreeding depression. Of the 194 studies in the data, 187 studies included information about both pollinator and the breeding system. Although there was a high number of studies including information both, I also evaluated the influence of pollinators and breeding system separately. The pollinator only dataset included 187 studies, while body size was reduced to 169 studies once wind-pollinated species and generalists were omitted, and the dataset with only breeding system included 194 studies. The effect of pollinators on inbreeding depression was not significantly different from the null, indicating that there are no differences ($F_{9,177}$= 0.51, P= 0.87; Figure 1.6). Similar results were obtained by classifying pollinators according to their body size ($F_{4,164}$= 0.56, P= 0.69; Figure 1.7). The breeding system was significantly different from with self-incompatible populations have a higher inbreeding depression than self-compatible populations ($t_{78.6}$= -2.34, P= 0.021; Figure 1.8).

Considering other life-history traits, I also tested if there were any significant differences between mating system, growth forms and reproductive strategy on inbreeding depression. Taxa with a selfing mating system have reduced inbreeding depression compared to those with a mixed-mating or outcrossing mating system ($F_{2,101}$= 5.57, P= 0.005; Figure 1.9A). Annual taxa have reduced inbreeding depression compared to perennials ($F_{2,191}$ =7.65, P= 0.0006; Figure

1.9B) but no significant difference when compared to short-lived perennials. Although, species with an asexual reproductive strategy showed no significant difference ($F_{1,192}$ =3.06, P =0.08; Figure 1.9C), there was a trend to lower inbreeding depression in sexually reproductive taxa.

Discussion

The meta-analyses conducted partially support the hypotheses showing that breeding system influenced the inbreeding coefficient and inbreeding depression across taxa, where self-compatible taxa had higher F_{IS} and reduced inbreeding depression compared to self-incompatible taxa. However, pollinator functional group do not show any clear pattern to support the hypothesis that large pollinators will have reduced F_{IS} and high inbreeding depression. The results obtained on the meta-analysis of inbreeding coefficient indicate that the factors analyzed here, pollinators and breeding system, might not be enough to explain the heterogeneity observed on the data and that other factors that can influence within-population mating might be important such as, mating system, generational times, population size, etc. Including more studies in each of the breeding system and pollinator categories could help reduce the heterogeneity between studies, and allow for identifying interactions between these other factors. The combination of pollinators and breeding system can explain 13% of the observed heterogeneity between studies, there is still considerable heterogeneity that cannot be explained by the factors analyzed in this study and future analysis might need to incorporate additional factors, however, the inclusion of too many factors can also lead to obscure patterns of differentiation without been informative.

As was expected, the breeding system influenced the inbreeding coefficient and inbreeding depression, showing that taxa that can self-fertilize have higher levels of inbreeding compared to taxa that can not self-fertilize. This result was the same for the classic meta-analysis

approach and through the analysis of variance. Taxa that are self-incompatible express higher inbreeding depression on average that taxa that can self. The influence of pollinators on inbreeding coefficient or inbreeding depression is less clear than observed for the breeding system. These results suggest that the breeding system could be used to predict response in the inbreeding coefficient and the likelihood of experiencing inbreeding depression. However, it is important to notice the variation observed for F_{IS} and F in every category evaluated here, the variation observed suggests that the inbreeding coefficient and inbreeding depression varies within each group. Hence, we should be careful about making assumptions based only on breeding system or primary pollinator alone. Although there were some differences, there were no clear patterns among pollinator functional groups or pollinators categorized according to body size. The lack of significant differences between pollinators on F_{IS} or F could be due to the broad categories which do not account for the diversity of behavior among or between pollinators. Even though a pattern of behavior might be expected based on the size of a pollinator, the reality is that each species will act differently depending on their biology and the environment they lived in. Pollinator categories were also unequally represented in the datasets, both datasets had a higher representation of bees, bumblebees, or generalists while other pollinator groups were less represented. The unequal representation across functional groups is a limitation of the dataset. In addition, the lack of information to allow further division of the pollinators group, which recognizes the differences in behavior within each group (for example, bees only collecting pollen, nectar and pollen, solitary or social bees, etc.) could change the observed patterns presented here.

Breeding system relationship with inbreeding and inbreeding depression

The relationship between inbreeding, inbreeding depression and breeding systems has been well studied (Barrett and Harder, 1996; Charlesworth, 2006; Duminil et al., 2009; Angeloni et al., 2011a). These studies suggest that taxa that are self-incompatible are unable to self-fertilize and therefore have lower levels of inbreeding, hence lower inbreeding coefficients compare to taxa than can self fertilize. The results obtained here support this hypothesis, self-compatible taxa have an overall higher level of inbreeding within a population and reduced inbreeding depression compared to self-incompatible taxa. However, the observed variation within each category is important and suggest high variability within each breeding system category. Self-incompatibility is not a perfect system, and variation of self-incompatibility among taxa has been reported (Raduski et al., 2012). The same could also be said about self-compatible taxa, just because a taxon is able to self-fertilize does not mean they will a majority of the time. The frequency of self-fertilization or outcrossing, known as the mating system can vary within and between populations (Goodwillie et al., 2005; Whitehead et al., 2018). Allogamous taxa have a reduced inbreeding coefficient compared to mixed mating taxa (Duminil et al., 2007, 2009), while selfing taxa have reduced inbreeding depression compared to mixed mating or outcrossing taxa (Winn et al., 2011). This variation in the mating system, in relation to the breeding system, creates variation on the patterns of inbreeding and inbreeding depression expected across taxa, which could explain the variation observed in these datasets. In the only meta-analyses that directly evaluated differences between breeding system besides the one performed here, inbreeding depression has a significant effect size on self-compatible and self-incompatible taxa but no significant differences were found between both categories (Angeloni et al., 2011a), which differ from the results obtained here

Main pollinator related to inbreeding and inbreeding depression

Pollinators facilitate the transfer of pollen both within and between populations allowing for successful reproduction, and therefore directly influence population's mating dynamics (Devaux et al., 2014). Pollinator foraging behavior, including flight distances between plants, time between bouts, pollen carryover and other traits will also have an impact on realized mating events (Wilson and Thomson, 1991; Sahli and Conner, 2007; Ma et al., 2019) and the distribution and abundance of genetic diversity and gene flow (Brunet and Holmquist, 2009; Jabis et al., 2011; Skogen et al., 2019). Pollinator functional groups are collections of pollinators that share similar traits, and therefore, it is expected they will influence on plant mating dynamics in similar ways (Fenster et al., 2004). Large pollinators are expected to travel longer distances within and between populations, therefore reducing inbreeding levels within a population. The results found here, do not support the hypothesis that larger pollinators will result in reduced inbreeding coefficient. One reason that could influence the results found here is the broad pollinator categories used, without taking into consideration the variation within each pollinator group. Pollinators were either divided according to their body size or into main functional groups. Each pollinator group used here, is variable which can influence on the variation observed within each category for FIS and F, for example, bees can vary in their behavior, body size, and dispersal distance (Greenleaf et al., 2007; O'Connell et al., 2018; Chole et al., 2019; De Luca et al., 2019) and they might influence inbreeding differently. For example, small bees forage shorter distance visiting fewer plants between bouts which could lead to higher inbreeding rate. The same variation could be expected within other pollinator categories such as nectar-feeding birds (Brown et al., 1978) or others.

At this point in time, there is an important limitation on the information we know about certain pollinators behavior, traveling distance, and how they can influence on plant populations. Previous meta-analysis on the influence that different plant traits have on inbreeding showed that abiotically-pollinated taxa have reduced inbreeding coefficients compared to biotically-pollinated taxa (Duminil et al., 2009). Considering that not all pollinators within the biotic pollination category are the same, varying in size, foraging patterns, or general behavior, it is reasonable to expect that pollinators will influence plant mating differently. Pollinator mobility (mobile versus less mobile) had a significant effect size on the selfing rate of woody plants in fragmented landscapes in Australia, showing that pollination by mobile pollinators (birds, bats or large insects) results in lower selfing rates on average compared to less mobile pollinators (small moths and bees) (Breed et al., 2015). Small pollinators also can lead to increase genetic differentiation across populations compared to larger pollinators or even species pollinated by vertebrates (Gamba and Muchhala, 2020). A lot of research has been done to understand the role that bees have on inbreeding, genetic differentiation, plant mating, etc. (Castilla et al., 2017) but research in other pollinator groups might help fill up the gap we currently have to understand the variation and the influence that pollinators have on plant populations. Finally, another possible explanation to consider is that pollinators body size or pollinator functional group might not have an influence on genetic patterns across flowering plants, and more case-specific, taxonomic specific studies or more narrow meta-analysis should be carried to evaluate if at smaller scales there is a more clear pattern of pollinator influence, such as the one found in Breed et al. (2015). The dataset obtained here represents to my knowledge the only study that included pollinator functional groups, as a variable to consider while evaluating differences on inbreeding depression. However, one important limitation is that few studies evaluate inbreeding depression

in taxa with contrasting pollinator. More studies like this are needed to provide more insight into the role that pollinators play on inbreeding depression.

Relationship between the inbreeding coefficient and inbreeding depression

Inbreeding can lead to inbreeding depression, while the expression of inbreeding depression allows for purging, leading to changes on the populations genetic load. Constant inbreeding allows for selection to purge the genetic load (Crnokrak and Barrett, 2002) making it difficult to predict values of inbreeding depression in natural populations. This is likely more common in small populations, where a finite number of individuals, are subject to drift and genetic bottlenecks leading to an overall reduction of genetic diversity and even higher rates of inbreeding (Angeloni et al., 2011a). Therefore, in small populations purging is more likely as the inbreeding increases the expression of deleterious alleles (Crow, 1970; Keller and Waller, 2002; Pekkala et al., 2014). Despite the theoretical expectations that relate inbreeding and inbreeding depression, few of the studies on inbreeding depression reviewed here provide information about inbreeding levels in the populations. Among the studies that provide a value of inbreeding, F_{IS}, the values are used to corroborate or explain outcrossing rates of the population (t_m) but no further analysis or discussion is provided (Johnston and Schoen, 1996; Affre and Thompson, 1997; Hull-Sanders et al., 2005; Ishida, 2008). Although mating system evolution is closely related to the role of inbreeding depression, the mating system has the capability of showing year to year variation while the inbreeding coefficient measured in adult plants (not seedlings) should be more stable and provide information about inbreeding levels at an evolutionary time scale. In this chapter, I did not evaluate the relationship between F_{IS} and F, due to limitations on the number of studies which report both of these traits. More studies that evaluate both parameters

should be done to directly test how inbreeding coefficient and inbreeding depression relates within the same taxa, and if one parameter could help to predict the other.

Conclusions

The results obtained in this chapter support the hypotheses that self-compatible taxa show higher inbreeding coefficient and reduced inbreeding depression compared to self-incompatible taxa, but did not support the hypothesis that small pollinators will lead to higher inbreeding coefficient and reduced inbreeding depression compared to larger pollinators. The results obtained here are consistent with previous meta-analyses that focus on F_{IS} and F, although the datasets created here uses more specific and clear classifications for breeding system and pollinator functional groups and it only focus on angiosperms. The variability of inbreeding coefficient and inbreeding depression across taxa within each breeding system and pollinator category suggest that both estimates are species-specific and therefore efforts should be done to evaluated both estimates together. Doing this, would allow to test directly the relationship between inbreeding and inbreeding depression. The reduced number of studies that evaluated or presented both parameters for the same population did not allow to test directly the relationship between the inbreeding coefficient and inbreeding depression and this will be evaluated in the following chapters of my dissertation using species that fit into this variation as my study species.

CHAPTER 2

EVOLUTION OF SELFING SYNDROME AND ITS INFLUENCE ON GENETIC DIVERSITY AND INBREEDING: A RANGE-WIDE STUDY IN OENOTHERA PRIMIVERIS (ONAGRACEAE)

Abstract

Among flowering plants, self-pollination is a viable option for reproduction, despite the potential effects of inbreeding depression. To avoid the effects of inbreeding depression plants have evolved diverse breeding systems to favor outcrossing such as self-incompatibility. Changes in biotic and abiotic conditions can result in selective pressures that lead to a breakdown in self-incompatibility. The shift to increased self-pollination is commonly associated with reduced floral features, lower attractiveness to pollinators, and increased inbreeding. In this study, we examined how changes in breeding system can impact floral traits and population genetic structure in *Oenothera primiveris*. Across its range, this species exhibits a shift in its breeding system and floral traits from a self-incompatible population with large flower to self-compatible populations with small flower size. To test the role that changes in the breeding system and floral traits have on the evolution of selfing syndrome we evaluated floral traits in the field and under controlled conditions, and population genetic parameters using RADseq data. Our results indicate a transition towards the selfing syndrome in *O. primiveris* moving from west to east across its geographic range. This shift includes variation in the breeding system, reduction of floral traits (flower diameter, herkogamy, and scent production), and reduced genetic diversity with increased inbreeding. This shift towards facultatively autogamous self-pollination is also associated with a reduction in pollinator visitation. The observed variation

highlights the importance of range-wide studies to understand breeding system variation and the evolution of a selfing syndrome within a species.

Introduction

The cost of sexual reproduction is a well-established tenet of evolutionary biology (Maynard-Smith, 1978; Otto, 2009; Gibson et al., 2017). Yet for a majority of eukaryotic species, sex and recombination are the predominant form of reproduction (Charlesworth, 1989). Within the plant kingdom, the complete spectrum of sexual expression is found, from separate sexes to parthenogenesis, yet the most common form is hermaphroditism (Charlesworth, 1980). The main cost of sexual reproduction in hermaphrodites is associated with the cost of meiosis (Williams, 1975; Meirmans et al., 2012) and is dependent on the level of relatedness between parents and offspring (Uyenoyama, 1984). Outcrossing is expensive (Fisher, 1941; Barrett and Harder, 1996) due to the need to find a mate, and the fact that only one copy of an individual's chromosomes are passed to the next generation and recombination can break down important gene associations (Maynard-Smith, 1978; Otto, 2009). In contrast, inbreeding reduces the cost of meiosis as the parental genotype makes a higher contribution to the offspring (Uyenoyama, 1984). Species that can self fertilize pass on all their genetic information to the next generation and can also benefit from reproductive assurance (Charlesworth, 1989; Barrett and Harder, 1996). However, this short-term benefit of self-fertilization is offset by the increased potential for inbreeding depression, whereby fitness is reduced due the accumulation and expression of the genetic load (Charlesworth and Willis, 2009; Barrett and Harder, 2017). The avoidance of inbreeding depression is proposed as the main reason flowering plants have evolved a diversity

of breeding systems that promote outcrossing, despite the high cost of sexual reproduction (Stebbins, 1974; Barrett, 2002).

Plant breeding systems (per Neal and Anderson 2005) shape mating patterns within a population by determinating the extent to which selfing occurs (Charlesworth, 2006; Raduski et al., 2012). Self-incompatibility (SI) is one of the most common breeding systems in plants, occurring in over 50% of angiosperm families (de Nettancourt, 1977) and 50% of angiosperm species (Igic et al., 2008). There are two different main mechanisms of homomorphic self-incompatibility, sporophytic and gametophitic, that differ on their genetic basis and recognition site (Takayama and Isogai, 2005; Igic et al., 2008). The extent to which SI is expressed can vary within populations and species (e.g. Stephenson 2000; Nielsen et al. 2003; Theiss et al. 2010) and can be driven by selective forces that promote outcrossing or selfing, which themselves can vary in space and time (Whitehead et al., 2018). The transition to self-compatibility (SC) from self-incompatibility (SI) has occurred multiple times in angiosperms (Stebbins, 1974; Raven, 1979; Igic et al., 2008) and is thought to be facilitated by biotic and abiotic factors that can limit outcrossing. Changes in environmental factors (resources limitation, extremes in temperature, precipitation, etc.) and other selective pressures that can lead to changes in demographic patterns, reduction of population sizes, increased fragmentation, and isolation (Stephenson, 2000; Busch and Schoen, 2008; Voillemot and Pannell, 2017b) can limit opportunities for outcrossing. In addition, pollinator and pollen limitation and the subsequent reduction of available mates can also reduce the frequency of within-population outcrossing (Byers and Meagher, 1992; Burd, 1994; Schierup, 1998; Busch and Schoen, 2008). Under these conditions, individuals with leaky SI or a SC breeding systems can reproduce via self-pollination, and thereby have a fitness advantage over those that are strictly SI that need outcross pollen to reproduce. While the ability

to self-pollinate provides reproductive assurance under less than ideal environmental conditions (Charlesworth, 2006), selfing can also result in increased rates of inbreeding (Cheptou, 2019). The evolutionary conflict created between the advantage of self-fertilization for reproductive assurance in the short-term (Charlesworth, 2006) versus the disadvantages of increased inbreeding over longer evolutionary time frames is often referred as an evolutionary dead-end (Stebbins, 1974; Takebayashi and Morrell, 2001; Igic and Busch, 2013; Wright et al., 2013; Cheptou, 2019).

Increased selfing is commonly associated with highly reduced morphological features and lower attractiveness to pollinators and is referred to as the selfing syndrome (Darwin, 1876; Ornduff, 1969; Snell and Aarssen, 2005; Sicard and Lenhard, 2011; Shimizu and Tsuchimatsu, 2015). Overall, morphological changes associated with the selfing syndrome include reductions in flower size (Sicard and Lenhard, 2011; Duncan and Rausher, 2013b; Summers et al., 2015; Tedder et al., 2015), lower production of resources such as nectar (Sicard and Lenhard, 2011), pollen (Tedder et al., 2015), and scent (Raguso et al., 2007; Sicard et al., 2011; Doubleday et al., 2013) and changes in the intensity of petal colors (Button et al., 2012; Duncan and Rausher, 2013a). A classic example of selfing syndrome is the self-compatible species, *Capsella rubella*, which has reduced flower size and increased selfing rates compared with a self-incompatible, closely related species, *Capsella grandiflora* (Foxe et al., 2009; Eckardt, 2011; Sicard et al., 2011). Similar patterns and changes has also been found within the species *Camissoniopsis cheirantifolia* and *Oenothera flava* along their range of distribution (Dart et al., 2012; Summers et al., 2015; López-Villalobos and Eckert, 2018) suggesting that intraspecies evolution of the slefing syndrome can also occur. An important change in floral morphology that facilitates self-pollination is the reduction in herkogamy, the spatial separation between the anthers and stigma

(Webb and Lloyd, 1986; Bodbyl Roels and Kelly, 2011; Opedal, 2018; Cheptou, 2019). The ability to self-pollinate due to the proximity of anthers and stigma provides reproductive assurance under less than ideal environmental conditions, at the same time can increase inbreeding levels in the population (Charlesworth, 2006; Cheptou, 2019) and has been documented in other species (Duncan and Rausher 2013a,b).

Once self compatibility has evolved in a population, the frequency of outcrossing and selfing will vary depending not only on the biotic and abiotic factors acting on the population but also on the frequency of SC individuals in the population (Vallejo-Marín and Uyenoyama, 2004; Porcher and Lande, 2005; Busch and Schoen, 2008). Self-incompatible populations, are obligated outcrossers that rely on pollinator visitation for reproduction (Schoen and Lloyd, 1992), experiencing higher outcrossing rates under favorable biotic and abiotic conditions. As the number of SC individual's increases, populations are likely to transition to a mixed mating system, depending on the frequency of individuals that can self and that actually experience self-pollination. The extent to which self-pollination occurs will be determined by demographic factors (such as population size, density, etc.), pollinator limitation, pollen limitation, level of relatedness among individuals and the ability for autogamous reproduction (Karron et al., 1995, 2012; Goodwillie et al., 2005; Busch and Schoen, 2008; Eckert et al., 2010; Devaux et al., 2014; Voillemot and Pannell, 2017b; Whitehead et al., 2018). Together the frequency of SC individuals present in a populations and the rate of selfing will dictate the evolutionary trajectory of the population and will influence on population level changes on floral traits and in their genetic composition (Eckert et al., 2010; Wright et al., 2013; Cheptou, 2019).

Increased selfing can lead to genetic consequences, including a reduction in genetic diversity (Grueber et al., 2008; Tedder et al., 2015) and increased genetic differentiation among

populations (Barrett and Harder, 1996). These genetic changes can be accompanied by an increase in the expression of genetic load (deleterious alleles) in inbred individuals compared to outcross individuals (inbreeding depression) which can influence many aspects of reproduction and survival (Charlesworth and Willis 2009; Barrett and Harder 2017). The expression of the genetic load in inbred individuals might allow for the purging of deleterious alleles over time, after which the overall fitness of inbred individuals may increase (Crow, 1970; Dudash and Carr, 1998; Crnokrak and Barrett, 2002). However, before purging occurs, the fitness consequences of increased selfing will affect population viability and long-term persistence which will ultimately determine the extent to which the population can recover from a purging event (Frankham 2005). Furthermore, the consequences of selfing on genetic diversity and inbreeding will depend on many interacting factors, including life-history traits (Duminil et al., 2009) environmental conditions (Keller et al., 2002), population size (Angeloni et al., 2011b), and the number of founders and population growth rates (Biebach and Keller, 2010).

To date, most studies that evaluate changes in breeding system focus on comparisons between closely-related species or taxa that differ in key characters of interest (Charlesworth, 2006). However, a range-wide, multi-population assessment within the same species can provide a more robust understanding of the transition from SI to SC and the associated changes in mating system, floral traits, and genetic diversity that led to the evolution of the selfing syndrome (Foxe et al., 2010). In this study, we investigated the transition from mainly outcrossing (SI) to increased selfing (SC) and to the selfing syndrome across the range of the desert evening primrose, *Oenothera primiveris* A. Gray (Onagraceae). This species exhibits population-level variation in breeding system and floral traits important in pollinator attraction (flower size, herkogamy and scent production). Prior work on this species (Wagner, 2005) reveled that SI

occurred in the western portion of the range, where populations have large flowers. Populations in the center of the range have both SI and SC individuals and large flowers, and populations in the eastern portion are characterized by small, SC flowers. It is known that SI systems in other members of the family are variable and that leaky SI (Raven, 1979) and autogamous self-pollination has evolved in some taxa, but in Oenothera nearly all species are SI except for 7 of the 151 species that have shown to be both SI and SC (Klein 1964, 1970; Steiner and Stubbe 1984; Theiss et al. 2010; Wagner 2005).The variation observed across the range in the presence of SI individuals suggest that some populations of *O. primiveris* are transitioning to a SC breeding system. The clinal shift from SI to SC and associated changes in morphology that can facilitate self-pollination in *O. primiveris* provides an ideal system to investigate the evolution and consequences of the selfing syndrome within a species, as well as the importance of both the shift in the breeding system and the changes in floral morphology on patterns of genetic diversity. We test the hypothesis that the shift to the selfing syndrome (decreased herkogamy, reductions in floral traits and loss of SI) will result in higher rates of autogamous self-pollination, increased inbreeding, lower genetic diversity, and greater population differentiation. Floral trait data, pollinator observations, and genetic parameters were measured for naturally-occurring populations and a breeding system assessment was conducted on plants grown under controlled growth-chamber conditions.

Methods

Study system

Oenothera primiveris A. Gray., the only member of *Oenothera* sect. *Eremia*, is a herbaceous annual or short-lived perennial that occurs in small, patchy populations in

sandy/rocky soils of dry washes (ephemeral streams/river systems) in the Mojave, Sonoran and Chihuahuan Deserts of the US and Mexico (Wagner, 2005). Populations that occur in dune systems are large, likely due to the greater availability of suitable habitat compared to those in the center and east of the distribution which occur in sandy/gravel washes that are more limited in size and therefore have smaller population sizes. Fruits are likely dispersed along washes during rain events. Floral traits vary across the distribution, but all flowers are pale yellow with evening anthesis, remaining open until the next morning when they senesce. Populations in the western and central portions of the range exhibit floral traits associated with pollination by hawkmoths (large, fragrant flowers that produce nectar) while those in the eastern portion of the range have flowers with reduced floral characters (small flowers and anthers surrounding the stigma at anthesis), commonly associated with a transition to predominately selfing and are presumed to have little or no cross pollination (Wagner 2005). In addition, a range-wide breeding system assessment showed that SI is restricted to the western-most populations, namely those in Eureka Dunes (Inyo County, California), with other populations elsewhere in the distribution consisting of SI and SC individuals or entirely of SC individuals (Wagner 2005).

Eight populations of *Oenothera primiveris* were included in this project and spanned the geographic range of the species in the United States, representing the expected spectrum of breeding system and morphological variation in flower size (Figure 2.1; Table 2.1). Populations included Eureka Dunes (Pop 1), the only known populations to exhibit completely SI (Wagner 2005), two populations in the center of the distribution with large flowers (Pop 2: Nipton Road., Pop 3: T-Bone Hill) and five populations in the eastern area of the distribution with small flowers (Pop 4: Hackberry Road, Pop 5: Whetstone Mountains, Pop 6: Dona Ana, Pop 7: White Box Canyon, and Pop 8: Aguirre Springs). Census population size was estimated in each

population, by an eye estimation of number of plants present and classified into large (> 250 plants), medium (~100 – 250 plants) and small (<100 plants) (Table 2.2). For the year of collection, Pop 4 and 3 were classified as having large population size, while Pop 1 and 2 were considered to have a medium size and Pop 6 and 7 were considered small. Voucher specimens from each population were deposited at the Nancy Rich Poole Herbarium (Chicago Botanic Garden, CHIC) and the United States National Herbarium at the Smithsonian Institution (US). Data collected for each population varied due to the limited number of available individuals flowering at the time of visit. Information about the specific data collected for each population is described below and summarized in Table 2.1.

Floral traits in natural populations

Floral traits and pollinator visitation rates were measured in 5 of 8 natural populations in March and April 2015 and March 2016 (Table 2.1). In the Eureka Dunes population (Pop 1), two populations in the center of the distribution (Pop 2 and 3) and 2 populations in the east (Pop 4 and 5). Populations in the east of the distribution (Pop 4 and 5) were small, which limited data and sample collection. Floral morphology was collected on 9 to 30 individuals/population. Flowers were excised from the plants at the base of the ovary and the following morphological traits were measured (to the nearest 0.01mm using digital callipers) on one flower per plant: corolla diameter, floral tube length, floral flare and herkogamy (stigma-anther separation). Corolla diameter was measured in two dimensions, along the two longest petal axes in the same plane, perpendicular to the hypanthium. Floral tube length was measured from the top of the ovary to point of sepal insertion at the end of the floral tube. The floral flare was measured as the width of corolla opening. Herkogamy was calculated from separate measures of style and

filament lengths. Because filaments are adnate to the hypanthium, filament and floral tube lengths were summed and subtracted from the style length to calculate herkogamy. Floral morphology was also measured on a subset of plants grown under controlled conditions (Breeding System Assessment, below) and compared to measurements in natural populations.

Floral scent was collected in situ on 9 to 30 individuals/population from 5 populations using dynamic headspace collection methods (Raguso and Pellmyr, 1998) at floral anthesis (Galen et al., 2011). Measurements of floral morphology and floral scent were collected from the same individual flowers. We sampled one flower per plant and collected floral scent immediately after anthesis, between 18:00 and 20:00 hrs. Each flower was enclosed in a Reynolds (nylon resin) oven bag (12 x 15 cm, 270 ml volume) and affixed to the floral stem with plastic ties. Floral volatiles were collected in a cartridge containing an adsorbent material (10 mg of 80–100 mesh Super Q, Alltech Associates, Waukegan, Illinois, U.S.A.), packed into a Pasteur pipette with silanized glass wool. Air from the floral headspace, concentrated in the enclosing bag, was pulled at the flow rate of 200 ml/min through the cartridge using a personal air sampler vacuum pump (Supelco, Berwick, Pennsylvania, U.S.A.). After 60 minutes of sampling, the cartridges were removed and volatiles were eluted with 200 microliters of hexane into Teflon-capped borosilicate glass vials. Samples were stored on ice while in the field and then at -20°C until they were processed to avoid evaporation. Before analysis, we concentrated the samples to a uniform volume of 50 µl using gaseous N_2 and added 5 µl of 0.03% toluene in hexane (= 23 ng) as an internal standard. One µl aliquot of each sample was injected into a Shimadzu GC-17A gas chromatograph equipped with a Shimadzu QP5000 quadrupole, electron ionization (EI) mass spectrometer (Shimadzu Scientific Instruments, Columbia, Maryland, U.S.A.) as a detector. All analyses were made using splitless injections on a polar GC column (diameter 0.25 mm, length

30 m, film thickness 0.25 µm (Econo Cap's carbowax coating, known as EC WAX); Alltech Associates), using ultra-high purity (99.999%) helium as a mobile phase (split ratio 12:1, a constant flow of 1ml/min.). The GC temperature and pressure parameters (injection port temp. 240 °C, detector temp 260 °C, initial temp. 40 °C, hold time 2 min, increased at 15 °C/min to 260 °C, hold time 2.38 min) were optimized to resolve floral volatiles common to Oenothera species with a total run time of 19 min. per sample, allowing us to efficiently process high sample replicates. EI mass spectra (70 eV) were collected from m/z 40-350 (daltons) at a detector voltage of 70 eV, with a scan speed of 1000 and a scan-interval of 0.29 seconds. Compounds were tentatively identified using computerized mass spectral libraries (Wiley Registry of Mass Spectral Data, National Institute of Standards and Technology, and Adams (> 120,000 mass spectra) and were verified whenever possible by comparing mass spectra and standardized retention indices with those of authentic standars. Peak areas were integrated using Shimadzu's GCMSolutions software, were normalized for slight differences in final sample volume using the internal standard. Emission rates were calculated using the internal standard and were expressed as ng toluene equivalents per flower, per hour.

Pollinator observations and assessments of hawkmoth visitation

Pollinator visitation rates were recorded in 4 of the 5 populations for which floral trait data were collected (Populations 1 to 4) and a pollinator was recorded as a visitor if it contacted the petals, anthers or stigma. Pollinator observations were conducted at two time periods, once at dusk just before floral anthesis (between 18:30 and 20:30) for 60 minutes, and again the following morning (between 8:30 and 11:00) before flower senescence for 30 to 60 minutes. During each observation period, between 2 and 4 human observers and/or 2 to 4 GoPro cameras (GoPro Hero, San Mateo, California, USA) simultaneously monitored flowers on 15 to 56 plants.

Red LED lights were used during evening observation periods to minimize disturbance to floral visitors. Pollinators included the hawkmoth, Hyles lineata (Sphingidae), and small solitary bees.

Breeding system assessment

Breeding system assessments were conducted under controlled conditions in growth chambers (Conviron CMP6050) at the Chicago Botanic Garden (Glencoe, Illinois). Plants were grown from field-collected fruits, which were dried to 20% relative humidity and stored at 4°C before germination. Seeds were surface sterilized by submerging for 5 minutes in a 20% bleach solution followed by a rinse in clean deionized water before being placed on Petri dishes with 1.5% agar. Plates were then placed in cold stratification at 4°C for 7 days followed by 5-7 days in an incubator with diurnal cyclic conditions of 25°C for 12 hours (day) and 15°C for 12 hours (night). Seeds were nicked with a forceps to facilitate higher germination success and were then surface-sterilized by submerging in bleach for 5 minutes and rinsed with deionized water before being placed on fresh plates of 1.5% agar with 1 ml of Plant Preservative Mixture (PPM, Plant Cell Technology) (Guri and Patel, 1998). Plates were then returned to the incubator under the same conditions mentioned above and were inspected every three days for germination. Seedlings were planted in a germination potting mix and watered every 3 days for the first three weeks of establishment and then twice a week thereafter. When rosettes reached ~3 cm in diameter, they were transplanted into larger pots (6.4 cm square pots) using 3:1 of regular potting soil and perlite. We aimed for 20 maternal lines for each of the eight populations, although the limited numbers of fruits collected from populations 6 and 7 and poor germination of all populations reduced final sample sizes (Appendix Table S2.1). Floral morphology (see above; floral flare was not measured to avoid breaking the flower and with controlled crosses) was also

measured on the greenhouse-grown plants to determine if patterns observed in the field are maintained under common, controlled conditions.

Breeding system and autogamous self-pollination was assessed once plants reached flowering. Controlled crosses were conducted on newly opened flowers to ensure maximum pollen viability and stigma receptivity. Flowers that were used as pollen recipients were emasculated using forceps prior to hand-pollination to reduce accidental contamination. Within population outcrosses consisted of moving pollen between plants from different maternal lines of the same source population. Anthers were removed from pollen donors using forceps and then used to saturate the stigma of the flower on the recipient plant with pollen. Self-pollination crosses were performed by transferring pollen from the anthers to the stigma of the same flower. Forceps where cleaned with a 70% ethanol solution between crosses to prevent unintentional pollen transfer. Unmanipulated flowers were used to assess autogamous self-pollination. Jewellers tags were used to record cross-type and, once flowers had senesced, a color wire was used to tie the top of the capsule closed to prevent the release of seeds as the fruit matured. Fruits were collected when mature and seed number was recorded. Crosses were considered incompatible if no seeds were produced. To assess SI at the population level, we calculated the Bawa index (Bawa 1974), or the ratio of fruits producing seeds via self crosses to within-population crosses. Populations with a Bawa index of zero were determined to be SI where those with a Bawa index closer to one were deemed SC, while intermediate values can indicate the presence of SI and SC individual within a population. A self-compatibility index (SCI) was also calculated for each maternal line included in the study and for each population (Appendix Table S2.2). SCI was calculated as the ratio of seeds produced by self crosses to seeds produced by within-population crosses for each maternal line (Zapata and Arroyo, 1978; Ruhsam et al.,

2010). SCI was calculated for each maternal line in a populations and then average across maternal lines in the population to obtain a population level estimate. This index represents the variation of self-compatibility across maternal lines of a single population. SCI ranges from 0 to 1, were 0 represents full SI while 1 represents fully SC.

Next-generation sequencing and genetic parameters

Total genomic DNA was extracted from leaf tissue collected in the field following a modified cetyltrimethylammonium (CTAB) protocol developed by Doyle and Doyle (1987). Restriction-site Associated DNA sequencing (RADseq) was used to identify Single Nucleotide Polymorphism (SNP's) across 6 populations of *Oenothera primiveris* (Pop 1, 2, 3, 4, 6 and 7). RADseq allows for a cost-effective random representation of the genome when there is no previous sequence information available (Davey and Blaxter, 2010). The samples were prepared using the RADseq method developed by Elshire et al. (2011). Two genomic libraries of 96 unique barcodes were constructed through the digestion, ligation and PCR of each sample. To avoid any batch effect, each genomic library contained half of the samples from each population and included samples of a species run previously with this technique as a positive control (O. harringtonii). For the digestion, the genome was cut with ApeKI (#R0643, New England Biolabs, Ipswich, MA) and then ligated with oligos, which included specific Illumina primers and a unique barcode. Unique 96 barcodes were obtained according to the specification of the protocol from Integrated DNA technology (IDT Coralville, IA). These barcodes ensured that only fragments containing the specific primers were amplified. Each PCR was carried out independently for all samples, each library was then quantified using High sensitivity QubitTM kit (dsDNA HS Assay Kit, Thermo Fisher Scientific) and then pooled in the final step before sequencing to assure a equivalent amounts of each sample was present in the final genomic

library. Sequencing was performed using Illumina 1.5 at the Center for Genetic Medicine from Northwestern Medicine.

To build loci and detect haplotypes for each individual, we used the denovo.map.pl pipeline of Stacks v1.28 (Catchen et al., 2013). Different combinations of filtering parameter used in Ustacks and Cstacks were tested following the recommendation by Paris et al. (2017). The parameter combination that provided the highest and more conserved number of alleles included minimum depth of coverage to create a stack (m = 3), maximum distance to create a stacks (M = 3) and number of mismatches allowed while creating the catalog (n = 2). We used a subset of individuals with the highest number of reads from each population to build the catalog. Finally, we used populations to identify Single Nucleotide Polymorphism (SNP) restricting the data to only the first locus per read. Only SNPs that were present in 70% of the individuals were considered in the final data set. Genepop and Structure outputs were obtained from Stacks and used for population genetic analysis.

Statistical analysis

A one-way ANOVA was used to assess population-level differences in floral traits from measurements collected in the field and growth chamber: flower diameter (calculated as the mean of the two measurements), herkogamy, floral flare, floral tube length and total scent emission rates. Differences in overall scent composition and morphology between populations were visualized using non-metric multidimensional scaling (NMDS) of Bray-Curtis distance metrics, and statistical differences were determined using non-parametric analysis of similarities (anosim; Clarke 1993). A one-way ANOVA was also used to test for among populations differences in the number of seeds produced. Mean values of autogamous seeds produced were correlated with mean herkogamy and flower diameter of each population, to evaluate if flower

size and herkogamy were correlated with the number of seeds produced through autogamy (values used to perform the analysis can be found in Appendix Table S2.3). We also calculated pollinator visitation rates (number of visits per flower per hour) per population but visits were not frequent enough for statistical analysis.

The following population genetic parameters were estimated in GenAlEx (Peakall and Smouse, 2012): percentage of polymorphic loci (%P), mean number of alleles per locus (N), number of effective alleles (N_A), and observed and expected heterozygosity (H_O and H_E). Inbreeding coefficient (F_{IS}) were obtained through Genepop (Rousset, 2008) and estimations of effective population size (N_E) were obtained in NeEstimator v2 (Do et al., 2014) using the linkage-disequilibrium method. To infer population differentiation between populations we used Bayesian clustering analysis in STRUCTURE (Pritchard et al., 2000). We ran simulations using a model that infers population structure with admixture from 1 to 8 clusters using 100.000 MCMC iterations followed by 100.000.000 burn-in chains for 20 independent replicates. To identify the most likely number of K clusters, delta K was calculated as described in Evanno et al. (2005).

To test the role of breeding system and flower size on genetic diversity, we used the Bawa index of self-incompatibility and mean flower diameter as explanatory variables to evaluate differences between genetic parameters (%P, N, N_A, H_O, H_E and F_{IS}), the number of autogamous seeds produced (mean number of autogamous seeds produced per population), mean herkogamy and the estimated number of population size, N_E (Appendix Table S2.4). We chose Bawa index and flower diameter as the two main traits that vary across populations of *O. primiveris*. All analyses were performed in R version 3.3.3 (R Core Team, 2017).

Results

Floral traits and pollinator observations

Populations of *O. primiveris* sampled in the field showed a bimodal distribution in floral traits and overall scent emission, with populations in the west of the distribution (Pop 1, 2 and 3) showing a two fold difference in floral diameter (mean = 61.63 mm, SE = 1.23), a four-fold difference in herkogamy (mean = 9.81 mm, SE = 0.43), a two-fold differences in floral flare (mean = 4.01 mm, SE = 0.08; Figure 2.3C), longer floral tube (mean = 44.5 mm, SE = 1.37; Figure 2.3D), and a ten-fold difference in scent emission rates (mean = 25,210 ng per flower, SE = 2,785) (Table 2.1) compared to populations on the east of the distribution. Populations in the east of the distribution (Pop 4 and 5) consisted of smaller floral diameter (mean= 33.18 mm, SE = 1.1) with little to reverse herkogamy (mean= 2.06 mm, SE = 0.6), small floral flare (mean = 2.54 mm, SE = 0.11; Figure 2.3C), shorter floral tube (mean = 34.43 mm, SE = 0.62; Figure 2.3D), and lower scent emission rates (mean = 2,769 ng per flower, SE = 398) (Table 2.1). All differences in floral traits between populations in the west of the distribution (Pop 1, 2 and 3) and the east (Pop 4 and 5) were statistically significant (flower diameter: $F_{4,121} = 107.8$, $P < 0.0001$; herkogamy: $F_{4, 114} = 30.24$, $P < 0.0001$; floral flare: $F_{4, 121} = 40.8$, $P < 0.0001$; floral tube: $F_{4, 114} = 77.16$, $P < 0.0001$; floral scent emission rate: $F_{4, 121} = 68.13$, $P < 0.0001$; Figure 2.2A).

Floral traits measured in the growth chamber showed the same pattern as the field-collected data, suggesting that these differences are genetically determined although with less differences than when evaluated in the field. In the growth chamber, floral diameter, herkogamy and floral tube length showed a binomial distribution. Populations on the west (Pop 1, 2 and 3) had less than a two-fold differences in flower diameter (mean = 59.03 mm, SE = 1.21), 1.3 times longer hypanthium (mean = 60.53 mm, SE = 1.37) and a 1.3-fold difference in herkogamy (mean

= 4.3 mm, SE = 0.47) compared to populations on the east of the distribution. Populations on the east (Pop 4, 6, 7 and 8) had on average flowers with smaller diameters (mean = 34.63 mm, SE = 0.62), reduced or reverse herkogamy (Mean = 3.08 mm, SE = 0.2) and shorter hypanthia (mean = 46.55 mm, SE = 0.84). All differences in floral traits between populations in the west of the distribution (Pop 1, 2 and 3) and the east (Pop 4, 6, 7 and 8) were statistically significant (flower diameter: $F_{6, 318} = 69.8$, $P < 0.0001$, Figure 2.3A, herkogamy: $F_{6, 317} = 39.37$, $P < 0.0001$, Figure 2.3C, and floral tube length: $F_{6, 319} = 30.29$, $P < 0.0001$, Figure 2.3D).

Floral scent composition was different between western and eastern populations of *O. primiveris* (anosim R= 0.81, P = 0.001, Figure 2.2B) and was dominated by trans-ß-ocimene, nitrogenous aldoximes (3 methyl butyl aldoxime, 2 methyl butyl aldoxime) and sesquiterpenes (alpha farnesenes and trans-ß-caryophyllene) and emission rates were more than 33-fold higher on average in western populations.

We recorded visits from the hawkmoth, *Hyles lineata*, to all larger flowered western populations (Pop 1, 2, 3) but not in the eastern populations. Visitation rates were highest in Pop 3 (3.88 visits per flower per hour), followed by Pop 2 (2.38 visits/flower/hour) and then Pop 1 (0.277 visits/flower/hour). Small bees were recorded from Pop 3 (0.15 visits/flower/hour) and Pop 4 (0.44 visits/flower/hour).

Breeding system assessment

All seven populations that we examined where determinated to be self compatible with variable Bawa index values demostrating that self-incompatibility is still present in the species (Table 2.1; Appendix Table S2.1). The Bawa index across populations ranged from 0.22 (Pop 1) to 1.24 (Pop 8). Considering the Bawa index of each population and the ratio of self crosses that produced seed, Pop 1can be considered mainly as self-incompatible, it is not exclusively so, as

some individuals within the population produced seeds after self-fertilization (Appendix Table S2.1). The converse case was observed for populations 2 and 3, because not all self-pollination produced seeds (Appendix Table S2.1), these populations are not completely SC.

The self-compatibility index indicated that self-incompatible and self-compatible maternal lines are both present in 5 of the 7 populations examined, indicating that self-compatibility has not been fully established in these populations. While self-incompatibility is not present in the remaining 2 populations. The self-incompatibility index across maternal lines in Pop 1, 2, 3, 4, and 8 ranged from 0 to 1, indicating that individuals within each population can be SI or SC (Appendix Table S2.2). While in Pop 6 and 7, the SCI value across maternal lines ranged from 0.28 to 1 (in Pop 6) and from 0.35 to 1 (in Pop 7), indicating that self-incompatibility is not present in these populations (Appendix Table S2.2). The average SCI value across maternal lines within each population show that SCI varied from 0.127 in Pop 1 to 0.811 in Pop 7 (Figure 2.4). Significant differences were found on SCI among maternal lines across populations ($F_{58, 64}$= 4.3, P= 0.001), and a Tukey post-hoc test show that significant differences are only found between Pop 1 and the rest of the populations while no significant differences were found among the other population comparisons.

Autogamous self-pollination differed significantly between populations ($F_{6, 654}$ =11.34, P < 0.0001). The western-most population (Pop 1) produced no seeds through autogamous self-pollination, while the two populations in the center of the distribution with large flowers (Pop 2 and 3) produced 13 and 2 seeds on average, respectively (Appendix Table S2.3). Populations in the east with small flowers produced a higher number of autogamous seeds, ranging from 20.2 to 26.3 seeds on average (Appendix Table S2.3). Flower diameter (R^2 = -0.854, P = 0.014) and herkogamy (R^2 = -0.968, P = 0.0003; Figure 2.5), showed significant negative correlations with

mean number of seeds produced through autogamy. Populations with large flowers (Pop 1, 2 and 3) produced few or no autogamous seeds compared to populations with small flowers and reduced or reverse herkogamy. Populations with small flowers and higher Bawa index had a higher number of autogamous seeds than populations with large flowers and lower Bawa index ($F_{2, 4}$ =18.55, P = 0.009).

Next-generation sequencing and genetic parameters

Populations of *O. primiveris* showed different genetic diversity estimates across populations. Based on 601 SNP's loci obtained and the genetic patterns observed across populations, results were divided according to flower size. Populations with large flowers (Pop 1, 2, and 3) had on average higher genetic diversity measured as %P, N, N_A, H_O, and H_E than populations with small flowers (Table 2.2). Bawa index and flower diameter were use to assess differences between the genetic parameters estimated. Genetic diversity estimators N, H_E and %P show a significant interaction between flower size and the Bawa index ($F_{3, 2}$ = 214.8, P = 0.004 for N; F3, 2 = 24.6, P = 0.039 for H_E and F3,2 = 217.5, P = 0.004 for %P), while N_A did not ($F_{2,3}$ = 6.13, P = 0.087 for N_A). N, HE and %P estimators were reduced with an increased in the Bawa index and a reduction of floral diameter. Genetic diversity estimators N, H_E and %P also show a significant interaction between flower size and the self-incompatibility index ($F_{3, 2}$ = 1602, P <0.001) for N; $F_{3, 2}$ = 38, P = 0.026 for H_E and $F_{3,2}$ = 1663, P <0.001 for %P), while NA did not ($F_{3,2}$ = 3.3, P = 0.24 for N_A). N, H_E and %P estimators were reduced with an increased in the self-incompatibility index and a reduction of floral diameter.

F_{IS} varied from -0.15 to 0.58 across populations of *O. primiveris*. Populations with large flowers (Pop 1, 2 and 3) had lower inbreeding coefficients than populations with small flowers (Pop, 6 and 7), while the mainly SI populations (Pop 1) had the lowest F_{IS} value. There was no

interaction between the inbreeding coefficient, Bawa index and flower size ($F_{3, 2} = 1.55$, P =0.41). While, there was an interaction between the inbreeding coefficient, self-incompatibility index and flower size ($F_{3, 3} = 16.5$, P =0.02), showing that populations with low SCI and bigger flowers had lower F_{IS} compared to populations with high SCI and small flowers. N_E varied from 3.8 to 47.4 across populations and no interaction was found between N_E, Bawa index and flower size ($F_{2,3} = 4.12$, P =0.20 for N_E) or between N_E, self-incompatibility index and flower size (F3,2 = 4.9, P =0.17 for N_E). While all populations evaluated showed low values of N_E, populations with large flowers (Pop 1, 2 and 3) had higher values of N_E than populations with small flowers. One of the evaluated populations, Pop 4 had the lowest estimate of N_E, but also a large census population size and was the only population with a negative F_{IS} value (N_E: 7.6, F_{IS}: -0.15).

The high likelihood found using ΔK statistics revealed a clustering of individuals into 2 groups (Figure 2.6), with some individuals showing genetic differentiation but without an obvious pattern of the differentiation across populations. Because an incipient peak was also observed at ΔK = 3 we considered the differentiation between populations using this information, showing that populations with larger flowers are differentiated from those with small flowers (Figure 2.6). The pattern observed in the results from STRUCTURE was also supported through the F_{ST} values across populations indicating that populations with large flower have an insipient genetic differentiation from populations with small flower size (Mean $F_{ST} = 0.18$). Genetic differentiation among populations with large flower size was low (Mean $F_{ST} = 0.08$) while differentiation among populations with small flower size was slightly higher (Mean F_{ST} =0.14).

Discussion

Our evaluation of floral traits and genetic diversity patterns across the distribution of *Oenothera primiveris* partially supports the hypothesis that populations capable of self-fertilization will evolve to have reduced floral traits, increased autogamous self-pollination, higher inbreeding, and greater population differentiation. Populations of *Oenothera primiveris* show variable rates of self-compatibility, indicating that SI individuals are still be present across populations but at different frequencies. We confirmed the observations by Wagner (2005) that *O. primiveris* populations in the west of the distribution have larger flowers compared with those in the east and that the western-most population, Eureka Dunes, had the highest incidence of self-incompatibility. Moving from west to east across the distribution, populations exhibited higher Bawa index, and a higher self-compatibility index, showing higher success of self crosses, an increase in autogamous self-pollination and a reduction in floral traits important for pollinator attraction, including smaller floral diameter, less herkogamy, smaller floral flare, reduced hypanthium length, and lower floral scent emission rates. Interestingly, these changes were not exclusively associated with changes in the breeding system. The shift from large to smaller flower size in eastern populations was associated with low pollinator visitation rates and higher autogamous seed set. Hence it was not surprising that the reduction in floral display, and not the changes in breeding system, was associated with reduced genetic diversity, increased inbreeding, and higher genetic differentiation. These changes documented across the range of *O. primiveris* are consistent with the expectations of an evolution toward the selfing syndrome, from mainly self-incompatible to self-compatible populations and associated mixed mating, the reduction of floral traits, and reduced genetic diversity and increased differentiation (Sicard and Lenhard, 2011; Duncan and Rausher, 2013b; Shimizu and Tsuchimatsu, 2015; Tedder et al., 2015).

The results of our hand pollinations and unmanipulated autogamous treatments document a variable breeding system in *O. primiveris* as successful self-pollination occurred in all populations but to varying degrees across maternal lines. SI individuals appears to be maintained to a higher degree in western populations. Pops 1, 2, and 3 show reduced seed production after self-pollination (less than 50% successful) compared to populations with small flower size (more than 70% successful). Pop 1 represented the lowest rates of successful self-pollination (11%) and lower number of seeds produced after self pollination (Average= 2.7), suggesting a higher frequency of SI individuals present in the population than in the other populations investigated. In addition, these results demonstrate that Pop 1 is not exclusively SI, as reported by Wagner (2005). This variation suggests that while some populations can be considered primarily to be self-compatible there are still self-incompatible individuals in these populations. Variation in breeding system within species and populations has been documented in a variety of angiosperms (Tsukamoto et al., 2003; Voillemot and Pannell, 2017b; Shao et al., 2019) and while common in the Onagraceae, it is known for seven species of Oenothera, (Klein, 1970; Straley, 1977; Raven, 1979; Erich Steiner and Stubbe, 1984; Wagner, 2005; Theiss et al., 2010). For example, variable rates of self-compatibility within and among populations have also been found in *Oenothera pallida* subsp. *pallida* and across populations of *Oenothera californica* subsp. *californica* showing a wide range of variability in their Bawa index (Theiss et al., 2010). The variation of SI across populations might reflect an evolutionary mating strategy in the face of outcrossing limitations (Porcher and Lande, 2005; Busch and Schoen, 2008) and might be common in many taxa with SI systems.

Breeding and mating systems determine the extent of selfing occuring in a population (Charlesworth, 2006; Raduski et al., 2012) and mixed mating is an important dynamic in

populations that are SC (Goodwillie et al., 2005). The increase frequency of SC individuals across population of *Oenothera primiveris* along with subsequent selective forces favoring selfing, are likely promoting a mixed mating within these populations. Resulting in the the observed patterns of reductions in floral traits and genetic parameters, particularly in populations in the east. For example, the occurrence of populations with both SI and SC individuals but no measurable morphological or genetic changes (Pops 1 - 3) suggest that outcrossing has a selective advantage over self-pollination in these locations. Populations of Camissoniopsis cheiranthifolia also show variation on breeding system and flower size across its range, associated selfing rates were low in populations that are SI with large flowers but increases with higher frequency of SC in the populations and changes in flower size (Dart et al., 2012). The maintenance of breeding system variation within and between populations suggests there are conflicting selective forces acting to maintain this polymorphism (Fisher, 1958; Byers et al., 2005, Raduski et al. 2011) that differ across the range of *O. primiveris*. Wagner (2005) suggested that the variation on the breeding system in *Oenothera primiveris* depended on the frequcency of SC individuals in the populations and in the establishment of SC individuals in new habitat on the east. From an ecological perspective, the evolution of selfing allows for reproductive assurance when when populations are small and pollinators are limited (Fausto et al., 2001b). Furthermore, when selfing rates are variable, mixed mating can be evolutionarily stable (Holtsford and Ellstrand, 1990; Rausher and Chang, 1999).

Shifts in breeding system are common with environmental changes and can occur more than once within a species or even within populations of the same species (Charlesworth, 2006; Igic et al., 2008; Shao et al., 2019). In addition, self-compatibility and reproductive assurance can facilitate range expansion, especially for populations initiated with only a few individuals

(Baker, 1955; Husband and Barrett, 1991; Cheptou, 2012). As SI is likely the ancestral state (Raven, 1979) in Onagraceae, the loss of SI in *O. primiveris* was likely the first step towards the evolution of the selfing syndrome. Indeed, Wagner (2005) suggested that SC and progressive autogamy were favored with the spread into desert habitats. Populations in western extent of the distribution are large and occur on sand dunes, habitat that is more continuous and larger in scope than the dry desert washes where populations occur in the rest of the distribution. The movement to a habitat that is smaller in size, with more frequent disturbances (rain events that can wash away individuals on a regular basis) results in a different demographic landscape. Under these circumstances, populations are likely too small to maintain sufficient S-allele diversity for compatible mating events to occur (Busch and Schoen, 2008). This demographic change could also be related to lower pollinator services in small or fragmented populations, resulting on a reduction of seed production (Allee effect) (Lamont et al., 1993; Groom, 1998). Conditions that will favor reproductive assurance and changes in morphological traits that could lead to an increase in seed set and a reduction of traits to attract pollinators. The demographic change from one ecosystem to another was likely an intial driver in the loss of SI in this species (Wagner, 2005), as well as remaining an important factor in the distribution of SI individuals in contemporary populations as fluctuations in population size are a defining future of most populations. Indeed, populations of *O. primiveris* have overall lower effective population sizes, especially compared with other more widespread (Suárez-Montes et al., 2016). Similar patterns of high census size but low effective population size could be found in other annual species that live in similarly environmental conditions to *O. primiveris*, where favorable environmental conditions show increased population size in the population without reflecting on levels of genetic diversity or effective population sizes (Husband and Barrett, 1992; Ellstrand and Elam,

1993). Smaller effective population sizes, as found in the eastern populations studied, suggest that range expansion may have been facilitated by reproductive assurance. This is supported by one of the center populations (Pop 4) that had the lowest effective population size and a negative FIS, but the highest census population size, which suggest that this population experienced a rapid expansion after a bottleneck (Grueber et al., 2008). While demographic factors may explain the transition towards a SC breeding system and small effective population sizes, they alone do not explain the reduction in floral traits and increase in autogamous self-pollination observed across the distribution.

Consistent with the evolution of the selfing syndrome, we found morphological changes that facilitate an increase in the frequency of self-pollination and are less attractive to pollinators, both contributing to reduced outcrossing events. Populations in the east had smaller floral diameters, reduced or no herkogamy, lower scent emission rates, and were capable of producing high seed numbers through autogamy, which was also reflected in the high values of the inbreeding coefficient in these populations. Of the morphological changes, is likely that the reduction in herkogamy was a critical step towards increased autogamous seed set (Opedal 2018). As seen in other species (e.g. *Ipomoeae lacunosa*, Duncan and Rausher, 2013a, *Camissoniopsis cheiranthifolia*, Dart et al. 2012, *Arabis alpine*, Tedder et al. 2015; *Linaria cavanillesii*, Voillemot and Pannell 2017), a transition to reduced herkogamy can facilitate an increase in autogamous seed set by increasing selfing rates and inbreeding. Variation in flower size and herogamy suggest a mating system continuum in *Oenothera flava*, were subspecies with reduced herkogamy experienced higher selfing rates than subspecies with herkogamy (Summers et al., 2015). Changes in herkogamy and corolla width lead to variable outcrossing rates in populations of Camissoniopsis cheiranthifolia, were SC populations with reduced flower

size had reduce outcrossing compared to populations with large flower size that were either SI or SC (Dart et al., 2012). These examples support the evidence that selfing rate is most likely determined by morphological modifications rather than transition in breeding system.

Hawkmoths are known to be variable in space and time (Miller, 1981; Campbell et al., 1997) and while they are the assumed the primary pollinator of *O. primiveris*, visitations rates were variable and not frequent enough for statistical analyses, though our observations were limited to just 2 nights and only included the three western-most populations, all of which have large flowers with pronounced herkogamy and high scent emission rates. Despite this, moth scales have been found on stigmas in a population with small flowers in an earlier assessment (Levin and Raguso, pers comm.) and hawkmoths are important pollinators for many taxa in the habitats where *O. primiveris* occurs in the center and east of its range (Sonoran and Chihuahuan Deserts). Sporadic visitation by hawkmoths in populations with small flower size could effectively move pollen within or between populations and maintain genetic diversity within populations, as has been shown for other *Oenothera* species in the Chihuahuan Desert (Lewis, 2015). Increased and consistent pollinator observation effort paired with light trapping surveys of hawkmoths range-wide would provide better comparative data to definitively determine the extent to which pollinator limitation has been an important driver in this system and helps to maintain reduced floral traits, higher rates of inbreeding and low genetic diversity.

While reproductive assurance allows for reproduction under unfavorable environmental conditions and/or in the absence of pollinators (Lloyd, 1979), in the long-run increased selfing can lead to higher inbreeding, reduced genetic diversity, which can lower adaptability to environmental changes, increasing extinction risk (Frankham, 2005; Frankham et al., 2017; Cheptou, 2019). However, self fertilization can have a genetic advantage over outcrossing

because individuals can transmit both copies of their genes to the progeny without the need to finding a mate (Fisher, 1941; Lloyd, 1979). In the short-term, self fertilization increases the expression of the genetic load and in large populations can reduce the effects of inbreeding depression due to selection against these deleterious alleles (purging) (Dudash and Carr, 1998). The limited genetic diversity of the eastern populations of *O. primiveris*, along with the higher levels of historic inbreeding and small census and effective population size, suggests that the eastern populations might be at higher risk of extinction that the populations from the west.

Conclusions

The combination of reduction or loss of self-incompatibility, reduced genetic diversity, increased inbreeding levels, increased autogamous seed set, and reduced floral traits across the range of *O. primiveris* represent many of the traits linked to the evolution of the selfing syndrome. Even though mating system was not directly evaluated here, the increased selfing and reduction of genetic diversity of the populations in the east, suggest that selfing might be the predominant mating system in these populations. Populations in the west show variation in the breeding system from mainly SI to SC with SI individuals, maintained high genetic diversity and floral resources to attract pollinators suggesting that outcrossing or mixed mating might be the predominant mating system compared to the populations in the east. The pattern observed in the western populations suggests that there is conflicting selection for SI within these populations maintaining the polymorphism, likely associated with variability in pollinator community and fluctuation in population size. The changes across the distribution can explain the greater genetic differentiation between east and west populations, differences that can continue to accumulate over the species evolutionary time. The hallmark of a species which has evolved the selfing

syndrome is high levels of inbreeding and low genetic diversity. Although we did see low genetic diversity and high inbreeding coefficient to the east, it remains to be determined if the fitness consequences of these changes will influence long-term population persistence and further evolutionary change. Overall our data suggest that the processes driving this evolutionary shift are likely ongoing and future work in this system, should focus on identifying the relative contribution that demographic factors and pollinator limitation have on the breeding system transition within and between populations of the species.

CHAPTER 3

CAN POPULATION REPRODUCTIVE SYSTEM AND HISTORIC INBREEDING INFLUENCE EXPRESSION OF INBREEDING DEPRESSION? A COMPARISON OF OENOTHERA PRIMIVERIS (ONAGRACEAE) POPULATIONS

"That any evil follows from the closest interbreeding has been denied by many persons, but rarely by a practical breeder, and never, as far as I know, by one who has largely bred animals which propagated their kind quickly…" The Variation of Animals and Plants under Domestication, Charles Darwin (1868).

Abstract

An increase in the rates of inbreeding can lead to a reduction in fitness due to the expression of deleterious alleles previously hidden in the heterozygotes state. The reduction in fitness, known as inbreeding depression, can vary between populations depending on the history of purging and inbreeding. This will depend on the species trait that directly influences the amount of inbreeding. For example, species that are self-incompatible or have a flower morphology that promotes outcrossing, should experience limited inbreeding and therefore limited opportunities to purge recessive deleterious alleles. As a consequence, those populations which do not have a history of inbreeding are expected to show strong fitness reduction when the inbreeding rates increase. In this chapter, I tested how variation on the breeding system and the inbreeding coefficient in *Oenothera primiveris* can impact the expression of inbreeding depression. To evaluate the changes in fitness between self and outcross pollination, I performed controlled crosses across 7 populations of the species in the growth chamber and evaluated fitness in the greenhouse. The results of this chapter do not support the hypothesis that

populations with self-incompatible individuals will have higher inbreeding depression than self-compatible populations. Populations that maintained a higher frequency of self-incompatibility showed reduced inbreeding depression in the first-generation compared to populations that have a reduce frequency of self-incompatibility. This difference may be explained by recent purging in populations with self-incompatible individuals therefore reducing the genetic load. While populations that are self-compatible might express a fitness decline due to deleterious alleles of mild effect which are harder to eliminate from the population or due to the fixation of deleterious alleles due to the effects of drift. The results obtained here suggest that inbreeding depression is a population-specific trait that needs to be evaluated to obtained accurate results.

Introduction

The negative impacts of inbreeding on fitness have been known for some time by Darwin (Darwin, 1876). The reduction in fitness associated with increased inbreeding is attributed to the expression of recessive deleterious alleles, and is known as inbreeding depression (Lloyd, 1979; Lande and Schemske, 1985; Schemske and Lande, 1985). The two main factors which will determine if a population will experience inbreeding depression are the standing genetic load of the population, and changes in the levels of inbreeding. Genetic (or mutational) load describes the frequency of recessive deleterious mutations present in the genome, maintained in their heterozygous state (Crnokrak and Barrett, 2002; Haliburton, 2004; Wright et al., 2008), while an elevation in the levels of inbreeding can occur through changes in population size, fragmentation, and shifts in the mating system (Ouborg and Treuren, 1995; Reed and Frankham, 2003; Leimu and Mutikainen, 2005). The impact of these changes on inbreeding

and therefore the expression of inbreeding depression will depend on the amount of genetic load present in a population.

Inbreeding, often simplified as the mating between relatives, is the increased likelihood that two allele that are identical by descent meet in an individual (e.g. Charlesworth and Charlesworth, 1987, 1999; Keller and Waller, 2002; Frankham, 2005). The probability that two alleles are identical increases when mating between relatives and when there is a reduction in the gene pool associated with a decrease in effective population size (Frankham et al., 2002). The most accepted theory on how increased inbreeding can reduce fitness is known as the dominance hypothesis (Charlesworth and Charlesworth, 1999; Charlesworth and Willis, 2009; Larsen et al., 2011; Hedrick et al., 2016), which predicts that an increase in homozygosity can lead to the expression of the genetic load (Keller and Waller, 2002; Hedrick et al., 2016). Genetic load is usually hidden (in the heterozygous state) and subsequently expressed infrequently in a large and genetically diverse population (Pekkala et al., 2012), however, when populations become small, an increase in inbreeding can allow these deleterious alleles to come together as homozygotes and therefore expressed more frequently in the population. Consequently, the average fitness in small populations is expected to decrease from generation to generation as the level of inbreeding (homozygosity) increases (Crow and Kimura, 1970; Keller and Waller, 2002; Pekkala et al., 2014). If the fitness reduction observed is strong or has severe consequences for the fitness of the next generation, then selection will act, eliminating these alleles from the populations (otherwise known as purging). Purging, will result in a fitness rebound of the population in subsequent generations (Crow, 1970; Crnokrak and Barrett, 2002), although the fitness levels do not necessarily return to pre-inbreeding conditions. The restoration of fitness depends not only on the intensity of inbreeding but also on the type of deleterious alleles. Inbreeding depression

due to strongly deleterious alleles (such as lethal or semi lethal) will be eliminated quickly from the population while alleles which are only mildly deleterious can be difficult to purge (Crnokrak and Barrett, 2002). The degree of fitness loss from inbreeding depression is likely to be the product of both allele types acting and the history of inbreeding within the population (Dudash et al., 1997; Picó et al., 2004; Angeloni et al., 2014).

In plants, inbreeding depression has been studied extensively, comparing populations or species with contrasting population size (Newman and Pilson, 1997), breeding systems (Vogler et al., 1999; Glemin et al., 2001; Busch, 2005b; Voillemot and Pannell, 2017a), mating systems (Kalisz, 1989; Dudash and Carr, 1998; Goodwillie, 2000; Fishman, 2001; Porcher and Lande, 2016), and under different environments (Dudash, 1990). From these studies, we know that inbreeding depression can be associated with different plant traits, especially those associated with increased rates of inbreeding. These traits allow us to speculate about a species previous experience with inbreeding and purging (Angeloni et al., 2011a). However, these studies have also demonstrated that variation in responses exists within and between populations. Considering that inbreeding depression is usually evaluated on a few populations that represent the extreme variation of a trait (for example, large versus small population sizes, or outcross versus selfing mating system, etc.), it is difficult to predict how inbreeding depression might vary across populations that do not represent the extremes. Understanding inbreeding depression across populations that share characteristics and not only the extremes representation of a trait can help us better understand the impact inbreeding depression have across populations of a species.

Plant breeding systems can be described as the collection of physiological and morphological traits that determine the likelihood that any two gametes will unite (Raduski et al., 2012). The breeding systems (dioecy, heterostyly, and self-incompatibility) can shape mating

patterns within a population, promoting outcrossing, and therefore influence the amount of selfing that occurs (Charlesworth, 2006). Self-incompatibility, where a plant will not accept self or related pollen, is one of the more common breeding systems among angiosperms. Self-incompatibility, while desirable in large populations with abundant pollinators, will breakdown when reproductive assurance is low. Comparisons of inbreeding depression between species or populations with contrasting breeding systems have shown that populations that can self-fertilize (SC) will generally exhibit lower levels of inbreeding depression due to purging (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). The magnitude of inbreeding depression is lower in self-compatible populations (*Linaria cavanillesii*, Voillemot and Pannell, 2017 and *Leavenworthia alabamica*, Busch, 2005), and populations with weaker SI (*Campanula rapunculoides*, Vogler et al., 1999). Although some studies have found no differences in inbreeding depression among populations (Rathcke and Real, 1993).

The expression of inbreeding depression will vary with life stage evaluated (Husband and Schemske, 1996; Angeloni et al., 2011; Winn et al., 2011) and across populations (Byers and Waller, 1999). It is expected that species with higher frequency of inbreeding will often display inbreeding depression in later life stages rather than in early stages, while in mainly outcrossing species it is often expressed in both early and later stages (Husband and Schemske, 1996; Angeloni et al., 2011a; Winn et al., 2011). This highlights the importance of measuring multiple life-stages to estimate overall fitness, as a more accurate estimate of inbreeding depression (e.g. Dudash, 1990). Similarly, inbreeding depression varies across population depending on their genetic load and the history of inbreeding in the population. The genetic load can vary by population depending on their origins and if they have experienced purging in the past (Dudash et al., 1997; Picó et al., 2004; Ouborg et al., 2006). Populations with lower background levels of

inbreeding (F_{IS} is low) might have expressed their genetic load less frequently than those with a higher F_{IS} (> 0.25), and had less opportunity to purge. However, purging can be unreliable and inconsistent in natural populations (Byers and Waller, 1999; Crnokrak and Barrett, 2002), and will likely depend on those factors that influence inbreeding rates (Pekkala et al., 2012) such as population size, historic bottlenecks, pollinator services, breeding system, etc. In large outcrossing populations, if inbreeding depression is expressed in a few individuals, there is no noticeable decline in the population fitness (Ellstrand and Elam, 1993; Byers and Waller, 1999). In species with a limited number of populations and small population sizes, the reduction in fitness will have a greater impact (Frankham et al., 2002; Frankham, 2005, 2015) and is recognized as one of the factors contributing to population extinction. Considering the possible variation when evaluating inbreeding depression among populations or by different life-stages it is important to evaluate inbreeding depression on different species, across multiple populations, and at different life-stages (Byers and Waller, 1999).

In this chapter, I compare the impact of breeding system and historic inbreeding associated with increased autogamy, on inbreeding depression within the species, *Oenothera primiveris*. I will do this by evaluating multiple life-stages and populations that show variation on breeding system, flower size and history of inbreeding. In the previous chapter, I demonstrated that populations of the species show variable breeding system, where both SI and SC individuals are present in the population but at different rates. Populations also show variation on the inbreeding coefficient, genetic diversity, and flower size that influence the amount of autogamous selfing a population experience. Based on these differences, I propose to evaluate inbreeding depression in these populations and see how it relates to the different traits expressed across the populations. In doing so, I will test the following hypothesis, (1) that

inbreeding depression will be determined by breeding system, where individuals that have a greater capacity to self (SC populations) will exhibit less inbreeding depression, due to previous purging of the genetic load. (2) Inbreeding depression across populations will differ depending on the population traits associated with elevated rates of self-pollination (flower size). (3) That inbreeding depression will be related to the history of inbreeding in the population, as reflected in the inbreeding coefficient (F_{IS}). And Finally, (4) I propose that as genetic diversity and effective populations size are associated with historic bottlenecks, and therefore potential genetic load, they will be correlated with the population level of inbreeding depression. Overall, this chapter will provide information about the relationship that plant traits and population history have on the expression of inbreeding depression.

Methods

Study system

Oenothera primiveris is an annual or short-lived perennial species with a wide distribution, spanning the three North American deserts. Populations across the range exhibit variation in the breeding system, flower size, and levels of inbreeding (Chapter 2), with populations to the west having larger flowers, higher frequency of SI individuals, and reduced inbreeding coefficient compared to those in the east (Table 3.1). Seeds were collected from seven populations that span the range of observed variation recorded for the species. Sampling included three populations with large flower sizes, which also had reduced inbreeding coefficient (Pop 1, 2, and 3). These three populations also had a mixed breeding system with Bawa index ranging from (0.22 - 0.74) and self compatibility Index (SCI) from 0.13 - 0.39, suggesting a higher ratio of SI/SC individuals. The four remaining populations have reduced flower size and variable level

of inbreeding, had a higher Bawa Index (0.73 - 1.24) and SCI (0.67 - 0.81) suggesting a lower ratio of SI/SC individuals.

Establishment of controlled crosses (Generation 0)

Mature fruits were haphazardly collected from seven populations, were dried, then seeds were removed from fruits and stored in cold conditions (4°C). Seed grown from each pod were assigned the same maternal line, which varied from 5 to 24 (average = 13.29; Table 3.2). The reduced number of maternal lines in some populations (Pop 6 and 7) were due to the limited number of fruits found in natural populations. Populations of the species can be found in desert washes, where they are carried away or deposited under sand making them difficult to find. Seeds were germinated under the experimental conditions described in chapter 2 where germination was facilitated by nicking the seed coat, before being plated out on agar plates. Seedlings were then transplanted to a mix of 3:1 of regular potting soil and perlite and maintained in the growth chamber (Conviron CMP6050) until fruits maturation. Between 5 and 15 of the maternal lines grown produced flowers and were used to perform controlled crosses (average = 10.42; Table 3.2).

To generate lines to evaluate inbreeding depression, flowers were either selfed, using pollen from same flower, or crossed to another maternal line from same population. No crosses were conducted between individuals of the same maternal line (biparental). Due to low number of flowers per plant, and non-synchronous flowering, cross treatments were not able to be performed on the same individual (Table 3.2). All crosses were conducted using forceps to transfer the pollen to the recipient's maternal plant stigma. The forceps were cleaned after each cross using a 70% ethanol solution to prevent movement of unwanted pollen. As flowers of *Oenothera primiveris* open at dusk and senesce the next morning, we changed the day-night

conditions in the growth chamber (Conviron CMP6050) so that crosses could be conducted on recently opened flowers during the daylight hours. The crosses done on any given day depended on which flowers recently opened, favoring whenever possible within-population outcross. After a cross was performed, color wired was placed over the developing fruit to keep track of the cross and allow it to develop until collection without losing seeds. Flowers that were missed or had pollen covered stigma upon opening, were kept unmanipulated and used to evaluate autogamous self-pollination. As the degree of self-compatibility varied across population this limited the self-pollination treatment, especially in Pop 1, 2 and 3, hence I augmented this treatment by using seeds obtained through autogamous self-pollination whenever possible. After pollination, the fruits were allowed four to eight weeks to mature depending on their size, then they were collected and placed in coin envelopes and stored at 4°C until the seed number was counted and then seeds were stored at 4°C.

Measuring inbreeding in first generation

Seeds were sterilized in a solution with 5% bleach with 5µL of 10% solution of TWEEN soap and maintained there for 24 hours before plating in 1.5% agar plates. After 24 hours, seeds were washed with distilled water and put in 1.5% agar plates. For each cross, we aimed for 25 seeds, but low seed set meant some treatments were as low as 3 seed, (Average = 20.31). Seed numbers were most limited in populations were self-incompatibility alleles are present. Plates were maintained in cold conditions (4º C) for 7 days followed by 5-7 days in an incubator with diurnal cyclic conditions of 25°C for 12 hours (day) and 15°C for 12 hours (night), this protocol allowed the seeds to imbibe water and facilitate the removal of the seed coat without damaging the embryo. The seed coat was then nicked using forceps to check for endosperm and the number of viable seeds were counted and placed on fresh plates of 1.5% agar with 1 ml of Plant

Preservative Mixture (PPM, Plant Cell Technology) (Guri and Patel, 1998) to prevent pathogens growth.

Germination was recorded every three days and seedlings were moved to the soil immediately. Seedlings were planted in a germination potting mix and placed in the growth chamber at the Chicago Botanic Garden. The growth chamber was set at 25ºC for 12 hours (day) and 20ºC for 12 hours (night). Seedlings were watered every 3 days for the first three weeks of establishment and twice a week thereafter. After 2.5 weeks no new seedlings emerged but plates were maintained in case new germination occurred. Early survival was evaluated after 4 weeks in the germination trays and before moving plants to bigger pots. Plants were up-planted into larger pots (6.4 cm square pots) using 3:1 of regular soil and perlite. Plants were then haphazardly placed between the trays and moved to the greenhouses at the Chicago Botanic Garden. In the greenhouse, plants were watered twice a week and allowed to grow for 16 weeks (for a total of 20 weeks in soil), after 16 weeks' survival to flowering and if the plant was alive at the end of the experiment was recorded. Flower number was estimated at the end of the 16 weeks in soil by counting the number of flowers, dried ovaries on the plant and number of fruits produced. Fruits produced were collected and maintained in cold conditions. Since no crosses were done in the greenhouse, developed fruits are most likely the result of autogamous self-pollination or potentially outcross by rogue insects. Considering that SI and herkogamy are present in some of the evaluated populations, seed number or fruit traits were not evaluated and were not used as an estimation of fitness. This experiment was repeated twice (Summer-Fall 2019 and Winter-Spring 2020) due to unexpected low viability of the seeds in the first round. The second round of fitness evaluation was done to corroborate some of the crosses evaluated in the first round and if possible, add new crosses.

Life-stages and plant fitness in Oenothera primiveris

Fitness of the crosses grown (Table 3.3) was evaluated at 5 different life stages: seed viability, seed germination, survival after 4 weeks (before moving seedlings to bigger pots), survival to flowering and flower number (evaluated after ~16 weeks of the seedlings in soil). Seed viability was determined by the number of seed which had a white endosperm when the seed coat was partially removed. The number of viable seeds was recorded for each cross and ratio of viable seeds was calculated as the number of viable seeds divided by the number of seeds evaluated. Germination was recorded after radicle or cotyledon emergence and before moving to the soil. The ratio of germinated seeds was calculated as the number of seeds that germinated divided by the number of viable seeds. Early survival was evaluated after 4 weeks in soil, recording the number of plants alive after transplanting. The ratio of plants that survived until this stage was calculated as the number of plants alive divided by the number of seedlings moved to the soil. At the end of 16 weeks, survival to flowering and flower number data were collected. Each plant, alive or not, was evaluated for flower production, recording the total number of open flowers (if the plant was still alive and producing flowers), number of aborted ovaries remaining in the plant or number of developed fruits. Even when not pollinated a dry shrivelled ovary remains attached to the plants so it is easy to count total flowers produced. The ratio of plants that survived to the flowering stage (evaluated after 16 weeks in soil) was calculated as the number of plants that produce flowers divided by the number of plants that were alive at 4 weeks (early survival). The average number of flowers for each cross was calculated as the total number of flowers produced for the cross divided by the number of plants that survived to the flowering stage.

Analysis

Inbreeding depression calculations

Inbreeding depression was calculated for seed viability, germination, early survival, survival to flowering, average flower number and cumulative fitness. I calculated inbreeding depression following the recommendations of Agren and Schemske (1993) using the following equations where WS is the fitness of the self crosses and WO represent fitness of the outcross crosses.

Equation 1: Inbreeding depression when $W_O > W_S$

$$ID = 1 - \frac{W_S}{W_O}$$

Equation 2: Inbreeding depression when $W_O < W_S$

$$ID = \frac{W_O}{W_S} - 1$$

Test differences between populations at G0

Fitness measurements obtained in Generation G0 can help us to set a baseline of fitness using wild seeds, and evaluate if there is a difference in the next generation by crosses performed. Since this was not the initial goal for the chapter, only limited fitness data were collected for generation 0, as I did not evaluate fitness as extensively as in generation 1. Seed viability and seed germination were recorded for up to 15 maternal lines per population (except for Pop 6 and 7 that had 6 and 5 maternal lines respectively) and used to established generation 1. To analyze these data, I used a generalized linear model using a binomial distribution, to test differences across populations of *Oenothera primiveris*.

Influence of population and breeding system on the expression of inbreeding depression

As fitness data were collected in separate experiments, 2019 and 2020, I performed an analysis of variance using cumulative fitness and year of collection to test if there were significant differences between the year of collection. If there were no significant differences, I pooled the data. I evaluated differences between the cross types using populations or breeding system as a factor. As some individuals were from the same maternal line (G0), I used a mixed linear model using maternal line as a random factor to control for differences by maternal line. To evaluate differences based purely on breeding systems, I used the self-compatibility index (SCI) calculated in chapter 2. Each maternal line was assigned a SCI value for each maternal (Appendix Table S3.1), were a SCI value of 0, represented a SI maternal lines, a value of 1, represented a SC maternal line and values in between as maternal lines with variable breeding system. To evaluate differences between breeding system, I used a mixed linear model using maternal line as a random factor. Analyses at each level were done for each life-history stage and cumulative fitness.

As the dataset consisted of individuals were derived from the same maternal line, a fitness average was calculated between cross type by maternal line. By doing this, each trait was continuous and assumed normality (data were also transformed using arcsine) but the results were similar with or without transformation. Cumulative fitness for each maternal line was calculated by multiplying the average of each stage evaluated and if any trait had a fitness value of 0, cumulative fitness for the cross was also zero. All analyses were performed in R version 3.3.3 (R Core Team, 2017).

Life-fitness analysis (ASTER)

ASTER (Shaw et al., 2008) analysis has been identified as the more appropriate test for life-time fitness, as it allows for data that is conditional on previous stages, multimodal distribution with a discrete mode at 0 and highly skewed distributions and therefore not following any traditional parametric distribution. ASTER modeling generates the overall likelihood for a set of components expressed through the lives of individuals (Geyer et al., 2007). This analysis accounts for all fitness components even with different statistical distribution and the dependence of fitness components expressed later in the lifespan based on those earlier. Later life-stages, therefore, depend on the early fitness stages. To analyze the data, I used a fixed effect ASTER model to evaluate the influence of populations and crosses in final fitness. For this analysis, crosses that shared the same maternal line were incorporated together to avoid overrepresentation of the maternal line in the analysis. This analysis was done using the R Package ASTER (Geyer, 2015).

Evaluation of inbreeding depression based on flower size variation, the historic level of inbreeding (F_{IS}) and genetic diversity.

To evaluate differences in the expression of inbreeding depression across populations, I used information about the populations breeding system, floral size and genetic data (genetic diversity, effective population size and inbreeding coefficient) obtained in chapter 2 (Table 3.1). For flower size I used mean flower diameter which was shown to be correlated with low herkogamy and greater autogamous selfing. I used a two-way ANOVA to test if the interaction between flower size and breeding system influences inbreeding depression? . I repeated these analyses using genetic diversity (H_E), inbreeding coefficient (F_{IS}) and effective population size (N_E) obtained in chapter 2, to determine if the observed variation of inbreeding depression was

correlated with inbreeding depression using Pearson's correlation (R). This was done using cumulative fitness and the predicted values of fitness obtained through the ASTER model.

Results

Summary of the controlled crosses established and selected to evaluate fitness

Of the 93 maternal lines that were grown, 78.5% survived to the flowering stage. This represented over 620 individuals, although only 60% of those individuals produced flowers that could be used to performed controlled crosses (Table 3.2). The number of flowers produced varied by population, and these differences are reflected in the number of crosses done for each population. There was as total of 352 controlled crosses performed and near 66% of those crosses resulted in fruits producing seeds. Autogamous self-pollination was recorded for 663 flowers, and around 87% of those flowers produced seeds, which represented 65% of the total number of fruits, followed by self-pollination (22%) and outcross pollination (13%) (Table 3.2). Four populations (Pop 4, 6, 7, and 8) represent nearly 88% of the autogamous seed. In these populations herkogamy is absent, hence pollen can cover the stigma at opening not allowing for crosses to be done in these flowers.

I aimed to grow seeds from 15 to 20 fruits per cross type for each population, although loss of maternal lines, low flower number and lack of synchronous flowering ultimately limited number of successful crosses in some populations (Table 3.2). For two populations (1 and 3) many self crosses were unsuccessful hence all available crosses were used to evaluate fitness. For the remaining populations, I used all available outcrossed lines for the experiment and selected between 15 and 18 of the self crosses or autogamous from different maternal lines within each population.

Fitness differences between the type of cross and populations

No significant differences were found between year of experiment (F= 2.1, P= 0.12) hence data were pooled. Seed viability of selfed treatment varied from 46% to 56%, while in the outcross treatment ranged from 15% to 82% (Table 3.3). No significant differences were found between populations and cross type (Table 3.4). Seed viability of generation G1 evaluated in the greenhouse showed a decline compared to viability in generation G0 (91%, SE= 0.01; Figure 3.1). The proportion of seed germination, early survival and survival to flowering all showed a large range of responses (Table 3.3), but no significant differences were observed between generations, populations or between cross type (Table 3.4). The mean number of flowers produced varied from 0.5 to 6.33 (Table 3.3), did show a significant interaction between population and cross type ($F_{6, 26}$= 2.5, P= 0.048). Similarly, cumulative fitness also showed broad variation in responses, varying from 0.01 to 0.79 (Table 3.5) and also showed a significant interaction between populations and cross type ($F_{6, 26}$= 3.24, P= 0.016). The ASTER model showed a similar response, with the cross type and population having a significant influence on overall fitness (Table 3.7). The range of values from ASTER was similar to the reported for cumulative fitness (Figure 3.2). Inbreeding depression was calculated using both cumulative fitness and ASTER, the values spanned the entire range of possible outcomes from -0.91 to 0.74 with cumulative fitness and from -0.87 to 0.63 with ASTER.

Relationship between Inbreeding depression and breeding system, flower size, inbreeding and genetic diversity.

Estimations of fitness were also compared across breeding system based on the self-compatibility index (SCI). Overall, inbreeding depression values for each trait did not show significant differences between breeding system or cross types, except for cumulative fitness ($F_{2,30} = 3.26$, P= 0.05; Table 3.5), where inbreeding depression was higher in the populations with higher SCI (with higher rate of SC individuals) (Figure 3.3). No significant differences were found between inbreeding depression and average SCI for the population, when using cumulative fitness (F= 2.47, P= 0.18) or ASTER modeling (F= 5.86, P= 0.06) to calculate inbreeding depression. Higher inbreeding depression was associated with higher Bawa index in cumulative fitness (F= 7.78, P= 0.04), the same trend was found with ASTER but with only marginal differences (F= 5.86, P= 0.06). There were no significant differences between either estimates of inbreeding depression and floral diameter (cumulative fitness: F= 1.1, P= 0.34; ASTER modeling: F= 0.51, P= 0.5). There were no significant correlations between inbreeding depression and expected heterozygosity using cumulative fitness or ASTER (cumulative fitness R= -0.32, P= 0.53, Figure 3.4A; ASTER modeling R= -0.3, P= 0.57, Figure 3.4C). Similar results were found with effective population size (cumulative fitness R= -0.24, P= 0.64, Figure 3.4B; ASTER modeling R= -0.2, P= 0.71, Figure 3.4D). Finally, there was no significant differences across populations between inbreeding coefficient and inbreeding depression estimated using cumulative fitness (F= 0.04, P= 0.85), or ASTER modeling (F= 0.01, P= 0.99).

Discussion

The results of this chapter do not support the hypothesis that populations with a higher rate of self-incompatible will have higher inbreeding depression, and actually we found the reverse, that higher self-compatible populations had higher inbreeding depression. I also found no relationship between the inbreeding coefficient (F_{IS}) and inbreeding depression. Hence contrary to expectation F_{IS} does not help to predict the overall estimation of inbreeding depression in the populations and it is not negatively or positively correlated with inbreeding depression. Furthermore, inbreeding depression was not correlated with estimates of genetic diversity and effective population size. The wide variation in responses of inbreeding depression across populations for *Oenothera primiveris* demonstrated how inbreeding depression is a population dependent trait rather than being generalizable by species. Even within populations that share similar traits and have a similar genetic background (breeding system, floral size and inbreeding coefficient) did not show an equivalent response to increased inbreeding. The results obtained in this chapter provides an example of a species that does not fit into the expected pattern of inbreeding depression based on breeding system. Through this discussion, I will evaluate different reasons how the observed pattern may have come about and how the results obtained here can influence how we approach inbreeding depression in plant conservation.

Variation of inbreeding depression across the life fitness stage and overall fitness

Inbreeding depression showed variation across life-stages. Previous studies have suggested that later life-stages will show high inbreeding depression in mainly selfing species while mainly outcrossing species will express high inbreeding depression in all stages (early and late-life stages) (Husband and Schemske, 1996; Angeloni et al., 2014). The results obtained here

show that the response to inbreeding did not have any consistent pattern across life-stages. It is important to mention that increasing the sample size of maternal lines used might change the results obtained here, by helping to reduce the variation within each evaluated component of fitness. To include a higher number of populations, I had to compromise on sample sizes, needed to be limited to maintain all plants until the end of their life-cycle. Another limiting factor was the low number of crosses producing seeds in some populations, especially when there was self-incompatibility acting.

All of the components of fitness measured in this experiment contributed to overall life-time fitness, a measure of the final contribution an individual would make to the next generation. Two different estimates of life-time fitness were calculated here, cumulative fitness and life-time fitness estimated in ASTER, which uses the information obtained in early fitness stages. This is not done in cumulative fitness were each stage is analyzed separately and independently. The results obtained here using both approaches, however, show very similar results of fitness and inbreeding depression, not changing the overall patterns discussed below.

Relationship between inbreeding depression and breeding system

The plant breeding system will have a direct influence on an individual's capacity to self and therefore impact the expression of inbreeding depression in subsequent generations (Charlesworth, 2006). Populations with limited or no recent inbreeding are not expected to have purged their genetic load, and therefore are vulnerable to inbreeding depression (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987). Theory and experiments in diverse plant populations provide evidence that SI populations have higher inbreeding depression than SC but also provide evidence for the variability of inbreeding depression across populations within a taxon (Levin and Bulinska-Radomska, 1988; Johnston and Schoen, 1996; Vogler et al.,

1999; Hokanson and Hancock, 2000; Stone et al., 2010; Robertson et al., 2011). In this study, populations of *Oenothera primiveris* showed the complete spectrum of variability in the expression of inbreeding depression (-0.94 to 0.874). However, this variation was not explained by differences in the breeding system. Contrary to expectation, inbreeding depression was lowest among self-incompatible individuals and increased with higher rates of self-compatibility.

Western populations of *Oenothera primiveris*, have a higher frequency of self-incompatible individuals and they are expected to express the deleterious alleles that are hidden in the heterozygous state with increased inbreeding. The lack of loss of fitness observed in these populations could be due to low genetic load or insufficient generations of inbreeding to express and purged any genetic load. Finally, another reason for the observed pattern is sampling and experimental design. Self-pollination is not expected to produce seeds in SI individuals. Although I proposed to use a biparental inbreeding design, I could not maintain sufficient flowering plants to complete this design and as a consequence, we have a reduced number of individuals that are inbred. Hence the measure of selfing in these populations is more a reflection of the response only of maternal lines which were self-incompatible. Hence the frequency of SI or SC individuals will impact differently the expression of the population level inbreeding depression. This is most likely to influence the results for populations with lower self-incompatibility index rather than populations were self-compatibility is more ubiquitous. So, although some populations with low SCI did not show inbreeding depression, we cannot be sure if this would still be the case with biparental design, but we can say that populations with high self-compatibility, contrary to expectations, did show inbreeding depression.

Mixed mating or outcrossing mating system were expected to express higher inbreeding depression compared to populations with a selfing mating system, as these populations are likely

to maintain a higher genetic load and have fewer opportunities for purging (Dudash and Carr, 1998; Winn et al., 2011). However, despite our self-compatible populations also demonstrating higher autogamous self-pollination, and inbreeding (average F_{IS}= 0.33), they showed the greatest inbreeding depression. Which suggest these SC populations have maintained a high genetic load and not had an opportunity to purge. The results here show that purging may not occur even under high inbreeding scenarios, and supporting previous studies which suggest that purging is unreliable and unpredictable (Byers and Waller, 1999; Crnokrak and Barrett, 2002; Ralls et al., 2020). Purging is more likely to happen faster for alleles that produce a strong fitness reduction but might happen slowly or not at all if the fitness reduction is mild (Crnokrak and Barrett, 2002). The inbreeding depression we see in SC populations may be caused by mildly deleterious alleles that are not easily purged or that are fixed within lines in the populations (Wang et al., 1999).

Alternatively, if different self-lines within a population have fixed different deleterious alleles, the outcrossing between different self-lines, might lead to an increase in fitness through heterozygotes advantage (Charlesworth and Charlesworth, 1999; Charlesworth and Willis, 2009). Then the fitness differences observed between self and outcross is not because fitness of the self crosses was reduced but because the heterozygotes show an increase in fitness. This could be a possible explanation for the inbreeding depression results in the SC populations. Further generations of inbreeding and outcrossing might help to figure this out since outcross lines will reduce fitness in further generations since heterozygotes advantage tends to be ephemeral. Another interesting aspect of the observed pattern is that selfing has evolved in these populations despite the negative inbreeding depression observed. This suggests that the inbreeding depression is not sufficiently disadvantageous to prevent the evolution of selfing

(Lloyd, 1979; Lande and Schemske, 1985; Barrett and Harder, 1996; Goodwillie et al., 2005; among others). In this system, it seems like at some sites, reproductive assurance and seed production is more important for the population continuity than avoiding inbreeding depression. The rate of inbreeding, high inbreeding depression, reduced genetic diversity and small census and effective population size observed in the SC populations of *Oenothera primiveris*, suggest that these populations are at high risk of extinction.

Inbreeding coefficient and inbreeding depression in Oenothera primiveris

Within population inbreeding coefficient (F_{IS}) represents the degree of inbreeding averaged over the lifetime of the population, relative to the subpopulation (Wright, 1951). Even though F_{IS} does not necessarily reflect contemporary inbreeding levels, it can still provide information about the chances of purging the genetic load in the history of the population. In smaller populations, the average fitness it is expected to decrease from generation to generation as the level of inbreeding increases (Keller and Waller, 2002; Pekkala et al., 2014), which would allow selection to act. It was expected that populations with higher F_{IS} would experience lower inbreeding depression as they would have had the opportunity to purge their genetic load. In *Oenothera primiveris*, this was not the case, and there was no clear relationship between the inbreeding coefficient and inbreeding depression in this system. The lack of correlation between the two traits could be related to the high variation in inbreeding depression found across populations of the species. More examples where F_{IS} and inbreeding depression is evaluated should provide more accurate information on how strong the relationship between the two parameters is within populations.

Inbreeding depression associated with effective population size and genetic diversity

Small populations are expected to have reduced genetic diversity and increased inbreeding (Ellstrand and Elam, 1993; Reed and Frankham, 2003; Frankham, 2005; Leimu et al., 2006, Frankham et al 2017). In small populations, the effectiveness of selection is reduced relative to genetic drift allowing for the fixation of deleterious alleles instead of purging them (Hedrick and Miller, 1992; Frankham et al., 2017) overall decreasing the fitness of the populations. Populations in the east of the distribution in *Oenothera primiveris* had lower diversity and higher inbreeding, which would suggest smaller effective population sizes. This might suggest that the frequency of genetic load would be higher as a result of the reduced diversity or a bottleneck in the natural populations. The greater inbreeding depression supports the idea that the populations in the east might have a higher genetic load, which could be associated with a recent bottleneck (Ellstrand and Elam, 1993).

Conclusions

The results presented in this chapter show an unexpected pattern of inbreeding depression, with low inbreeding depression in populations with SI individuals and high inbreeding depression in fully SC populations with high autogamous seed set. The patterns observed could suggest that populations in the west have already expressed and purged some of the genetic load in the population therefore not showing inbreeding depression. While populations in the east might be expressing mild deleterious alleles which are harder to purge from the population. The populations or individuals breeding system does not seem like a good plant trait to predict inbreeding depression in this system, in fact the results show the opposite response under inbreeding. Future work should focus on determining the populations mating

system, to define the frequency of selfing and outcrossing in these populations. This information could help to understand the results obtained and determine the frequency of inbreeding in these populations. Finally, the result obtained here suggest that inbreeding depression is population-specific, given the variation across populations and that the expression of inbreeding depression even though it is related to the plant breeding system might not always lead to the expected outcome.

CHAPTER 4

INFLUENCE OF POLLINATORS ON INBREEDING DEPRESSION. A COMPARISON BETWEEN SPECIES WITH CONTRASTING POLLINATORS: CLARKIA BREWERI AND CLARKIA CONCINNA SUBSP. CONCINNA (ONAGRACEAE)

Abstract

Pollination will determine the amount of selfing or outcrossing that an individual experience, and ultimately a population's mating system. Pollinators will differ in how much pollen movement will occur between individuals based on their distribution, size, and behavior. Local and small range foragers might lead to higher rates of geitonogamy, and therefore self-pollination, compared to more sporadic pollinators and long distant pollinators. Consequently, populations pollinated by small range foragers might experience inbreeding more often and therefore could express and purge deleterious alleles more frequently. In this chapter, I am looking to test this hypothesis by evaluating fitness differences between two species of Clarkia that differ in their primary pollinator. *Clarkia concinna* subsp. *concinna* is mainly pollinated by insects with smaller ranges, while *Clarkia breweri* is mainly pollinated by hawkmoths which are known to migrate long distances and be sporadic foragers. To answer how pollinators can influence on the level of inbreeding and inbreeding depression, I performed controlled crosses to increase relatedness between individuals and then evaluated differences in fitness under two experimental settings, the growth chamber, and the greenhouse. Results show that inbreeding depression varies between species, populations, number of generations evaluated and environmental conditions. Inbreeding depression was overall higher in bee-pollinated

populations rather than in hawkmoth pollinated populations which do not agree with the expected pattern.

Introduction

A plant's mating system is determined by the gametes movement within and between individuals of a population. The mating system of a population can be classified by the frequency of outcrossing, ranging from predominately outcrossing (> 80%), mainly selfing (< 20%) or mix mating (<8 0% but > 20%). Although there are some examples of plants that exclusively self-pollinate or exclusively outcross, a majority of angiosperm species exhibit a range of mating systems, from self-pollination, mixed mating to outcrossing (Barrett, 2003; Goodwillie et al., 2005). Ultimately the mating system that a population experiences will depend on the species biology (breeding system), floral design (herkogamy, dichogamy, etc), and pollination (Barrett, 2003; Charlesworth, 2006; Devaux et al., 2014). Within plants, a wide diversity of reproductive strategies and pollination systems have evolved to facilitate pollination, which then ultimately interact to determine the frequency of outcrossing within a system. It has been estimated that nearly 85% of flowering plants rely on animal pollination for the transfer of pollen (Paton et al., 2008; Ollerton et al., 2011). The diversity of pollination syndromes range from generalist to highly specialized, where both pollinator and plants depend on each other for success (Nilsson, 1988; Pellmyr, 1994; Armbruster, 2017). Gene flow patterns are crucial for shaping the distribution of genetic diversity, determining rates of selection and minimizing drift and inbreeding (Slatkin, 1985). Hence, which animal vectors are moving pollen will play an important role in determining the mating system of a population, and the amount of inbreeding or outcrossing a population experiences (Eckert et al., 2010).

Studies of plant-pollinator interactions have often focused on reproductive assurance (Fenster and Martén-Rodríguez, 2007; Busch and Delph, 2012) and selection for floral traits (Schemske and Bradshaw, 1999; Teixido and Aizen, 2019), however less attention has been given to their role in determining the fitness of next-generation. Studies have focused on fitness changes in response to changes in the pollinator community or absence of pollinators (Bodbyl Roels and Kelly, 2011; Gervasi and Schiestl, 2017) but not on how different species of pollinators can influence inbreeding. It is well established that the fitness of progeny resulting from self-fertilization is more likely to be lower, due to inbreeding depression (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987, 1999). Pollinators play a crucial role in influencing the amount of outcrossing and selfing in a population, and therefore should also influence inbreeding depression within a population. This has been demonstrated in previous meta-analyses that have found that biotically pollinated species generally have higher inbreeding coefficients and greater variability in outcrossing rates compared to wind-pollinated species (Duminil et al., 2009; Whitehead et al., 2018). However, not all biotic pollinators are equivalent, hence it is important to understand the impact that different pollinators might have on plant fitness, inbreeding, and the expression of inbreeding depression.

Pollinators differ in size, abundance, foraging behavior, and their effectiveness at removing and transferring pollen (Wilson and Thomson, 1991; Sahli and Conner, 2007; Ma et al., 2019). All factors which will determine the mating system of a population. The few studies which have looked at the influence of pollinators on the mating system have supported this difference (Brunet and Sweet, 2006; Ma et al., 2019). Hawkmoths were linked to higher outcrossing rates (Brunet and Sweet, 2006; Rhodes et al 2017) as were bumblebees when compared to honeybees (Ma et al., 2019). How pollinators move between flowers will determine

the amount and quantity of pollen that is transferred, their influence on reproductive success (Wilson and Thomson, 1991; Brunet and Sweet, 2006; Sahli and Conner, 2007), and their influence on the genetic composition of the offspring (Rhodes et al., 2017; O'Connell et al., 2018). For example, species pollinated by local foragers with a short flight distance might result in greater geitonogamy, higher biparental inbreeding, and greater isolation (Lloyd and Schoen, 1992; Mitchell et al., 2009). Some molecular studies comparing inbreeding in plant species with different primary pollinators have supported this hypothesis (Jabis et al., 2011; Kramer et al., 2011; Howell and Jesson, 2013; Wessinger et al., 2019), while others have not (Collevatti et al., 2010; Torres-Vanegas et al., 2019).

Given that pollinator type and behavior can influence the rates of outcrossing and population connectivity, they will ultimately determine how common inbreeding is in a population (Devaux et al., 2014). As pollinator communities will also vary in space and time, it is expected that different populations will experience different rates of inbreeding depending on the year and community. However, it is also important to point out that other population traits are also important in determining the rate of inbreeding, including population size, distance to other populations, population density, etc. Populations pollinated by local foragers (like bees) will likely experience more frequent inbreeding than populations pollinated by more sporadic pollinators (like hawkmoths). Populations with higher rates of inbreeding will likely express their deleterious recessive alleles more often. This will allow for the purging of these alleles, restoring fitness to those populations. This is less likely to occur in populations with higher outcrossing rates. Hence, populations pollinated by local foragers are less likely to experience inbreeding depression since they have already expressed and purged some of their genetic load. In chapter 1, I tested how differences in pollinator might influence inbreeding and the expression

of inbreeding depression, while there were no significant differences, the inbreeding coefficient was higher in moth pollinated-species compared to bee pollinated-species. These results do not support the expectations for this chapter, however moths did have higher inbreeding depression which does support the expectation for this chapter. Nonetheless, it remains to be tested if the influence that hawkmoths and bees have on inbreeding and inbreeding depression.

To study the relationship between pollinators, inbreeding, and inbreeding depression, I will be comparing two sister species of Clarkia that differ in their main pollinator. Clarkia species are self-compatible annual species, easy to grow with a wide diversity of pollinators, floral forms, and mating systems. They are a highly studied group, being used in studies looking at reproductive assurance (Fausto et al., 2001a; Bontrager et al., 2019), the variation of floral traits (Raguso and Pichersky, 1995; Podolsky et al., 1997; Gamble et al., 2018), reproductive isolation (Briscoe Runquist et al., 2014; Bontrager and Angert, 2016; Kay et al., 2018), and inbreeding depression (Holtsford and Ellstrand, 1990; Holtsford, 1996; Barringer and Geber, 2008). Previous studies of inbreeding depression in Clarkia tembloriensis have shown no differences in inbreeding depression across life stages or populations with different outcrossing rates (Holtsford and Ellstrand, 1990). Holtsford (1996) found that populations with reduced outcrossing rate are more likely to purge lethal genes but still maintained mild deleterious alleles that can result in inbreeding depression.

In this chapter, I am comparing populations with different pollinators to determine if they can predict the expression of inbreeding depression in two sister species of *Clarkia*. One species, *Clarkia breweri*, is mainly pollinated by hawkmoths while *Clarkia concinna* subsp. *concinna* is mainly pollinated by small insects (Bees and bee flies). The purpose of this work is to determine if pollinators with different foraging behaviors impact the expression of inbreeding depression.

Based on known information about pollinator behavior, and using pollinator as a proxy for expected outcrossing rate, the expectations are that populations and species pollinated by small insects will have reduced inbreeding depression compared to populations and species pollinated by larger insects. With this expectation, I propose to test three hypotheses: (1) Hawkmoth-pollinated populations will exhibit greater inbreeding depression due to less frequent inbreeding than bee-pollinated populations. (2) Inbreeding depression will show variable results among populations of both species due to different population histories. (3) Inbreeding depression will increase faster with the level of relatedness among the individuals, biparental inbreeding will lead to moderate inbreeding depression compared to self-pollination, which will show higher fitness decline. Overall, this chapter will help to evaluate differences between populations and consider the role that pollinators have on inbreeding depression.

Methods

Species information

Clarkia concinna and *Clarkia breweri* are closely related species in the section Eucharidium of the family Onagraceae that differ in their primary pollinator (Groom, 1995; Miller et al., 2014). *Clarkia breweri*, is an outcrossing species pollinated primarily by nocturnal hawkmoths while *Clarkia concinna* is mainly pollinated by bees and small insects (Miller et al., 2014). *C. concinna* has a wider distribution, that goes from southern San Francisco to Humboldt county while, *C. breweri* has a more restricted distribution in central California (Figure 4.1) (Lewis, 1953; Kay et al., 2018). Both species are self-compatible and protandrous (Lewis and Lewis, 1955), which suggests that mixed mating or outcrossing is the predominant mating system. Clarkia concinna, has been divided into three different subspecies (subsp. concinna,

subsp. raichei and subsp. automixa) which vary in morphology, ecology, pollinators and mating system (Allen et al., 1990). Clarkia concinna subsp. concinna is outcrossing while the mating system in Clarkia concinna subsp. automixa and subsp. raichei is mainly self-pollination. In this work, I will compare inbreeding depression in 3 populations of both Clarkia breweri and *Clarkia concinna* subsp. *concinna*. Both species are interfertile, but morphological differences (including flower color, length of hypanthium tube and differences in stigma-anther position), habitat preference and differences in primary and most effective pollinator separate the species (Miller et al., 2014). Seed material was collected from 3 population of *Clarkia breweri* and 3 populations *Clarkia concinna* subsp. *concinna* along with the range of distribution for the species (Figure 4.1; Table 4.1). Between 25 to 35 maternal lines per population were collected and used in this study. The seeds were cleaned and kept in cold conditions before use.

Controlled crosses

Seeds were grown at the Chicago Botanic Garden. I aimed for 30 maternal lines per population but the final number varied from 26 to 36 (average = 31.3 for *C. concinna* and 33 for *C. breweri*; Table 4.1). For each maternal line, the average number of seeds grown varied from 17 to 23 (Average of 20 seed for *C. concinna* and 19 for *C. breweri*; Table 4.1). Seeds were germinated in 1.5% agar on Petri dishes and placed in an incubator with diurnal cyclic conditions of 15°C for 12 hours (day) and 12°C for 12 hours (night). Germinated seedlings were planted in small cone-tainers (2.5 x 12 cm) using 3:1 mix of regular potting soil and perlite and maintained in the growth chambers (Conviron CMP6050). The growth chamber was set at 20ºC for 12 hours (day) and 15ºC for 12 hours (night). Plants were watered by submerging the bottom of cone-tainers in water twice a week for the duration of their lifetime.

Maternal lines selected for each cross depended on which flowers had both female and male reproductive parts mature at the same time (there are 3 to 5 days' in-between pollen maturation and stigma receptivity). To perform the crosses, I used forceps to move pollen between pollen donors and maternal plants, which were washed in alcohol between crosses. I attached a color thread to the base of the ovary after each cross, and then when the flower was dropped a few days later, I used scotch tape to surround the top of the fruit and prevent losing seed when fruits matured. Fruits were collected when dried, seeds were cleaned and maintained under cold conditions until used. Half the seeds obtained in generation 0 (and up to 20) were selected to grow and to evaluate fitness differences in the next generations. The other half was stored and kept for the greenhouse experiment described later. The same growing conditions (germination and establishment) used to grow out generation 0 were used in all subsequent generations.

I aimed to generate 10 replicates of three cross types (Table 4.1). The first was self-treatment, which consisted of transferring pollen to the stigma of a receptive flower on the same plant for at least ten maternal tracked lines. This was repeated for every generation so that levels of inbreeding increased with each generation. The second treatment was outcrossing, which consisted of ten maternally tracked lines which were randomly crossed to a different maternal line each generation. Different paternal and maternal individuals was selected to ensure maximum outcrossing between maternal lines, however, this was not always possible and some crosses were half-sibs. This was intended to represent normal rates of outcrossing expected in the wild. For the final cross-treatment, I generated biparental lines. To do this, I created 10 families, each family was comprised of 5 plants, from which one line was selected as the parental donor to pollinate the remaining 4 maternal plants. Thus, creating a family of lines which were now

half-sibs. To create generation 2, pollen was collected from each of four maternal line, mixed, and then was used to pollinate each flower, hence each cross was either a self, full-sib or half-sib cross. As he biparental treatment did not start until generation 2, the first generation evaluated was treated as a regular outcrossing for the analysis.

Fitness data was collected on all plants that were grown up for crosses in the growth chamber, (one generation of *Clarkia concinna* subsp. *concinna* and two generations of *Clarkia breweri*). Once all crosses were complete, all generations, species and cross-types were grown together in a greenhouse experiment to compare their fitness under uniform conditions. I aimed to have up to 10 maternal lines of self-pollination, outcross, and biparental crosses (G2 and G3) for this experiment. I grew randomly selected 30 seeds per treatment combination and split between two different 1.5% agar plates. Seeds were x-ray to evaluate the viability and put in cold conditions (4°C) for 12 days, to promote germination. Plates were moved to natural light (in the greenhouse) for a week. Germination was recorded two times after that, allowing for more time for the seeds to germinate. If no more seed germinated after 3 weeks, plates were discarded. Up to 10 seedlings for every germinated cross were randomly selected and moved to the soil, where they were placed in medium-size cone-tainers (3.8 x14 cm) using 3:1 mix of regular potting soil and perlite. Plants were then randomized in the trays and allow to grow for 10 weeks.

Fitness traits measured

Fitness was evaluated at different life stages and as the cumulative measure (multiplicative factor of each stage). The fitness stages used were: seed viability, seed germination, survival after 4 weeks' survival to flowering, and flower number (evaluated after ~10 weeks of the seedlings in soil). The number of viable seeds was recorded for each cross and the ratio of viable seeds was calculated as the number of viable seeds divided by the number of

seeds evaluated. Seed viability was determined using an X-ray machine, each seed was recorded as full or not depending on visual inspection of an embryo, which glowed white. Germination was recorded after radicle or cotyledon emergence and before moving to the soil. The ratio of germinated seeds was calculated as the number of seeds that germinated divided by the number of viable seeds. Early survival was evaluated after 4 weeks of the seedling was placed in soil. The ratio of plants that survived was calculated as the number of plants alive divided by the number of seedlings moved to the soil. After 10 weeks in growth chamber and 16 weeks in greenhouse, survival to flowering, and flower number data was collected. Each plant was evaluated for flower production, recording the total number of open flowers, the number of dried ovaries remaining in the plant, or the number of developed fruits. Even when not pollinated the dry ovary remains attached to the plants so it is easy to count flowers produced. The ratio of plants that survived to the flowering stage was calculated as the number of plants that produce flowers divided by the number of plants that were alive at 4 weeks (early survival). Flower number for each cross was calculated as the total number of flowers produced for the cross divided by the number of plants that survived to the flowering stage. Cumulative fitness for each cross was calculated by multiplying the success ratio of each evaluated trait, and if the success ratio of any given trait was zero, cumulative fitness for the cross was also zero.

Analysis of fitness

Inbreeding depression calculations

Inbreeding depression was calculated for every trait and cumulative fitness in both experiments. I calculated inbreeding depression following the recommendations of Agren and

Schemske (1993) using the following equations where W_S is the fitness of the self crosses and W_O represent fitness of the outcross crosses (ID_{SO}).

Equation 1: Inbreeding depression when $W_O > W_S$

$$ID = 1 - \frac{W_S}{W_O}$$

Equation 2: Inbreeding depression when $W_O < W_S$

$$ID = \frac{W_O}{W_S} - 1$$

In generation 2, biparental treatment was separated from outcrossing and the two estimates of inbreeding depression were calculated. I used the same formulas of inbreeding depression, comparing biparental crosses to outcrosses (ID_{BO}), and selfed plants to biparental (ID_{SB}). In generation 1, as biparental were not different in practice to outcross plants, inbreeding depression was only calculated between self and outcross (ID_{SO}), same as above, by pooling maternal lines from biparental and outbreeding.

Variation of inbreeding depression

To answer the hypothesis that pollinator type will influence the amount of inbreeding depression in the populations, I evaluated fitness differences between cross-type, populations and generation for each species. This was done for each life-history stage separately and cumulative fitness. I performed a mixed linear model using maternal line and experimental conditions (growth chamber and greenhouse) as random effects. As the self and outcross treatments consisted of individuals from the same maternal lineages, a fitness average was calculated by maternal line. For the biparental treatment, data it was averaged by the paternal lineage since this was the common factor within each family lineage. Each trait was transformed using arcsine to

meet assumptions of normality, although but the results were similar with or without transformation. Cumulative fitness for each lineage was calculated by multiplying the average of each stage evaluated and if any trait had a fitness value of 0, cumulative fitness for the cross was also zero. All analyses were performed in R version 3.3.3 (R Core Team, 2017).

Life-fitness analysis (ASTER)

As described in the previous chapter, the ASTER model generates the overall likelihood for a set of components expresses through the lives of individuals (Geyer et al., 2007). This analysis accounts for fitness component with different statistical distribution and the dependence of fitness component express later in the lifespan of those express earlier (for example, to be able to produce flowers, you first need to survive until this stage, germinate and be a viable seed. Later life-stages, therefore, depend on the early stages). To analyze the data, I used a fixed effect ASTER model to evaluate the influence of populations, crosses and generation in final fitness, using maternal/paternal line as a fixed effect. This analysis was done using the R Package ASTER (Geyer, 2015). ASTER analysis was done for each species and experiments separately, testing the influence that cross-type, population and generation (if applicable) has on life-time fitness.

Inbreeding depression related to populations primary pollinator

To evaluate differences in inbreeding depression measured across pollinators type, I used known information about the populations' primary pollinator in one-way ANOVA to test how pollinators influence on inbreeding depression. This was done for IDS-O obtained through cumulative fitness or ASTER model and for the growth chamber and only for ASTER results in the greenhouse experiment.

Results

Crosses

For *Clarkia breweri*, two generations of crosses were completed in growth chamber (G0, G1 and G2), although the number of seed produced for the third generation was reduced. The number of maternal lines that were lost after generation 1 ranged from 10%-57%, but dropped to 50%-100% in the second generation. The decline in numbers of lines did not vary by cross type but rather by population, with population 2 only showing minor losses (10 - 18%) after first generation (G1 to G2) and population 4 the highest losses (50% - 58%). However, after second generation (G2 to G3) the losses jumped (>75%), with exception of self lines from Population 2 (only 50% lost). Regardless, for *Clarkia breweri*, I was able to evaluate 4 generations (0, 1, 2 and 3) in the greenhouse experiment. In *Clarkia concinna* subsp. *concinna* only one generation of crosses were completed in the growth chamber (G0 and G1), as there were large losses by the second generation (G1 to G2). This pattern was consistent across populations, although for population 2 and 3 the smallest losses were in the outcross treatments. Three generation (0, 1 and 2) were available to be used for greenhouse experiment, however there was poor germination in G0 and G1 stored seed, hence only generation 2 was evaluated in the greenhouse. The poor germination is likely due to dormancy being induced in storage conditions, as seed viability was high across generations.

Inbreeding depression in Clarkia breweri

Cumulative fitness in *Clarkia breweri* was much higher in the greenhouse than growth chamber for both generations 1 and 2, and all populations (Table 4.2 and 4.3). This can be mostly attributed to flower number and biomass, which was much greater in the greenhouse. Regardless of the absolute values the directionality of differences remained the same across the

generations and populations. The most significant indicators of fitness were populations ($F_{2,410}$= 29.62, P <0.0001; table 4.5) and generation ($F_{2,410}$= 122.63, P <0.0001; table 4.5), with population 4 consistently being less fit, and generation 2 showing lower fitness than generation 1. Although cross-type was significant ($F_{2,410}$= 2.99, P= 0.05; table 4.5) it did not show a consistent pattern. In the first generation, Population 4, which had the lowest cumulative fitness overall, the cumulative fitness was lowest in self lines, hence positive inbreeding depression in both growth chamber (ID_{SO}= 0.21) and greenhouse (ID_{SO}= 0.14). By contrast population 2 showed negative inbreeding depression in both growth chamber (ID_{SO}= -0.23) and greenhouse (ID_{SO}= -0.21), while Population 3 showed negative inbreeding depression in growth chamber (ID_{SO}= -0.25), but positive in the greenhouse (ID_{SO}= 0.16). However, all these values for inbreeding depression were low, and cumulative fitness had high variability (Table 4.2; Figure 4.2), suggesting that self and outcross lines performed similarly in most conditions.

In generation 2, the cumulative fitness was lower but for all populations and locations, the selfed lines performed better than the outcrossed lines (ID_{SO}), resulting in negative inbreeding depression for all populations except Pop 4 when grown in the growth chamber. By contrast when cumulative fitness of selfed lines was compared to the biparental lines (ID_{SB}), the pattern was less consistent, with biparental lines out performing selfed in Pop 2 and 3 in growth chamber and Pop 4 grown in the greenhouse, and reverse in opposite conditions. A similar result was found using the ASTER analysis which accounts for the dependency of fitness component express earlier in the lifespan (Figure 4.2B and D and Figure 4.3 B and D) showed that for the growth chamber and for the greenhouse data, there was a significant interaction between population, cross and generation on life time fitness (Table 4.7). The lack of trend, and high variation of response within lineages makes it hard to derive any meaningful pattern in this data

(Figure 4.3). One consistent results were that outcrossed lines often performed similar to selfed lines in the growth chamber (Fig 4.3 A and C), and biparental either did better than both (Pop 2 and 3) or worse (Pop 4). In greenhouse, however the differences were less pronounced, with all lines performing equally well (Fig 4.3 B and D). What is interesting is that the higher difference in fitness between Pop4 and the rest in generation 1, was significantly smaller by generation 2.

Breakdown the cumulative fitness by life history stage, we see that seed viability varied mostly by generation ($F_{3,410}$=85.62, P <0.0001; Table 4.5), ranging from 78-100% in generation 1 droping to 26-92% by generation 2. There was also a signficant interaction between population and generation ($F_{6,410}$=4.7, P=0.0001; Table 4.5), with Pop 4 showing a much large drop in viability by generation 2. Seed germination also varied by population ($F_{2,410}$=31.25, P <0.0001; Table 4.5), generation ($F_{3,165}$=71.2, P <0.0001; Table 4.5), and cross-type ($F_{2,410}$=5.69, P= 0.004; Table 4.5), being highest in Pop2, in generation 1 and selfed lines. The pattern was the same for Early survival and survival to flowering (Table 4.5), although there was a significant interaction between population and generation for early survival significant ($F_{6,410}$=4.74, P= 0.0001; Table 4.6), with population 4 having much lower survival in generation 2. Plants growing in the greenhouse show overall higher flower number than the ones evaluated in the growth chamber experiment, suggesting a response to growing conditions. Regardless the number of flowers produced also produced a significant interaction between generation and population ($F_{6,410}$=3.12, P= 0.01; Table 4.6), with Pop 2 in greenhouse showing only small decrease in number of flowers from generation 1 to 2, but all other populations showing much larger declines. Overall, populations 2 and 3, which showed the negative inbreeding depression, showed very variation in response across life history traits (Figure 4.5). By contrast, Pop 4 which is the only population to

show positive inbreeding depression (in growth chamber), showed largest deviations in the earlier life stages (Figure 4.5).

Inbreeding depression in Clarkia concinna subsp. concinna

In *Clarkia concinna* subsp. *concinna*, there was poor germination of generation 1 in the greenhouse so inbreeding depression could only be evaluated in the growth chamber for generation 1 and greenhouse for generation 2. As a consequence, we are unable to distinguish the effect of location and generation response in our models. In the growth chamber, the cumulative fitness was low for generation 1 ranging from 0.1 to 0.29 (Table 4.4; Figure 4.4 A), with exception of the outcross treatment in Population 3 which had the higher fitness $F_{2,165}$=15.05, P <0.0001; Table 4.6) in both generation and locations. For generation 2, which was grown in the greenhouse, the cumulative fitness was also significantly higher ($F_{1,165}$=21.6, P<0.0001; Table 4.6), ranging from 0.43 to 11.84, due mainly to much higher flower production (Table 4.4; Figure 4.4 B). There was also a significant interaction between population and generation (location) factor ($F_{2,165}$=5.59, P= 0.004; Table 4.6). The main difference being Population 2 in generation 2 performed better in the greenhouse than in the growth chamber (Fig 4.4). There was also a significant difference in cross-type ($F_{4,165}$=4.16, P= 0.02; Table 4.6), with self-pollination consisting underperforming compared to outcross and biparental crosses (Fig 4.4). A similar patterns was found using the ASTER analysis which accounts for the dependenancy of fitness component express earlier in the lifespan (Figure 4.4B) with the cross, pop and generation affecting lifetime fitness but no interactions between terms (Table 4.8).

Inbreeding depression was found by the first generation for both Population 1 (ID_{SO}= 0.62)) and Population 3 (ID_{SO}= 0.75), but none was found in Population 2 (ID_{SO}= -0.14),

although fitness was low overall in this population. By generation 2, many family and maternal lines had declined increasing variability but inbreeding was seen across all populations except for Population 1, where the two outcross lines remaining performed poorly. In population 2 and 3, the outcross lines outperformed both the biparental and self lines (ID_{SO} and ID_{SB}), with biparental performing better than selfed lines.

The life stages which had the largest impact on cumulative fitness differed by population (Figure 4.5), but each life stage did not always show significant difference by cross-type. Seed viability varied by population and generation, but not cross-type (significance values in Table 4.6) The seed viability was highest in generation 1 (90 - 98%) but dropped dramatically by generation 2 (7-56%) (Table 4.2). There was a significant interaction between generation and population ($F_{2,165}$= 5.79, P= 0.004; Table 4.6), with the largest drop in Population 1 by the second generation (7 - 19%). Seed germination showed a similar pattern to seed viability, with significant differences between population and generation (significance values in Table 4.6), with population 3 showing consistently higher germination regardless of generation (Table 4.6). Cross-type was also significant ($F_{2,165}$=4.16, P= 0.02; Table 4.6), with self crossing consistently performing worse than outcross and biparental, except for Pop 1 in generation 2. For early survival and survival to flowering, the highest significant predictor was population ($F_{2,165}$=8.73, P<0.0001, and $F_{2,165}$=8.57, P<0.0001 respectively; Table 4.6) with Population 1 having the lowest survival rates (Table 4.2). To a lesser extent cross-type was also significant ($F_{2,165}$=2.90, P= 0.06, and $F_{2,165}$=3.65, P= 0.03 respectively; Table 4.6), with selfed plants performing worse. Finally, mean flower number showed significant variation by both population and generation (experiment location) ($F_{2,165}$=11.9, P <0.0001, and $F_{1,165}$=24.85, P <0.0001 respectively; Table 4.6), with Population 1 flowering the least. Plants growing in the greenhouse show overall higher

flower number than the ones evaluated in the growth chamber experiment, a difference that could be associated with environmental conditions rather than generation. Again cross-type ($F_{2,165}$=3.6, P= 0.03; Table 4.6), was significant with selfed plants performing worse, except for Pop 2 in first generation, were selfed produced slightly more flowers (1.3 and 1.2). Overall, populations 2 and 3, which showed the highest inbreeding, the selfed lines showed largest change in latter life stages, while Population 1, which showed lower inbreeding depression, saw larger deviations in earlier life stages (Figure 4.5).

Inbreeding depression related to species and their main pollinator

I evaluated differences in inbreeding depression across populations and species for all traits evaluated and for cumulative fitness, this was done only for generation 1 evaluated in the growth chamber since it was present in both species and provided a complete dataset. For *Clarkia concinna*, inbreeding depression did not show significant differences across traits evaluated (F= 0.41, P= 0.08; Figure 4.5) but there were significant differences between populations (F= 0.02, P= 0.008; Figure 4.5). While for *Clarkia breweri* the interaction between populations and traits was significant (F= 0.27, P= 0.027; Figure 4.5).

Inbreeding depression show significant differences between pollinators (F= 9.8, P= 0.01; Figure 4.6) regardless of the method used to estimate fitness (either by using cumulative fitness values or by using aster modeling), there where no significant differences on inbreeding depression between method used (F= 0.02, P= 0.9; Figure 4.6). Inbreeding depression was overall higher in populations pollinated by bees and beeflies (*Clarkia concinna*) than in populations pollinated by hawkmoths (*Clarkia breweri*).

Discussion

The results obtained in this chapter show that inbreeding depression was more common in the beefly pollinated *Clarkia concinna* than hawkmoth pollinated *Clarkia breweri*. Inbreeding depression was expressed in one generation for two population of *Clarkia concinna*, but was only seen in one population of *Clarkia breweri* and only under certain conditions. Given that the populations pollinated by bees and beeflies had overall higher inbreeding depression compared to populations pollinated by hawkmoths, this would indicate we would reject our hypothesis that pollinators with larger body sizes and more sporadic foraging patterns result in higher inbreeding depression. The variation observed on inbreeding depression across population, generations, and experiments highlight the idea that inbreeding depression can be a population-specific trait and is a highly dynamic evolutionary process. This suggests that population factors other than shared plant traits can play an important role in ultimately determining if inbreeding depression is expressed. One important driver was the growing environment of the cross-type which will influence the degree to which inbreeding is expressed. In this experiment, the growth chamber and the greenhouse showed variable outcomes in inbreeding depression, with higher fitness overall being measured under the more benign greenhouse conditions.

Inbreeding depression in Clarkia breweri

Clarkia breweri showed large variations in response across generation, growing conditions and cross-type, but overall showed little or low levels of inbreeding depression across populations. In many cases self lines performed as good or better than outcross lines, although in two populations biparental lines performed the best. This was the same for both the growth chamber and the greenhouse experiment. Expectations were that populations of *Clarkia breweri* will express high inbreeding depression since they were pollinated by hawkmoths. This was not

observed, suggesting that populations carry a low genetic load, which might suggest they have experienced purging either through a bottleneck or elevated inbreeding. Pollinator type and abundance have been shown to vary among populations in several plant species (Miller, 1981; Herrera, 1988; Waser et al., 1996). And hawkmoths are known to vary from year to year (Miller, 1981; Campbell et al., 1997). The data obtained here suggest that populations of *Clarkia breweri* may have already experienced inbreeding than expected based on their main pollinator. This indicates that either hawkmoth are leading to an increase in selfing or that other factors, such as fluctuations in population size might have impact the expression of inbreeding depression in this species. Having pollinator observation over several years and determining the population mating system might help to set more realistic expectations of inbreeding depression for a particular population.

Inbreeding depression in Clarkia concinna subsp. concinna

For *Clarkia concinna*, which is predominately pollinated by bees and bee flies, we had proposed that inbreeding depression might be less obvious due to historically higher levels of inbreeding associated with the foraging behavior of their primary pollinator. In contrast, we found high inbreeding depression in two populations in the first generation in the growth chamber. By the second generation the highest inbreeding was observed when self were compared to outcross or biparental lines. In the greenhouse experiment, there was a large drop on crosses that germinated in generation 1, preventing a comparison of inbreeding depression with the results in the growth chamber. In generation 2, cumulative fitness showed high inbreeding depression compared to using the ASTER models number there was only moderate inbreeding depression with the self crosses. The differences in response between populations could be explained by fluctuations in popualtions size which vary based on environmental conditions.

Their patchy distribution, sporadic colonization events and fluctuating populations sizes (Groom, 1995, 1998) could lead to frequent bottlenecks. This would lead to mating system variation that can partially explain differences in inbreeding depression across populations.

In Groom and Preuninger (2000), they evaluated inbreeding depression of isolated and centralized populations of this species in both the greenhouse and the field. In the greenhouse, there was reduced inbreeding depression (0.13 and 0.19) and higher inbreeding depression when evaluated in the field (0.76). Interestingly, the values obtained in my growth chamber experiment were similar results to what they observed in the field. This response could be related to harsher conditions in less optimal growth chamber environment, differences in the collected populations or differences in fitness traits evaluated. The variation observed between my experiments (growth chamber and greenhouse) and previous results in the greenhouse and the field (Groom and Preuninger, 2000) suggest that inbreeding depression in *Clarkia concinna* can be highly variable and therefore it is not easy to generalize about the species.

Pollinators influence on inbreeding depression

The results obtained for *Clarkia concinna* and *Clarkia breweri* do not fit the expectations based on their pollinator. Populations pollinated by small insects overall did not express low inbreeding depression compared to populations pollinated by hawkmoths. As only one population of each species shows the expected pattern while the other two did not, might suggest that the pattern is opposite of expectations. It is possible that pollinators do not have been a predictive factor of inbreeding depression due to their impact on populations mating systems and other plant-life traits not considered here might be driving this pattern. As previously mentioned, *Clarkia* species are self-compatible and have protandry, to promote outcrossing. The data

obtained in this chapter suggest that *Clarkia breweri* is experiencing more self-pollination (either through self-pollination or geitonogamy) than expected, while *Clarkia concinna* it is not. Self-pollination would lead to the expression of the genetic load and therefore purging of the negative alleles in the population. that their behavior and relationship to inbreeding is opposite of our expectation. A molecular study of these populations will help to determine if inbreeding (Fis) is higher in one species than the other.

An alternative explanation for the results obtained here could be that the expectations need to be adjusted. Even though pollinators are very important in determining population genetic patterns, there is not enough information about how specific types of pollinators act in the populations, therefore I used pollinator behavior as a predictor for inbreeding and inbreeding depression here. However, in chapter 1, I showed how different pollinator types influence inbreeding and inbreeding depression. Even though the differences were not significant due to variation observed within each pollinator category the results suggest that the mean inbreeding coefficient is higher in moth-pollinated populations compared to bee-pollinated ones. Considering this, it could be expected that hawkmoth–pollinated populations of *Clarkia breweri* would express reduce inbreeding depression while bee-pollinated populations of *Clarkia concinna* would express high inbreeding depression. Which was the observed result in 2 out of 3 populations tested for each species, supporting the results observed in the meta-analysis of inbreeding depression presented in chapter 1. Based on this, pollinators might be driving inbreeding depression, just not in the way that was expected based only on the behavior of their main pollinator.

Pollinators determine pollen movement within and between populations. The bees and beeflies that pollinate *C. concinna* are more reliable while hawkmoths are more sporadic and

might show year to year variation. *Clarkia breweri* is pollinated by hawkmoths but it is also visited by other small insects (Miller et al., 2014), although these small pollinators are less efficient (Kay et al., 2018). These small insects might be pollinating *Clarkia breweri* more often than expected and therefore contributing to inbreeding levels and therefore the expression and purging of deleterious alleles. More pollinator observations in these populations and evaluations of progeny after a single visit might help to determine how efficient small pollinators are and how much are contributing to inbreeding levels in the populations. The high inbreeding depression observed for *Clarkia concinna* indicates that there is a high genetic load in these populations. The results obtained here suggest that bees and bee flies might be doing a great job maintaining inbreeding levels low within a population, and even between close populations. Future research should focus on understanding how pollinators are acting in both species and their influence on the mating system across different years. Understanding how pollinators are acting in the populations is important to determine the role they play in inbreeding and inbreeding depression.

Variation in the Inbreeding depression across populations

The variation across population suggest that traits other than pollinators can play important role in determining the expression of inbreeding depression. To understand the impact of inbreeding depression it is important to be familiar with the traits that can influence previous and current inbreeding rates within a population, including population size, effective population size, the proximity to other populations, historic bottlenecks etc. A population that has a small effective population size will have higher inbreeding than a population with large effective population size (Ellstrand and Elam, 1993; Newman and Pilson, 1997). In these Mediterranean annuals, populations size will vary from year to year, and this variation can lead to population

bottlenecks and differences in the mating system across generations. Small populations might experience more inbreeding and express inbreeding depression more often than large populations (Ellstrand and Elam, 1993; Frankham, 2005). Since census size does not always match effective population size, it is possible a large population in the year of the collection might be coming from a small inbred population, leading to the wrong expectations related to inbreeding and overall population genetic patterns. Self-pollination through geitonogamy can also become more frequent in small populations, increasing populations' selfing rate. Population differentiation might also lead to differences on the rates on inbreeding, isolated populations are more likely to have higher inbreeding, and therefore are more likely to have purged some of the genetic load (ElIstrand, 1992; Ellstrand and Elam, 1993; Theodorou and Couvet, 2002).

Species and population traits can also influence on inbreeding depression such as generational time, breeding system, mating system. Meta-analysis has evaluated how this traits influence on inbreeding depression showing that perennials and self-compatible have higher inbreeding depression (Angeloni et al., 2011a) and that mainly selfing populations have reduced inbreeding depression compared to mixed or outcross selling system (Winn et al., 2011). Different combination of plant traits might influence overall inbreeding depression, and often lead to differences between populations. More studies should focus on population comparisons to understand differences within a population and to understand how different factors can influence on inbreeding depression.

An alternative explanation for differences in plant mating

Clarkia species have been used as a model system in evolutionary biology. They have been used to understand color variation, genetics, mating system evolution, and there is extensive

literature related to *Clarkia* species and variation across populations. One of the early focus on *Clarkia* (along with other Onagraceae species) has been on the presence of reciprocal translocations present at least in *Clarkia williamsonii*, *Clarkia speciosa*, *Clarkia elegans* and *Clarkia unguiculata* (Lewis, 1951; Mooring, 1958; Wedberg et al., 1968; Bloom, 1974). Reciprocal translocations, which is the transfer of genetic material between homologous chromosomes are somewhat common in plants within Onagraceae (Wedberg et al., 1968; Grant, 1975; Raven, 1979) and can vary in frequency within and between populations or even they can get fixated in the populations. For example, in *Clarkia unguiculata*, populations have translocations for four, six or eight chromosomes, with variation across different populations and within populations (Mooring, 1958). Translocations will complicate mating between individuals. If two individuals with different translocation are crossed, they might have set less seed due to unbalance chromosome arrangements, while the mating between two individuals (or the same individual), with the same chromosomal arrangement, will not have this issue. Even though translocations have not been evaluated in *Clarkia concinna* or *Clarkia breweri*, the poor performance of some outcross lines in some populations could indicate incompatibilities might exist within these species. This is somewhat supported by the lack of consistent and meaningful results in population genetic patterns using RADseq data for both species (Diaz-Martin, Cisternas, Fant personal communication). If translocations are present in these species, this could help us to understand some of the fitness differences observed here. More research trying to understand the influence that translocations have on plant mating it is necessary to understand how they could influence inbreeding depression.

Conclusions

Inbreeding depression is a fascinating subject. Differences in inbreeding depression can be observed between populations of the same species, within families of the same population, and even between different environmental conditions. Understanding and recognizing what produces inbreeding depression and if any factors will influence its expression is important. Meta-analyses focusing on inbreeding depression (see chapter 1) have helped us to understand how different factors influence inbreeding depression, but there remains high variability across different factors. A combination of interacting factors is likely what leads to the differences in inbreeding depression, might help to understand the variation. More studies focusing on multiple populations of the same species and providing background information of those populations should be done to understand the variability and to find overall patterns for a species. Pollinators move the pollen between individuals of a population therefore determining the amount of selfing or outcrossing. Here I evaluated inbreeding depression across populations with different main pollinators. It was expected that bee-pollinated populations would express reduced inbreeding depression since they have experience more inbreeding than hawkmoth-pollinated populations, however, that was not what I found. *Clarkia concinna* had higher levels of inbreeding depression compared to *Clarkia breweri*, in the greenhouse and in the growth chamber, although with no significant differences. These results do not match the expectations based on pollinator behavior but they do match the results obtained in Chapter 1, were moth-pollinated populations accumulated more inbreeding on average than bee-pollinated populations. Populations show different results of inbreeding depression, contributing to the idea that inbreeding depression is population-specific. To understand the role that pollinators play on inbreeding depression pollinator observation and mating system evaluation should be done.

FIGURES AND TABLES

CHAPTER 1

Table 1. 1 Summary table of the number of studies in each of the datasets in this chapter.

Data set	Subdivision	Category	Number of studies
Inbreeding coefficient (F_{IS})	Pollinators	Bees	53
		Beetles	6
		Flies	5
		Wasp	11
		Thrips	4
		Bumblebees	23
		Moths	14
		Birds	13
		Bats	12
		Generalist	28
		Abiotic	23
	Pollinators by body size	Extra small	15
		Small	61
		Medium	10
		Large	31
		Extra large	24
	Breeding system	Self-incompatible	67
		Self-compatible	98
Inbreeding depression (F)	Pollinators	Bees	79
		Beetles	4
		Flies	24
		Wasp	1
		Bumblebees	23
		Butterflies	9
		Moths	17
		Birds	12
		Generalist	16
		Wind	2
	Pollinators by body size	Extra small	1
		Small	107
		Medium	27
		Large	22
		Extra large	12
	Breeding system	Self-incompatible	47
		Self-compatible	147

Table 1. 2 Table of model outcomes for F_{IS} dataset representing 151 studies with information about pollinator and breeding system. Definition of each term can be found in the methods.

Factor	**Df**	**F**	**P-value**
Pollinator	9, 177	0.51	0.869
Breeding system	1, 185	4.95	0.027
Pollinator + Breeding system	10, 176	1.03	0.416
Pollinator * Breeding system	17, 169	1.10	0.356

Table 1. 3 Table of model outcomes for inbreeding depression (F) dataset representing 187 studies with information about pollinator and breeding system.

Factor	**Df**	**Q_E**	**P-value**	**τ^2**	**I^2**	**Amount of heterogeneity explained by factors**
No moderators	150	158,378.48	<0.001	0.0689	99.96%	-
Pollinator	141	43,392.75	<0.0001	0.0651	99.88%	5.5%
Breeding system	143	45,411.53	<0.0001	0.0613	99.92%	11%
Pollinator + Breeding system	140	21,221.58	<0.0001	0.0588	99.85%	15%
Pollinator * Breeding system	131	15,020.71	<0.0001	0.0595	99.79%	13.6%

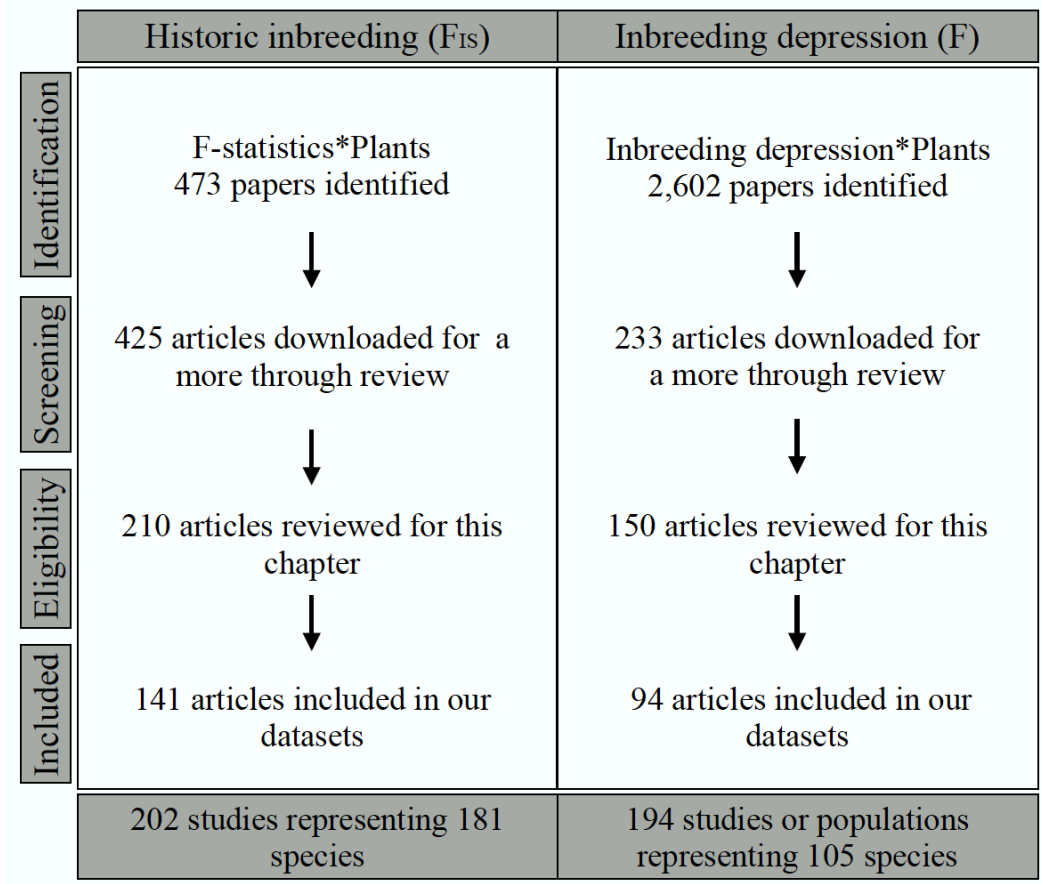

Figure 1. 1 PRISMA flow diagram for study selection, panel in the left represents the diagram for historic inbreeding (F_{IS}) and panel in the right represents the diagram for Inbreeding depression dataset (F).

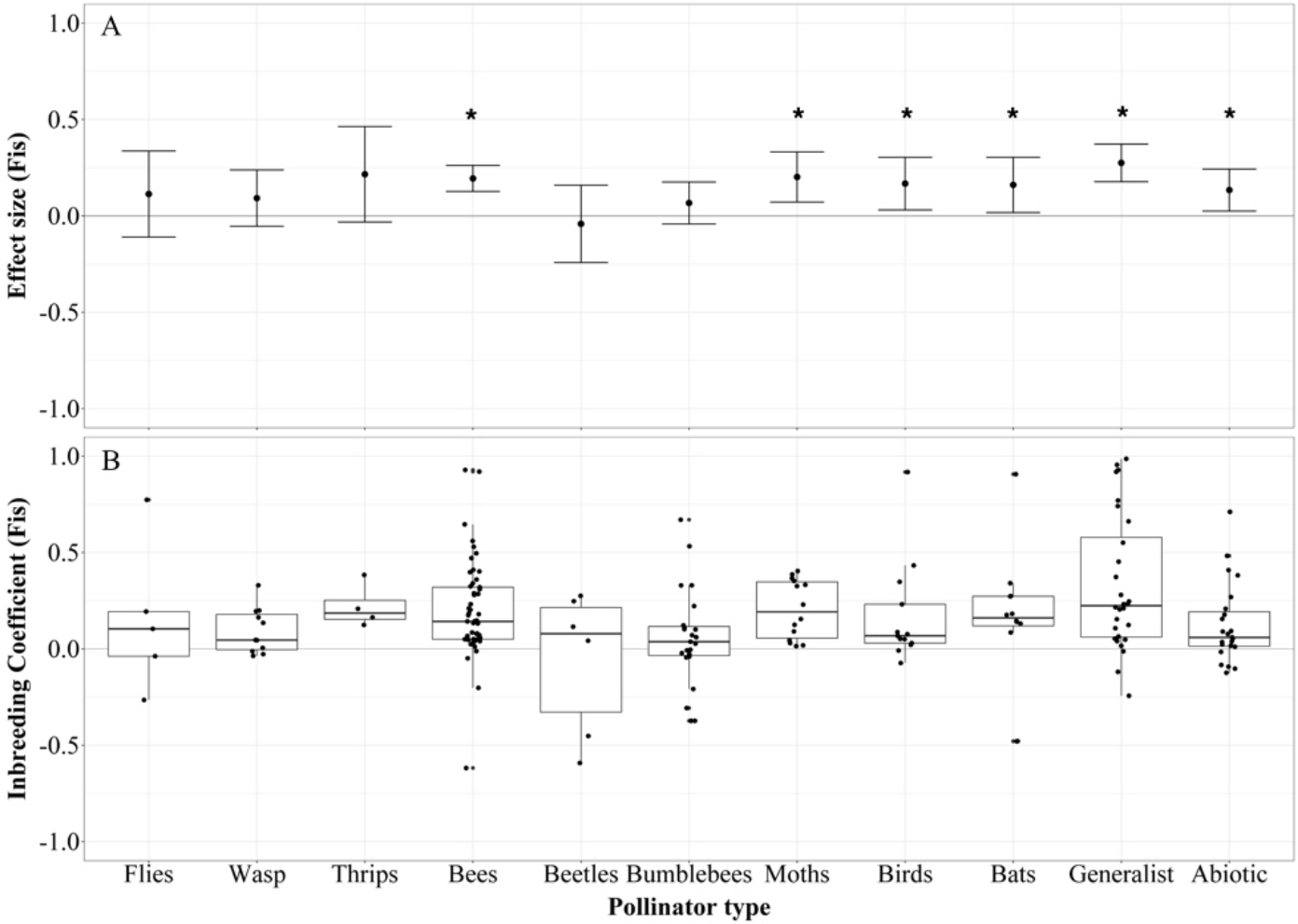

Figure 1. 2 Influence of pollinators on inbreeding coefficient (F_{IS}) using a meta-analysis approach (A) and analysis of variance (B). Asterisk indicate categories with an estimate value and confidence intervals different from zero.

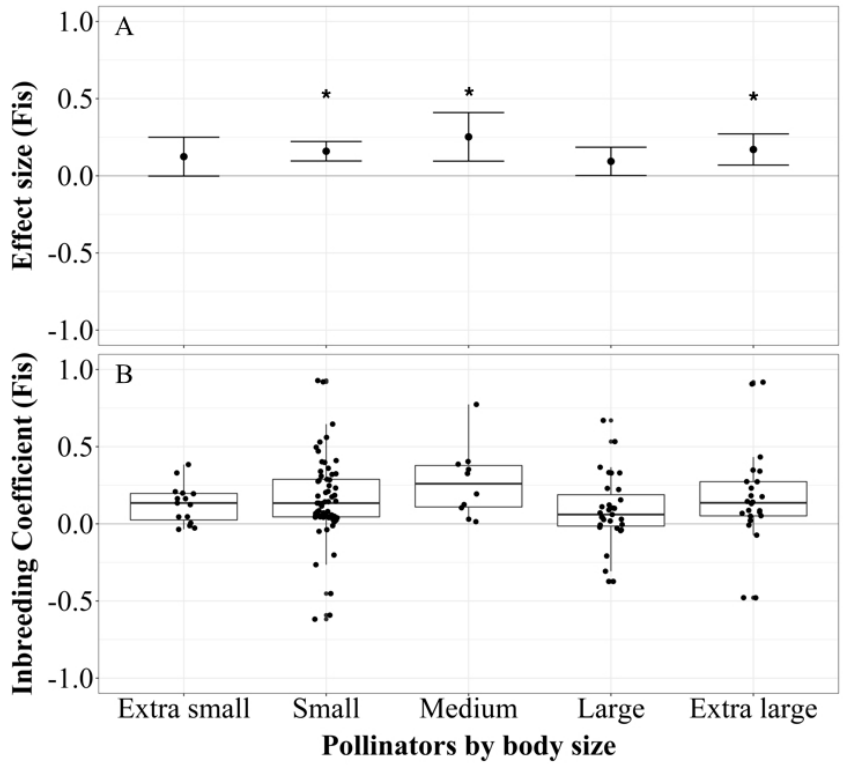

Figure 1. 3 Influence of pollinators classified according their body size on inbreeding coefficient (F_{IS}) using a meta-analysis approach (A) and analysis of variance (B).

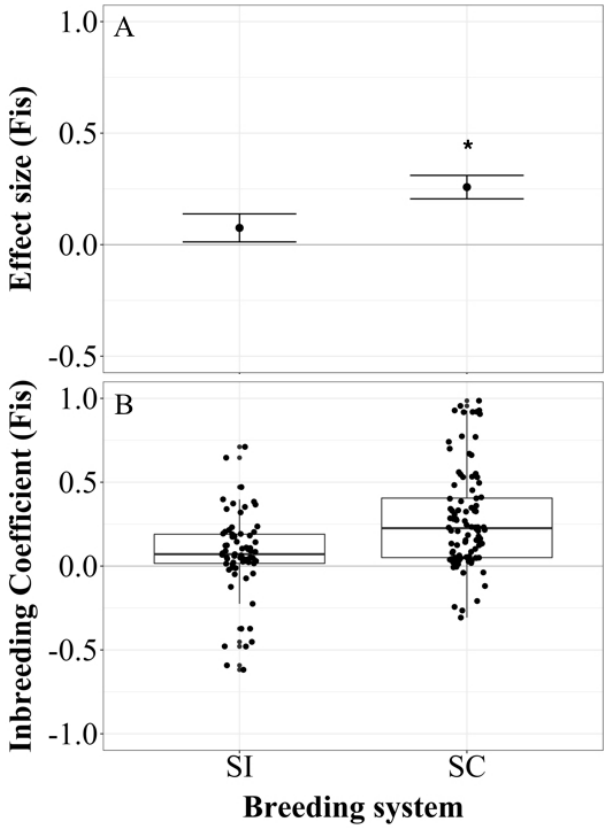

Figure 1. 4 Influence of breeding system on inbreeding coefficient (F_{IS}) using a classic meta-analysis (A) and analysis of variance (B).

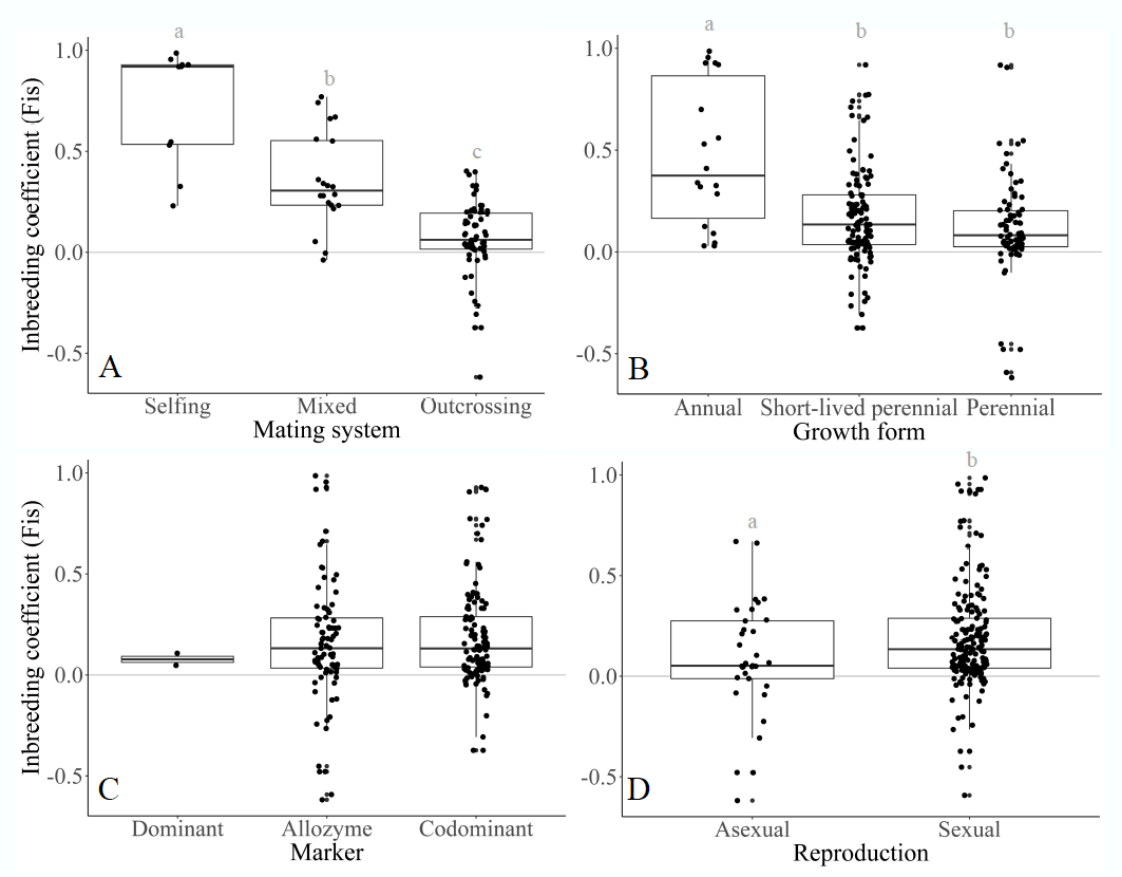

Figure 1. 5 Inbreeding coefficient (F_{IS}) related to other life-traits used in this study: mating system (A), growth form (B), type of molecular marker (C) and reproductive strategy (D)

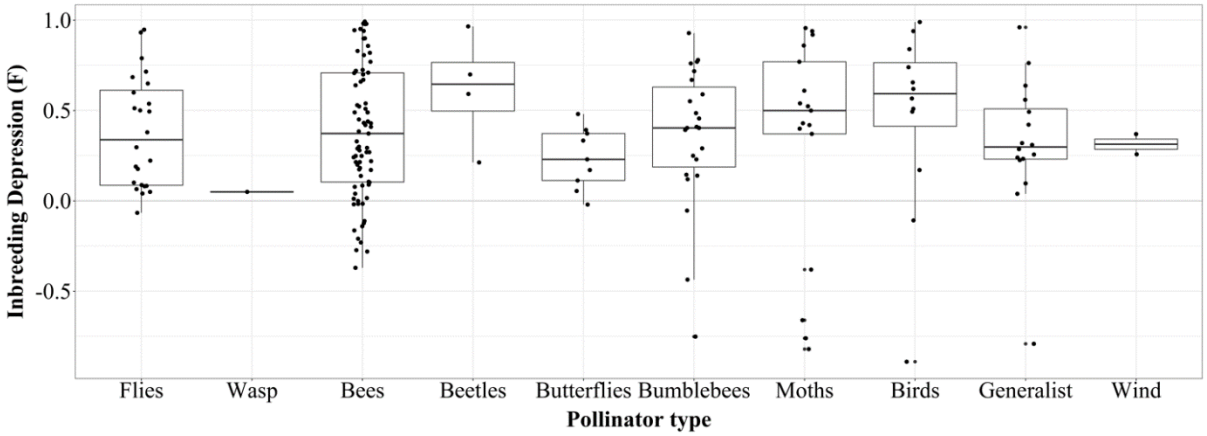

Figure 1. 6 Influence of pollinator categories on inbreeding depression (F).

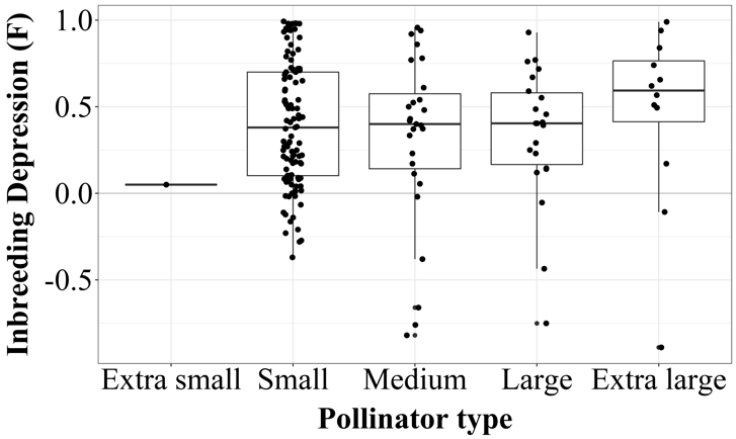

Figure 1. 7 Influence of pollinator classified according their body size on inbreeding depression (F).

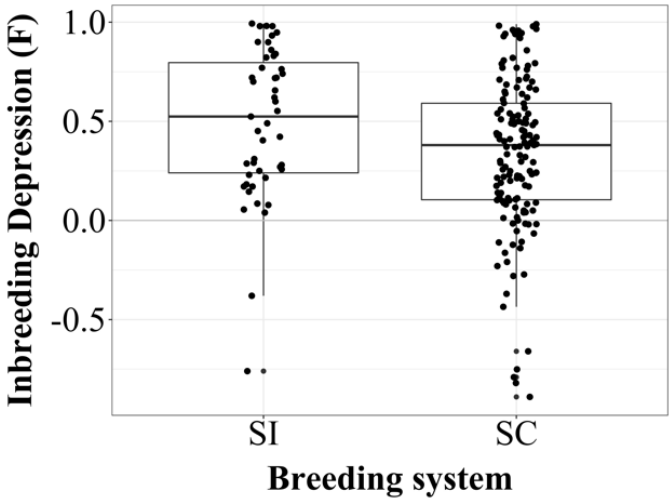

Figure 1. 8 Influence of breeding system on inbreeding depression (F).

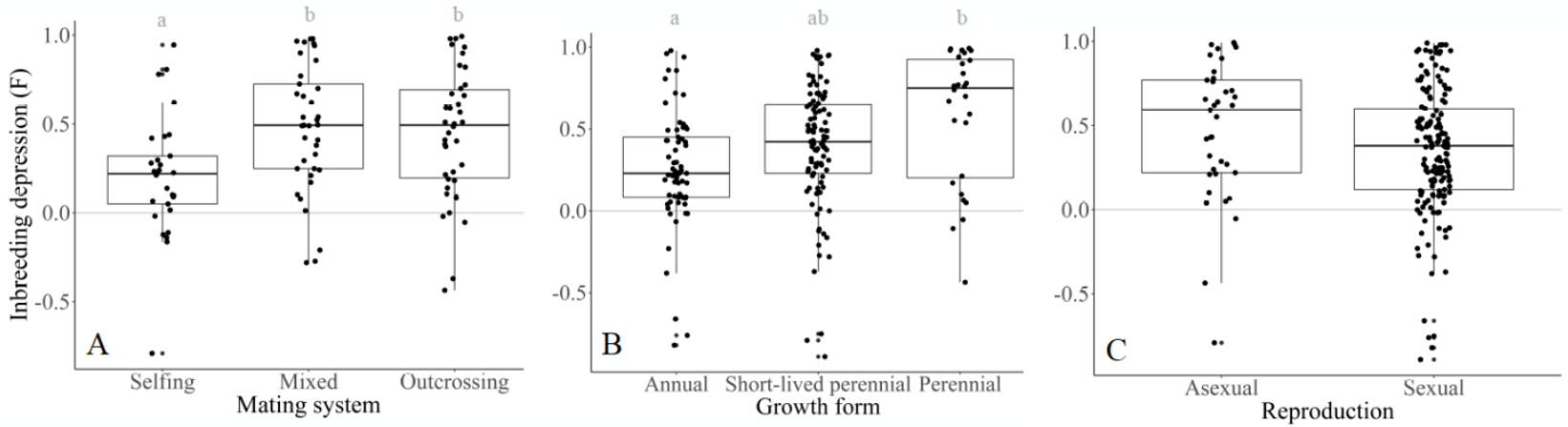

Figure 1. 9 Inbreeding depression (F) related to other life-traits used in this study: mating system (A), growth form (B) and reproductive strategy (C)

CHAPTER 2

Table 2. 1 *Oenothera primiveris* populations included in the study and summary of data that were collected in the field (F) or in growth-chamber experiments (G): floral scent (S), floral morphology (M) and pollinator observations (PO), population genetic parameters (PG), and breeding system assessments (BS). Flower diameter and herkogamy are reported in mm and standard error is provided in parentheses.

Population ID	Location	Latitude and Longitude	Collectors number	Data collected	Flower diameter (mm)	Herkogamy (mm)	Bawa index	SCI	Nature of the breeding system
Pop 1	Eureka Dunes, CA	Lat: 37.1189 Long:-117.672	LOL 590	F: S, M, PO G: PG, BS, M	F: 53.83 (1.3) G: 57.47 (1.36)	F: 7.98 (0.63) G: 7.6 (0.6)	0.22	0.13	SI/SC
Pop 2	Nipton Rd, NV	Lat:35.463 Long: -115.319	LOL 183	F: S, M, PO G: PG, BS, M	F: 60.25 (1.58) G: 59.58 (1.8)	F:10.53 (0.51) G:5.51 (0.57)	0.77	0.39	SI/SC
Pop 3	T-Bone Hill, UT	Lat: 37.133 Long: -113.582	LOL 201	F: S, M, PO G: PG, BS, M	F: 71.45 (2.05) G: 59.88 (3.27)	F: 10.91 (0.94) G: 9.04 (2.06)	0.49	0.286	SI/SC
Pop 4	Hackberry Rd, AZ	Lat: 35.186 Long: -113.627	LOL 240	F: S, M, PO G: PG, BS, M	F: 31.33 (0.83) G: 30.47	F: 2.07 (0.65) G:0.6 (0.37)	1.02	0.69	SI/SC

					(1.02)				
Pop 5	Whetstone Mt, AZ	Lat: 31.814 Long: -110.551	LOL 584	F: S, M	F: 36.02 (2.05)	F: 1.19 (1.32)	NA	NA	NA
Pop 6	Dona Ana, NM	Lat: 32.472 Long: -106.799	LOL 579	G: PG, BS, M	G: 38.47 (1.23)	G: 0.85 (0.49)	0.73	0.69	SC
Pop 7	White Box canyon, NM	Lat: 32.587 Long: -108.817	LOL 583	G: PG, BS, M	G: 38.84 (1.41)	G: -0.31 (0.51)	0.94	0.81	SC
Pop 8	Aguirre Springs, NM	Lat: 32.391 Long: -106.535	LOL 604	G: BS, M	G: 34.38 (1.12)	G: -0.28 (0.3)	1.24	0.77	SC

Table 2. 2 Genetic diversity table of *O. primiveris* by population.

Population ID	Population size	Sample size	% P	N	N_A	H_O	H_E	F_{IS}	N_E	*CI 95%*
Pop 1	Medium	20	61.7	1.62	1.21	0.13	0.13	0.03	47.4	39 - 60
Pop 2	Medium	20	45.9	1.46	1.18	0.09	0.11	0.13	32.3	27 - 40
Pop 3	Large	20	54.2	1.54	1.19	0.10	0.12	0.12	28.2	24 – 34
Pop 4	Large	20	21.5	1.22	1.11	0.08	0.06	-0.15	11.8	9 - 16
Pop 6	Small	19	16.6	1.17	1.08	0.02	0.05	0.58	3.8	3 - 6
Pop 7	Small	20	18.3	1.18	1.08	0.02	0.05	0.55	16.1	12 - 22

%P: percentage of polymorphism, mean number of alleles per locus (N), N_A: Number of effective alleles, H_O: Observe heterozygosity, H_E: Expected heterozygosity, F_{IS}: Inbreeding coefficient, N_E: Estimated effective population size, CI 95%: confidence interval for estimated effective population size.

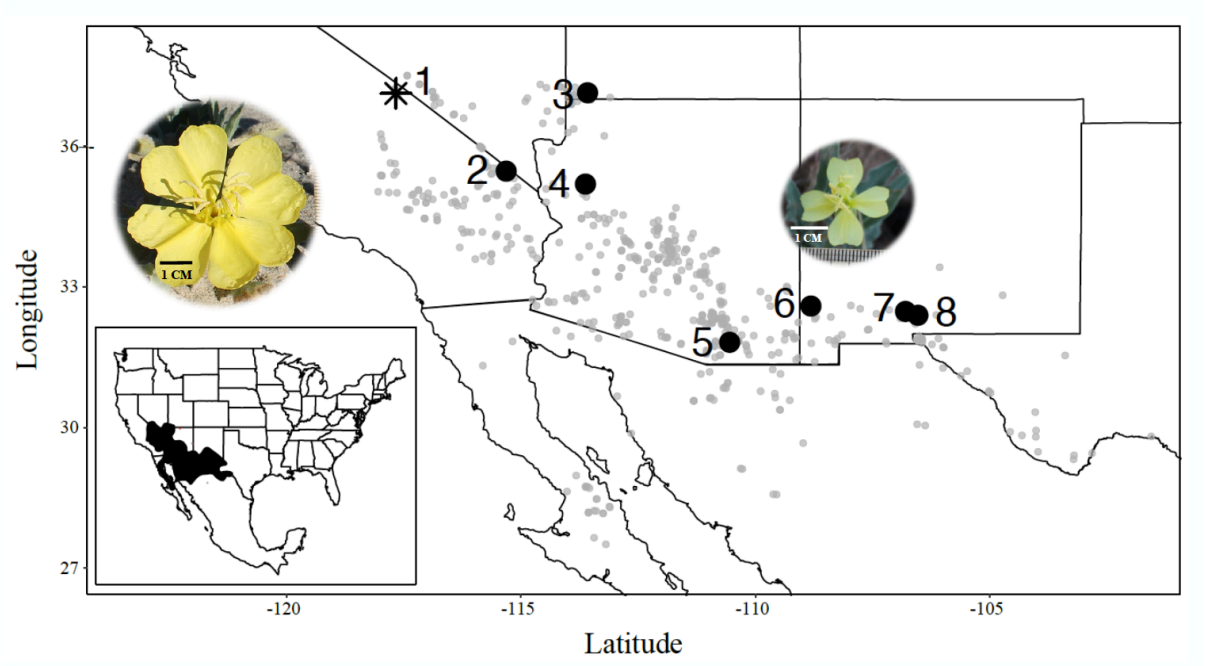

Figure 2. 1 Distribution of *Oenothera primiveris* in western United States and northern Mexico. Gray circles represent herbarium records for the species (Global Biodiversity Information Facility, 2020). Black circles and the asterisk represent sampled populations. The asterisk represents Eureka Dunes, the only population described to be fully self-incompatible.

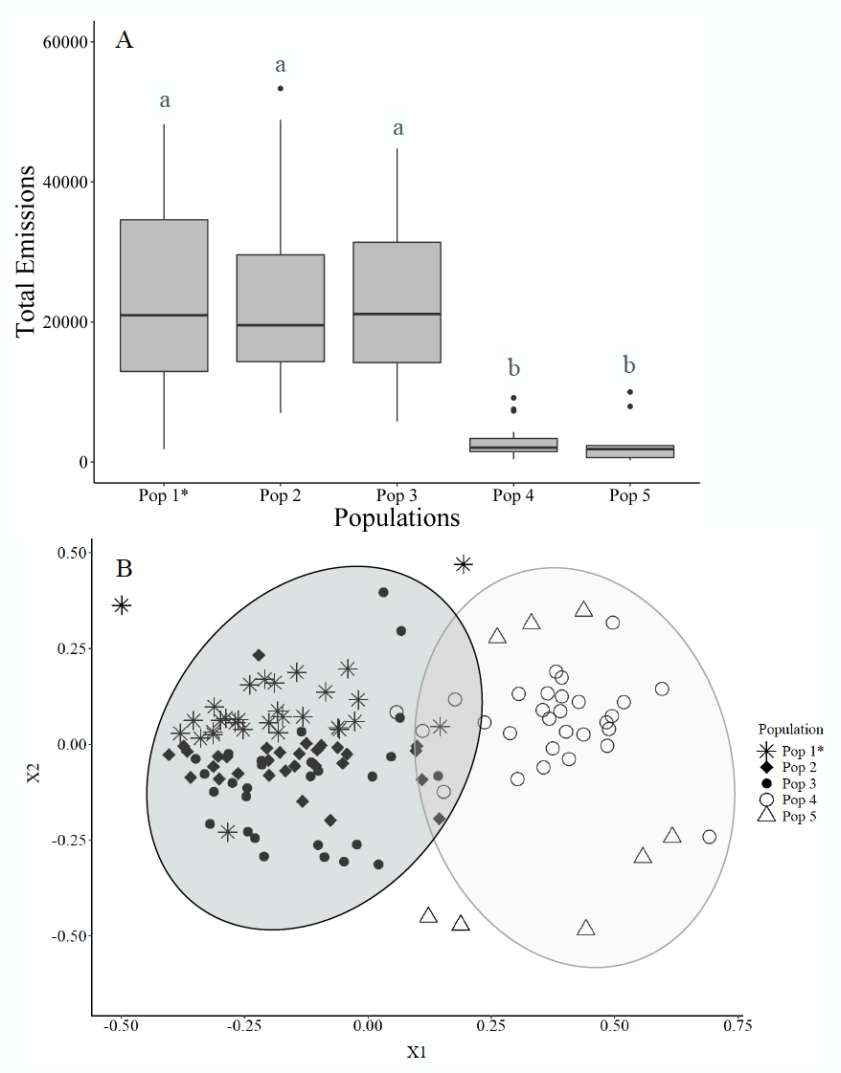

Figure 2. 2 Population variation in total amount of scent emission and scent composition from plants sampled in the field. (A) Total amount of scent produced, measured as toluene equivalents. (B) Scent compositions graphed on two axes, different symbols represent the different populations and different color indicate flower diameters (Large flowers: grey ellipses, small flowers: white ellipses).

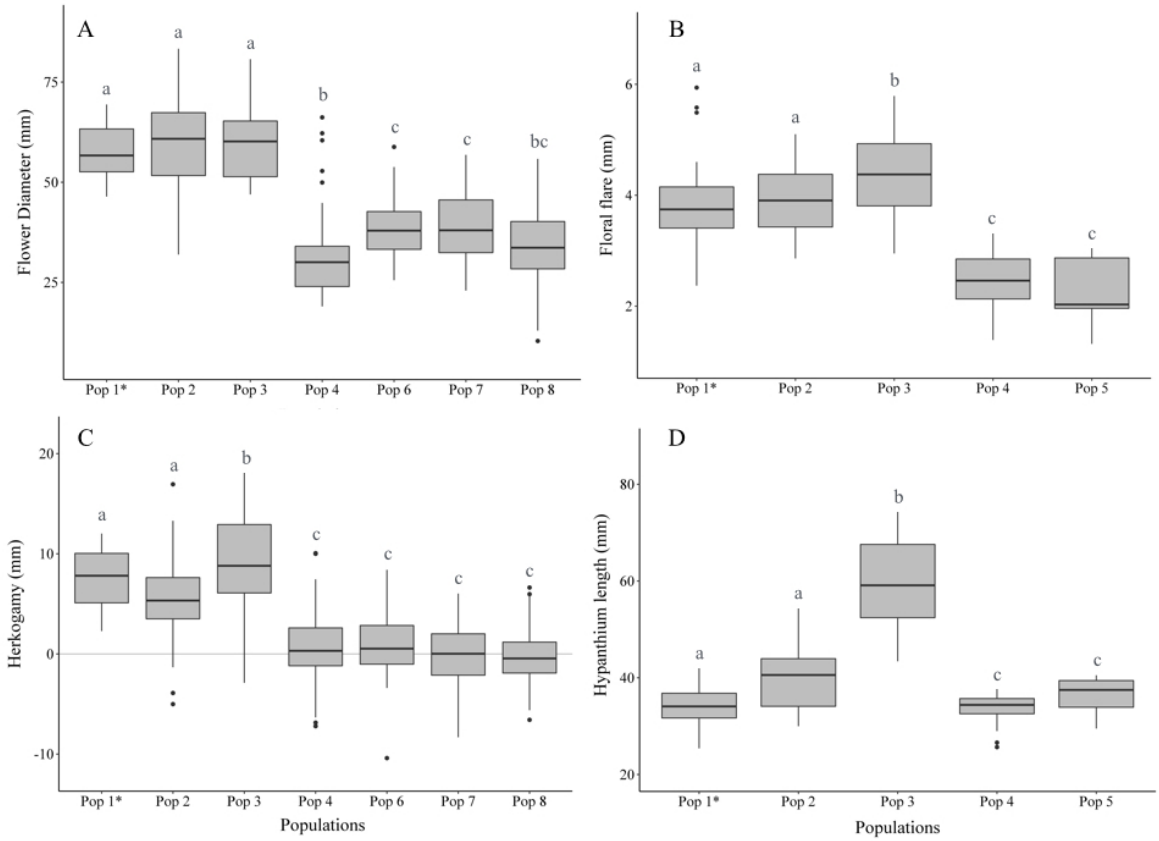

Figure 2. 3 Variation in (A) floral diameter and (B) herkogamy of populations evaluated in the growth chamber experiment. Variation in (C) floral flare and (D) floral tube length measured in the field. Distribution that shares a letter are not statistically significally different according to Tukey post-hoc test.

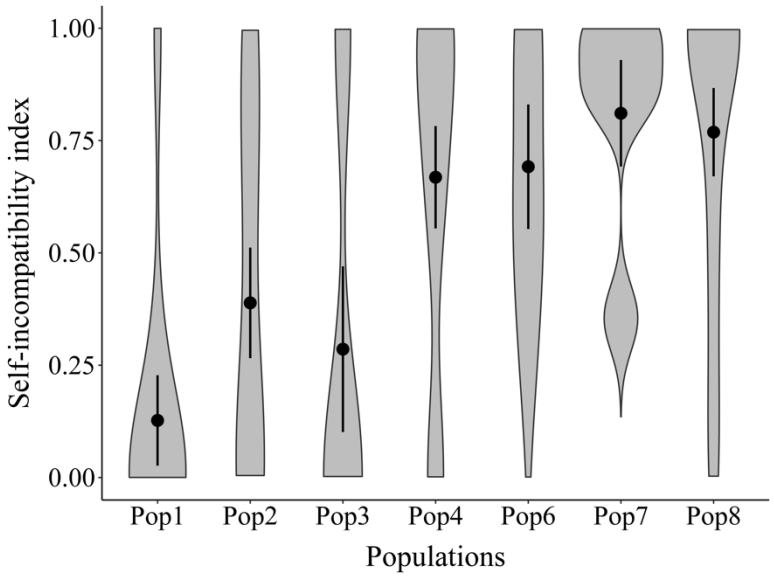

Figure 2. 4 Variation of the self-incompatibility index across populations of *Oenothera primiveris*. Mean SCI and standard error are represented in the figure.

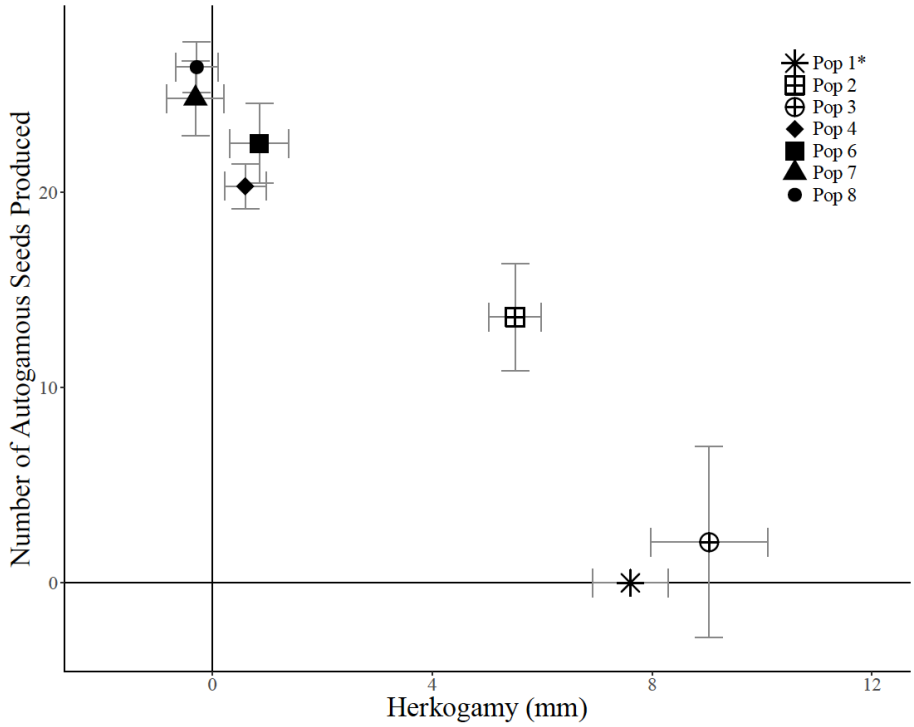

Figure 2. 5 Correlation between number of seeds produce through autogamous self-pollination and mean herkogamy value for each population, represented by different symbols. Error bars show SE for both traits evaluated. Pearson's correlation coefficient= -0.968, P= 0.0003.

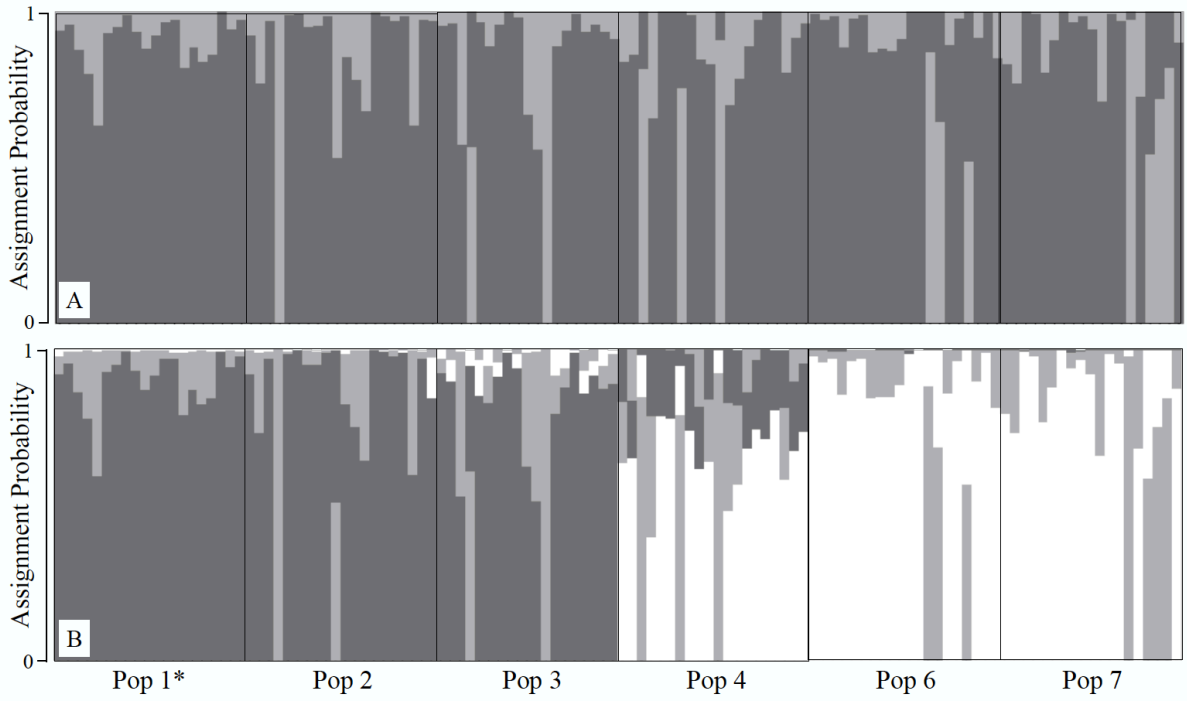

Figure 2. 6 Population differentiation detected among 6 populations of *O. primiveris*. (A) ΔK=2 represent the most likely differentiation although (B) ΔK=3 showed a lower peak and represents an incipient differentiation.

CHAPTER 3

Table 3. 1 Summary of results obtained in Chapter 2 by populations of *Oenothera primiveris*. Including, maternal lines used to evaluate populations breeding system, breeding system estimators (Bawa index, and self-compatibility index), mean floral diameter (SE), and genetic parameters, (inbreeding coefficient (Fis), expected heterozygosity (He) and effective population size (Ne).

Population ID	**Maternal lines**	**Bawa index**	**Self-compatibility index (+/- SE)**	**Flower size**	**Inbreeding coefficient (F_{IS})**	**Expected heterozygosity**	**Effective population size**
Pop 1	10	0.22	0.13 ± 0.1	57.5 ± 1.4	0.03	0.13	47.4
Pop 2	12	0.77	0.39 ± 0.12	59.6 ± 1.8	0.13	0.11	32.3
Pop 3	7	0.49	0.29 ± 0.18	59.9 ± 3.3	0.12	0.12	28.2
Pop 4	13	1.02	0.67 ± 0.1	30.5 ± 1.0	-0.15	0.06	11.8
Pop 6	5	0.73	0.69 ± 0.3	38.5 ± 1.2	0.58	0.05	3.8
Pop 7	5	0.94	0.81 ± 0.12	38.8 ± 1.4	0.55	0.05	16.1
Pop 8	13	1.24	0.77 ± 0.1	34.4 ± 1.1	NA	NA	NA

Table 3. 2 Summary of number of maternal lines and individuals grow in generation G0 and G1, including number of flowering individuals in G0, cross type, success (as percentage and seed number), and number of G1 maternal lines (and individuals) used to evaluate inbreeding depression. Different maternal lines were used for self and outcross treatments.

Population ID	**G0 Lines (# individuals)**	**Lines flowered (# individuals)**	**Ave # flowers**	**Cross**	**Cross success (# crosses)**	**Ave # seed (± SE)**	**G1**	**G1 Lines (# individuals)**
Pop 1	24 (109)	11 (24)	1.96	Self	11% (18)	24.5 ± 6.0	Self/Autogamous	2 (2)
				Autogamous	0% (19)			
				Outcross	50% (8)	29.5 ± 14.8	Outcross	3 (4)
Pop 2	14 (68)	14 (44)	2.29	Self	44% (27)	23.8 ± 4.6	Self/Autogamous	11 (17)
				Autogamous	48% (46)	25.9 ± 5.5		
				Outcross	55% (22)	28.2 ± 8.2	Outcross	6 (12)
Pop 3	13 (64)	7 (13)	2.08	Self	29% (7)	71.5 ± 50.6	Self/Autogamous	4 (4)
				Autogamous	14% (14)	13.5 ± 9.6		
				Outcross	67% (6)	60 ± 30.0	Outcross	4 (4)
Pop 4	15 (138)	15 (95)	3.63	Self	46% (67)	28.9 ± 5.2	Self/Autogamous	10 (18)
				Autogamous	100% (229)	21.3 ± 1.4		
				Outcross	79% (34)	34.1 ± 6.6	Outcross	8 (15)
Pop 6	7 (54)	6 (48)	2.48	Self	73% (30)	30.2 ± 6.4	Self/Autogamous	6 (18)
				Autogamous	87% (75)	26.0 ± 3.2		
				Outcross	100% (8)	31.1 ± 11.0	Outcross	4 (8)
Pop 7	5 (73)	5 (54)	2.74	Self	77% (31)	27.0 ± 5.5	Self/Autogamous	5 (18)
				Autogamous	89% (90)	26.4 ± 2.9		
				Outcross	86% (21)	29.9 ± 7.0	Outcross	5 (13)
Pop 8	15 (116)	15 (95)	2.95	Self	100% (42)	27.5 ± 4.3	Self/Autogamous	10 (18)

Autogamous	94% (188)	28.2 ± 2.1		
Outcross	77% (31)	27.7 ± 5.7	Outcross	9 (15)

Table 3. 3 Measure of Cumulative fitness and independent traits (% viability, % germination, % early survival, % survival to flower and flower number) of selfed and outcrossed maternal lines by populations for *Oenothera primiveris*. Mean fitness value and standard error for 5 fitness stages and estimates of cumulative fitness (see methods for a description on how this was calculated). Inbreeding depression (ID) was calculated for every evaluated stage and values can be found in bold.

Population ID	Cross type	Maternal Lines (# ind)	Cumulative fitness	Seed Viability	Germination	Early survival	Survival to flowering	Flower number
Pop 1	Self	2 (2)	0.79 (0.11)	51% (6%)	56% (0%)	7% (1%)	75% (25%)	6.3 (2.7)
	Out	3 (4)	0.07 (0.07)	57% (15%)	11% (11%)	11% (11%)	33% (33%)	1.7 (1.7)
	$\sigma_{(ID)}$		-0.91	0.11	-0.80	-0.84	-0.56	-0.74
Pop 2	Self	11 (17)	0.49 (0.15)	56% (1%)	36% (7%)	57% (11%)	65% (13%)	3.5 (0.7)
	Out	6 (12)	0.52 (0.30)	56% (13%)	26% (6%)	48% (17%)	61% (2%)	3.0 (1.3)
	$\sigma_{(ID)}$		0.06	-0.001	-0.29	-0.15	-0.05	-0.15
Pop 3	Self	4 (4)	0.20 (0.07)	46% (16%)	44% (16%)	64% (22%)	34% (14%)	1.7 (0.7)
	Out	4 (4)	0.20 (0.15)	65% (13%)	24% (17%)	41% (25%)	24% (14%)	1.4 (0.8)
	$\sigma_{(ID)}$		-0.01	0.3	-0.45	-0.35	-0.3	-0.17
Pop 4	Self	10 (18)	0.14 (0.10)	56% (8%)	32% (9%)	42% (12%)	35 (12)	0.8 (0.3)
	Out	8 (15)	0.24 (0.05)	66% (12%)	34% (8%)	68% (12)	57 (12)	2.72 (0.6)
	$\sigma_{(ID)}$		0.42	0.14	0.07	0.39	0.39	0.72
Pop 6	Self	6 (18)	0.14 (0.06)	47% (14%)	24% (7%)	40% (14%)	51% (17%)	2.2 (0.8)
	Out	4 (8)	0.01 (0.01)	15% (7%)	6% (4%)	13% (13%)	25% (25%)	0.5 (0.5)
	$\sigma_{(ID)}$		-0.94	-0.69	-0.75	-0.69	-0.50	-0.77
Pop 7	Self	5 (18)	0.07 (0.04)	53% (9%)	23% (12%)	42% (12%)	40% (19%)	2.8 (1.7)
	Out	5 (13)	0.24 (0.09)	62% (9%)	31% (9%)	53% (16%)	55% (18%)	2.57 (0.7)
	$\sigma_{(ID)}$		0.70	0.15	0.25	0.21	0.27	-0.1
Pop 8	Self	10 (18)	0.19 (0.08)	52% (8%)	29% (8%)	42% (12%)	31% (1%)	1.9 (0.7)
	Out	9 (15)	0.74 (0.17)	82% (4%)	43% (6%)	61% (9%)	73% (11%)	3.5 (0.7)
	$\sigma_{(ID)}$		0.74	0.36	0.32	0.32	0.58	-0.1

Table 3. 4 Summary statistic for traits measured and cumulative fitness against population, cross type (Self/Out) and interaction. Significant values are in bold.

Life stage	Source of variation	Df	F	P-value
Seed viability	Cross	1, 26	1.99	0.17
	Population	6, 47	1.24	0.30
	Cross*Population	6, 26	1.62	0.18
Germination	Cross	1, 26	0.35	0.56
	Population	6, 47	0.65	0.68
	Cross*Population	6, 26	1.5	0.23
Early survival	Cross	1, 26	0.01	0.93
	Population	6, 47	0.78	0.59
	Cross*Population	6, 26	1.45	0.23
Survival to flowering	Cross	1, 26	0.54	0.47
	Population	6, 47	0.99	0.44
	Cross*Population	6, 26	1.38	0.26
Flower number	Cross	1, 26	0.34	0.56
	Population	6, 47	1.04	0.41
	Cross*Population	6, 26	2.5	**0.05 ***
Cumulative fitness	Cross	1, 26	1.76	0.19
	Population	6, 47	2.16	**0.06**
	Cross*Population	6, 26	3.24	**0.03 ***

Table 3. 5 Summary statistic for traits measured and cumulative fitness against breeding system (SCI), cross type (Self/Out) and interaction. Significant values are in bold.

Life stage	Source of variation	Df	F	P-value
Seed viability	Cross	1, 30	2.33	0.14
	Breeding system	2, 50	0.16	0.85
	Cross*Breeding system	2, 30	1.58	0.22
Germination	Cross	1, 30	0.55	0.46
	Breeding system	2, 50	0.27	0.76
	Cross*Breeding system	2, 30	1.65	0.21
Early survival	Cross	1, 30	0.001	0.97
	Breeding system	2, 50	1.68	0.19
	Cross*Breeding system	2, 50	0.43	0.65
Survival to flowering	Cross	1, 30	0.37	0.55
	Breeding system	2, 50	1.32	0.27
	Cross*Breeding system	2, 30	0.96	0.39
Flower number	Cross	1, 30	0.2	0.66
	Breeding system	2, 50	1.91	0.16
	Cross*Breeding system	2, 30	1.63	0.21
Cumulative fitness	Cross	1, 30	1.22	0.27
	Breeding system	2, 50	0.33	0.72
	Cross*Breeding system	2, 30	3.26	**0.05 ***

Table 3. 6 Aster model comparisons for *O. primiveris.*

Models	Df	Model deviance	Test Df	Test deviance	Test P-value
Population					
Model 1: varb + fn + (cross * pop): fn Model 2: varb + fn + (cross + pop): fn					
1	11	-3225.4			
2	17	-3207.9	6	17.57	0.0074

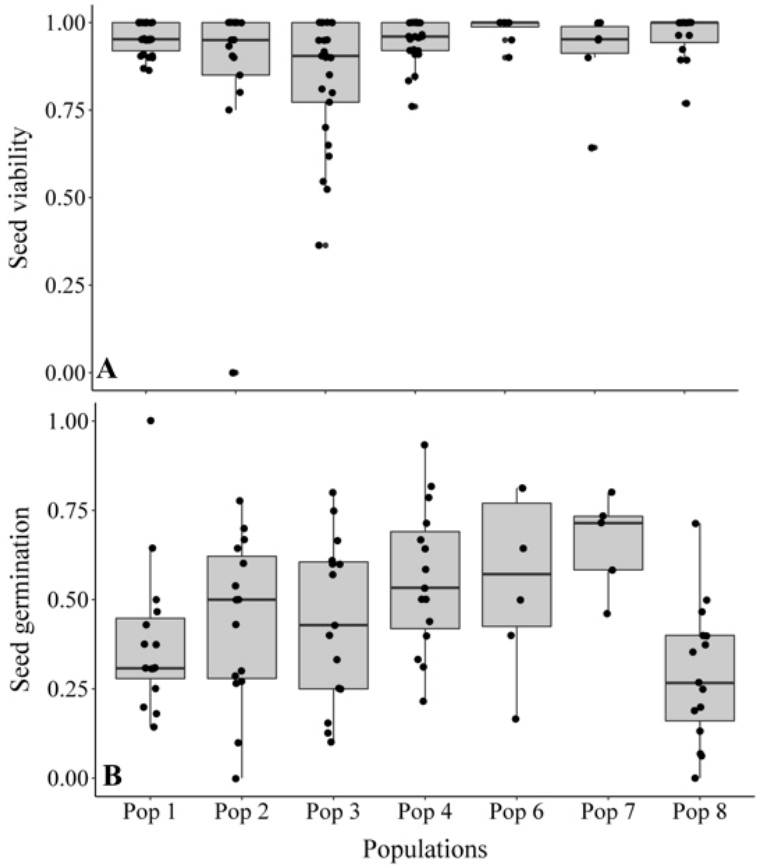

Figure 3. 1 Differences across populations of *Oenothera primiveris* for seed viability and seed germination evaluated in Generation 0.

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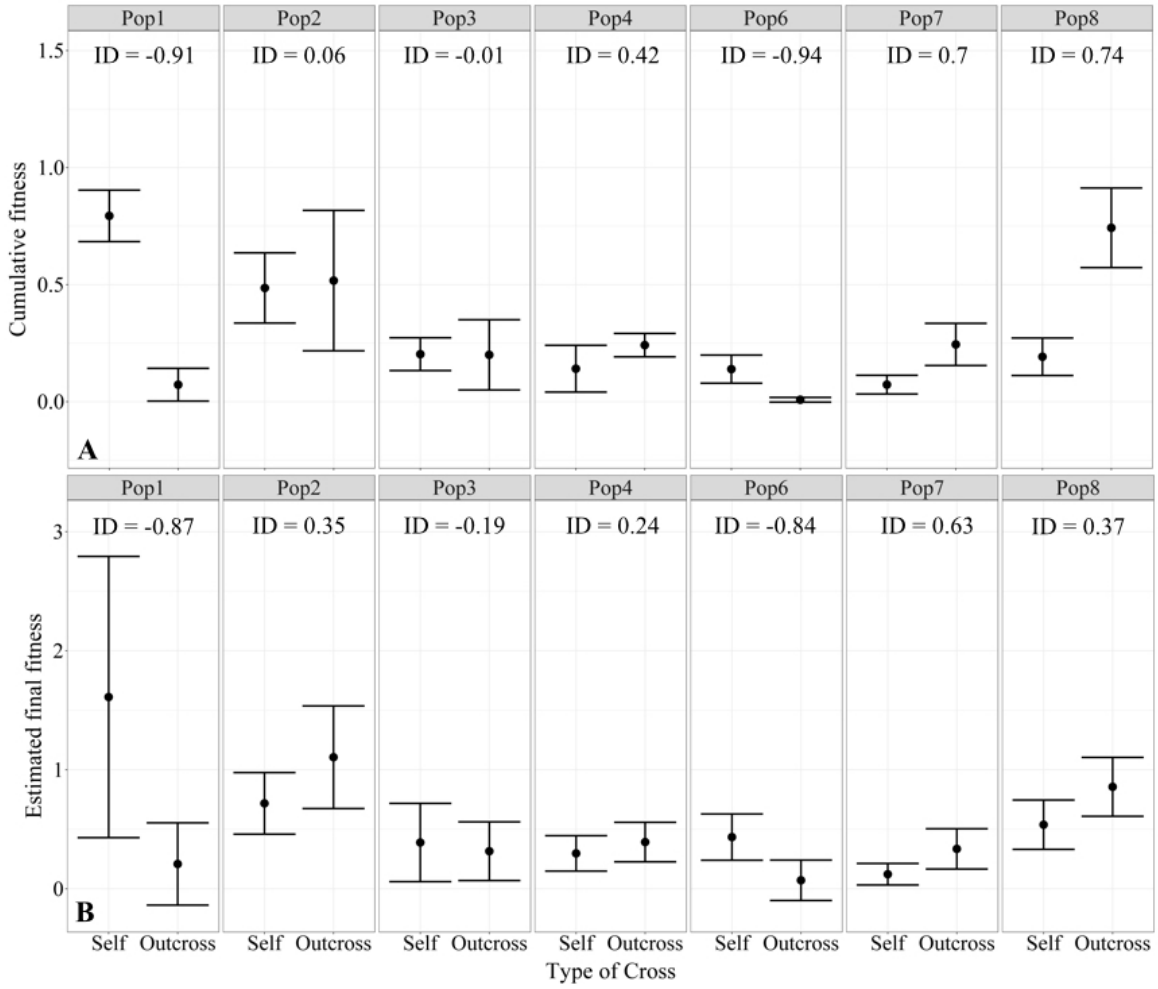

Figure 3. 2 Fitness comparisons between self and outcross lines by populations of *Oenothera primiveris* for A) cumulative fitness and B) ASTER. Estimates of inbreeding depression were calculated (ID).

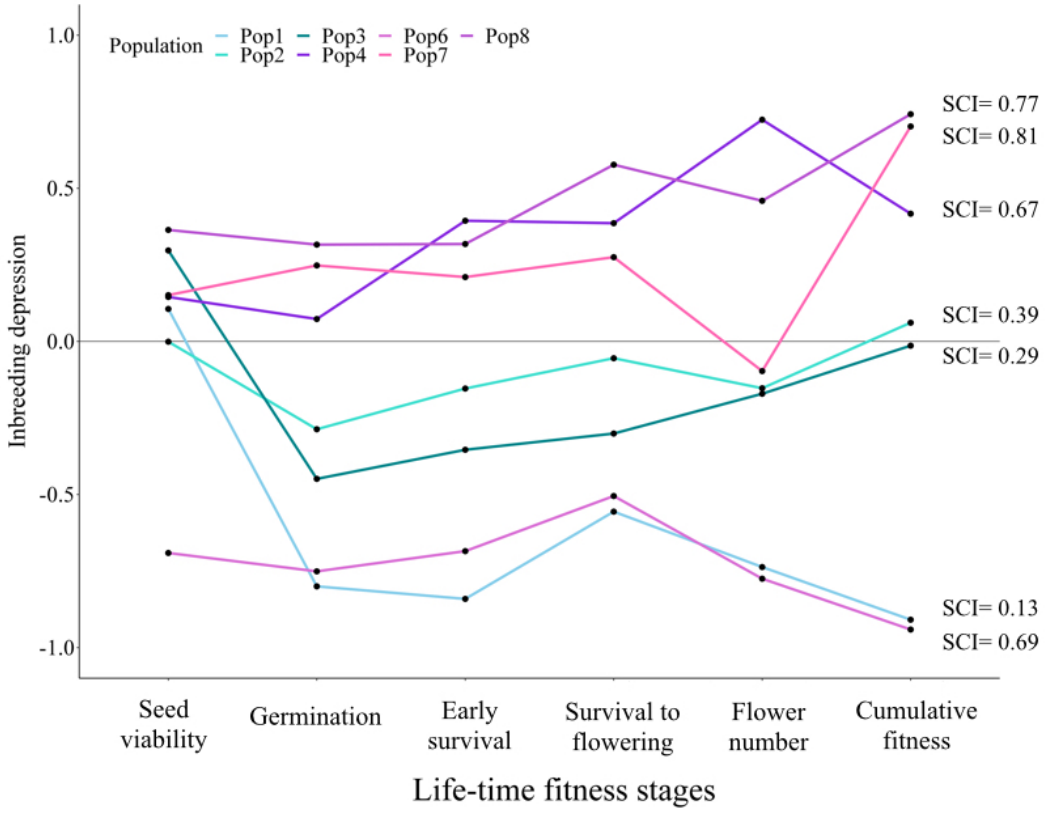

Figure 3. 3 Estimated of inbreeding depression for each fitness stage evaluated in *Oenothera primiveris.* Lines in blue tones represent populations with self-incompatible and self-compatible individuals with large flower size (Pop 1, 2 and 3) and lines with pink tones represent populations that are mainly self-compatible with small flower size (Pop 4, 6, 7 and 8).

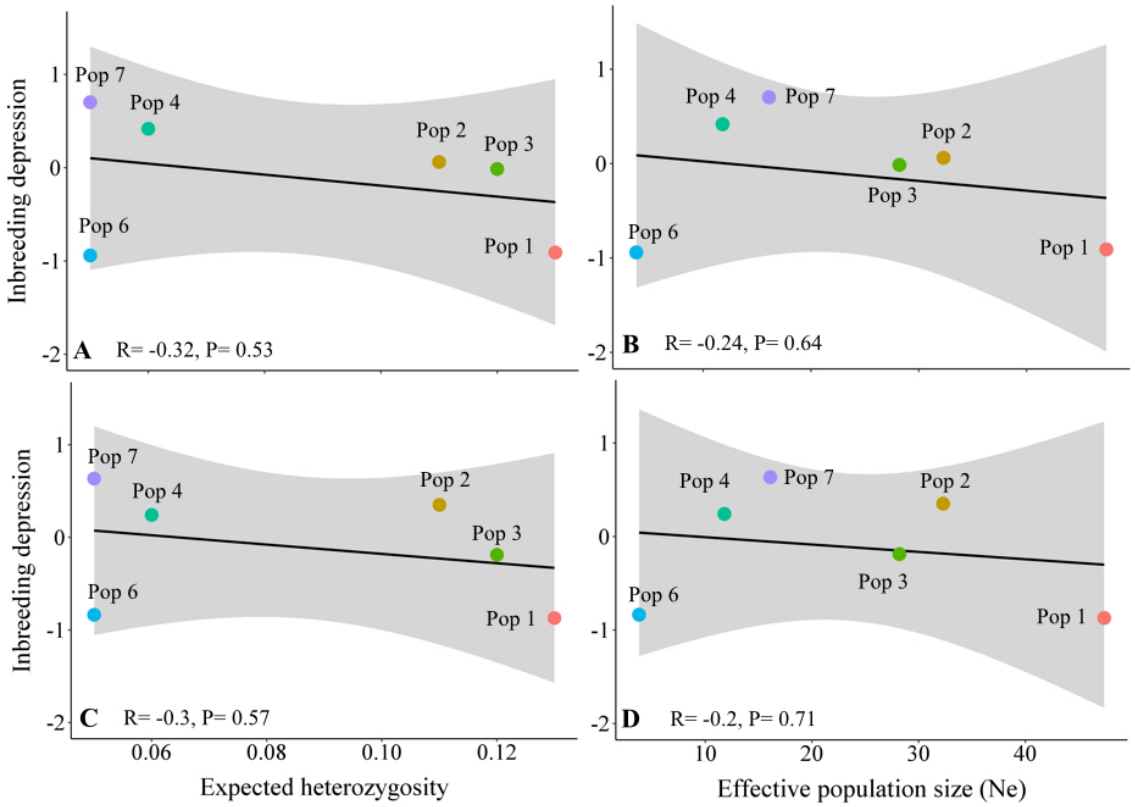

Figure 3. 4 Correlations between inbreeding depression and expected heterozygosity (A, C) and Effective Population Size (B, D) for inbreeding depression obtained through cumulative fitness (A, B) and ASTER estimates of fitness (C, D). Pearson's correlation index (R) and respective P values reported for each correlation.

CHAPTER 4

Table 4. 1 Species used in study, including population name, number, latitude, and longitude. In addition, number of maternal lines grow to generate all cross types, as well as number of lines generated each generation (G1, G2 ND G3), broken down by number of maternal lines (M) and individuals (n) per line in parenthesis, and breakdown of number of maternal lines lost each generation.

Species	Population name	Pop ID	Latitude and Longitude	Mat Lines	Cross	G1 Lines (Mat and #)	G2 Lines (Mat and #)	% Mat Lost	G3 Lines (Mat and #)	% Mat Lost
Clarkia breweri	Mile Marker 8, CA	Pop 2	37.354, -121.558	39	Self	10 (M=10 n=10)	9 (M= 9 n=15)	10%	5 (M=5 n=5)	50%
					Biparental	10 (M=39 n=39)	10 (M=33 n=50)	15%	4 (M=4 n=4)	90%
					Outcross	11 (M=11 n=14)	9 (M= 9 n=14)	18%	4 (M=4 n=4)	71%
	Pinnacles NP, CA	Pop 3	36.483, -121.167	35	Self	10 (M=10 n=10)	8 (M= 8 n=14)	20%	2 (M=2 n=2)	80%
					Biparental	10 (M=35 n=35)	10 (M=25 n=31)	29%	4 (M=4 n=4)	89%
					Outcross	11 (M=11 n=12)	7 (M= 6 n= 8)	45%	0 (M=0 n=0)	100%
	Coalinga Rd, CA	Pop 4	36.015, -120.473	26	Self	10 (M=10 n=10)	5 (M= 5 n= 9)	50%	3 (M=3 n=3)	70%
					Biparental	8 (M=26 n=26)	5 (M=11 n=12)	58%	0 (M=0 n=0)	100%
					Outcross	7 (M= 7 n= 8)	3 (M= 3 n= 3)	57%	2 (M=2 n=2)	75%

Clarkia concinna* subsp. *concinna	Devilhorn Rd, CA	Pop 1	38.823, -122.344	31	Self	10 (M=10 n=10)	2 (M= 2 n= 2)	80%	NA	NA
					Biparental	9 (M=31 n=31)	2 (M= 4 n= 4)	87%	NA	NA
					Outcross	12 (M=12 n=12)	2 (M= 2 n= 2)	83%	NA	NA
	Round Valley, CA	Pop 2	39.816, -122.65	27	Self	10 (M=10 n=10)	2 (M= 2 n= 2)	80%	NA	NA
					Biparental	8 (M=27 n=27)	4 (M= 4 n= 4)	85%	NA	NA
					Outcross	11 (M=11 n=12)	5 (M= 5 n= 5)	55%	NA	NA
	Lower Chiles, CA	Pop 3	38.533, -122.332	35	Self	9 (M= 9 n= 9)	4 (M= 4 n= 4)	56%	NA	NA
					Biparental	10 (M=35 n=35)	6 (M= 8 n= 8)	77%	NA	NA
					Outcross	12 (M=12 n=14)	5 (M= 8 n= 8)	33%	NA	NA

Table 4. 2 Fitness measurements for Generation 1 of three *Clarkia breweri* populations, broken down by cross type and experiment location. Experiment location (Exp) refers to the location that fitness was evaluated, by either being the growth chamber (GC) or greenhouse (GH). This includes, number of family and maternal lineages used. Measurements include, cumulative fitness, percent viable seed, percent germination, percent survival, percent survival to flowering and number of flowers. Inbreeding depression was calculated as described in the methods.

Pop ID	Exp	Cross	Family (& mat) Lines	Cumulative fitness	Viability	Germination	Early survival	Survival to flowering	Flower number
Pop2	GC	Self	10 (10)	1.01 (0.26)	91% (3%)	63% (13%)	70% (11%)	88% (10%)	2.07 (0.39)
		Outcross	21 (53)	0.78 (0.09)	93% (3%)	50% (6%)	67% (5%)	91% (5%)	2.44 (0.23)
		ID_{SO}		-0.23	0.02	-0.20	-0.05	0.03	0.15
	GH	Self	10 (10)	5.87 (1.03)	80% (6%)	93% (4%)	92% (3%)	90% (5%)	8.98 (0.69)
		Outcross	18 (2)	4.65 (0.95)	78% (9%)	76% (9%)	56% (9%)	79% (8%)	8.55 (0.93)
		ID_{SO}		-0.21	-0.04	-0.19	-0.38	-0.13	-0.05
Pop3	GC	Self	10 (10)	0.66 (0.17)	88% (8%)	36% (9%)	62% (12%)	80% (13%)	1.94 (0.41)
		Outcross	21 (47)	0.50 (0.1)	97% (1%)	40% (4%)	62% (8%)	79% (9%)	2.14 (0.3)
		ID_{SO}		-0.25	0.09	0.16	0.00	-0.01	0.10
	GH	Self	10 (10)	5.58 (1.08)	87% (6%)	81% (10%)	83% (10%)	80% (9%)	9.03 (1.40)
		Outcross	20 (20)	6.62 (0.69)	90% (5%)	77% (7%)	89% (6%)	75% (7%)	10.42 (0.82)
		ID_{SO}		0.16	0.04	-0.04	0.06	-0.06	0.13
Pop4	GC	Self	10 (10)	0.20 (0.1)	87% (9%)	19% (10%)	35% (13%)	46% (16%)	1.09 (0.48
		Outcross	17 (34)	0.25 (0.07)	100%	26% (7%)	73% (7%)	78% (9%)	1.11 (0.24)
		ID_{SO}		0.21	0.13	0.24	0.53	0.40	0.01
	GH	Self	10 (10)	2.22 (1.05)	94% (5%)	55% (12%)	65% (13%)	54% (13%)	4.57 (1.09)

Outcross	15 (17)	2.56 (0.54)	93% (3%)	53% (8%)	73% (8%)	63% (10%)	6 (0.99)
ID_{SO}		0.14	-0.01	-0.03	0.11	0.14	0.24

Table 4. 3 Fitness measurements for Generation 2 of three *Clarkia breweri* populations, broken down by cross type and experiment location. Experiment location (Exp) refers to the location that fitness was evaluated, by either being the growth chamber (GC) or greenhouse (GH). This includes, number of family and maternal lineages used. Measurements include, cumulative fitness, percent viable seed, percent germination, percent survival, percent survival to flowering and number of flowers. Inbreeding depression was calculated as defined in the methods.

Pop ID	Exp	Cross	Family (& mat) Lines	Cumulative fitness	Viability	Germination	Early survival	Survival to flowering	Flower number
Pop2	GC	Self	9 (9)	0.30 (0.09)	74% (10%)	80% (10%)	70% (9%)	73% (12%)	0.5 (0.11)
		Biparental	10 (33)	0.56 (0.06)	93% (2%)	85% (2%)	88% (3%)	91% (2%)	0.88 (0.08)
		Outcross	7 (8)	0.19 (0.06)	62% (13%)	61% (13%)	49% (11%)	62% (13%)	0.44 (0.11)
		ID_{SO}		-0.35	-0.17	-0.24	-0.30	-0.15	-0.12
		ID_{SB}		0.48	0.21	0.05	0.20	0.20	0.44
	GH	Self	8 (8)	3.25 (0.09)	69% (12%)	56% (11%)	63% (11%)	75% (13%)	7.11 (1.70)
		Biparental	9 (9)	2.99 (0.82)	58% (13%)	67% (14%)	67% (12%)	78% (13%)	7.31 (1.60)
		Outcross	9 (9)	1.64 (0.49)	65% (14%)	39% (10%)	45% (11%)	62% (13%)	7.23 (1.86)
		ID_{SO}		-0.50	-0.06	-0.31	-0.29	-0.17	0.02
		ID_{SB}		-0.08	-0.17	0.16	0.05	0.04	0.03
Pop3	GC	Self	8 (8)	0.23 (0.08)	65% (12%)	63% (13%)	55% (10%)	62% (11%)	0.63 (0.12)
		Biparental	10 (25)	0.51 (0.05)	92% (3%)	89% (5%)	82% (4%)	87% (4%)	0.88 (0.03)
		Outcross	6 (6)	0.18 (0.08)	39% (14%)	35% (13%)	32% (12%)	40% (14%)	0.46 (0.18)
		ID_{SO}		-0.20	-0.40	-0.45	-0.41	-0.36	-0.28
		ID_{SB}		0.56	0.29	0.29	0.33	0.29	0.28
	GH	Self	8 (8)	2.10 (1.00)	76% (13%)	38% (14%)	41% (14%)	43% (15%)	3.87 (1.4)

		Biparental	6 (6)	1.05 (0.61)	44% (16%)	27% (12%)	29% (12%)	26% (12%)	3.06 (1.27)
		Outcross	6 (6)	1.71 (0.67)	53% (15%)	28% (10%)	26% (10%)	44% (15%)	5.74 (2.04)
		ID_{SO}		-0.18	-0.31	-0.26	-0.37	0.02	0.33
		ID_{SB}		-0.59	-0.42	-0.29	-0.28	-0.40	-0.21
Pop4	**GC**	Self	5 (5)	0.11 (0.07)	37% (14%)	30% (15%)	24% (13%)	22% (12%)	0.31 (0.16)
		Biparental	5 (11)	0.05 (0.01)	56% (17%)	33% (13%)	17% (6%)	31% (13%)	0.48 (0.21)
		Outcross	3 (3)	0.19 (0.10)	36% (17%)	34% (16%)	31% (15%)	37% (18%)	0.42 (0.20)
		ID_{SO}		0.41	-0.04	0.13	0.21	0.42	0.28
		ID_{SB}		-0.50	0.34	0.10	-0.31	0.31	0.36
	GH	Self	5 (5)	1.01 (0.71)	26% (14%)	20% (13%)	17% (12%)	18% (12%)	1.73 (1.24)
		Biparental	3 (3)	1.14 (0.9)	36% (18%)	22% (13%)	21% (12%)	36% (18%)	2.6 (1.62)
		Outcross	3 (3)	0.72 (0.72)	41% (19%)	12% (9%)	26% (17%)	14% (14%)	1.43 (1.43)
		ID_{SO}		-0.28	0.36	-0.39	0.34	-0.19	-0.18
		ID_{SB}		0.12	0.27	0.06	0.19	0.51	0.33

Table 4. 4 Fitness measurements for three *Clarkia concinna* populations, broken down by cross type and experiment location. Experiment location (Exp) refers to the location that fitness was evaluated, by either being the growth chamber (GC) or greenhouse (GH). This includes, number of family and maternal lineages used. Measurements include, cumulative fitness, percent viable seed, percent germination, percent survival, percent survival to flowering and number of flowers. Inbreeding depression was calculated as described in the methods.

Gen	Exp	Pop ID	Cross	Family (& mat) Lines	Cumulative fitness	Viability	Germinate	Early survival	Survival to flowering	Flower number
G1	GC	Pop 1	Self	10 (10)	0.10 (0.06)	90% (4%)	37% (8%)	38% (12%)	29% (11%)	0.52 (0.29)
			Outcross	21 (45)	0.25 (0.10)	95% (1%)	43% (6%)	33% (5%)	27% (7%)	1.50 (0.57)
			ID_{SO}		0.62	0.06	0.13	-0.13	-0.09	0.66
		Pop 2	Self	10 (10)	0.12 (0.08)	98% (5%)	14% (4%)	17% (9%)	25% (13%)	1.3 (0.91)
			Outcross	19 (39)	0.10 (0.03)	93% (1%)	28% (4%)	53% (6%)	32% (7%)	1.22 (0.28)
			ID_{SO}		-0.17	-0.05	0.49	0.68	0.23	-0.06
		Pop 3	Self	9 (9)	0.29 (0.11)	95% (3%)	62% (10%)	51% (11%)	36% (10%)	2.32 (1.04)
			Outcross	22 (49)	1.19 (0.35)	94% (2%)	73% (4%)	35% (3%)	57% (7%)	5.77 (1.11)
			ID_{SO}		0.75	-0.01	0.15	-0.31	0.37	0.60
G2	GH	Pop 1	Self	2 (2)	0.43 (0.43)	7% (6%)	6% (6%)	7% (7%)	10% (10%)	1.50 (1.50)
			Biparental	2 (2)	1.50 (1.08)	19% (10%)	16% (10%)	13% (10%)	22% (15%)	5.98 (4.04)
			Outcross	2 (2)	0.25 (0.25)	12 % (9%)	2% (2%)	6% (6%)	8% (8%)	2.21 (2.2)
			ID_{SO}		-0.41	0.41	-0.66	-0.13	-0.23	0.32
			ID_{SB}		0.72	0.62	0.61	0.49	0.55	0.75
		Pop 2	Self	2 (2)	1.54 (1.15)	11% (8%)	13% (9%)	16% (11%)	18% (12%)	5.24 (3.86)
			Biparental	4 (4)	5.65 (3.17)	42% (16%)	31% (15%)	41% (16%)	50% (19%)	11.13 (4.32)

	Outcross	5 (5)	6.42 (2.30)	40% (13%)	30% (14%)	49% (16%)	49% (16%)	11.25 (3.76)
	ID_{SO}		0.76	0.73	0.68	0.67	0.63	0.53
	ID_{SB}		0.73	0.74	0.59	0.61	0.64	0.53
	Self	4 (4)	5.42 (3.63)	39% (16%)	30% (13%)	33% (17%)	27% (15%)	8.38 (5.05)
	Biparental	6 (8)	8.01 (2.56)	44% (13%)	52% (15%)	55% (15%)	57% (16%)	14.95 (4.27)
Pop 3	Outcross	5 (8)	11.84 (3.00)	56% (13%)	54% (13%)	59% (13%)	63% (14%)	22.37 (5.47)
	ID_{SO}		0.54	0.3	0.45	0.44	0.58	0.63
	ID_{SB}		0.32	0.1	0.42	0.39	0.53	0.44

Table 4. 5 Analysis table for *Clarkia breweri*. Results of mixed linear model for *Clarkia breweri*, using maternal line and experiment location as random effects. Asterisks refers to significance of the factors.

Life stage	**Source of variation**	**DF**	**F**	**P- values**
Cumulative fitness	Cross	2, 410	2.99	0.05 *
	Population	2, 410	29.62	<.0001 ***
	Gen	3, 410	122.63	<.0001 ***
	Cross*Population	4, 410	0.31	0.87
	Cross*Gen	6, 410	0.25	0.96
	Population*Gen	6, 410	1.77	0.10
	Cross*Population*Gen	12, 410	0.71	0.74
Seed viability	Cross	2, 410	2.44	0.09
	Population	2, 410	5.62	0.004 **
	Gen	3, 410	85.62	<.0001 ***
	Cross*Population	4, 410	1.19	0.32
	Cross*Gen	6, 410	0.43	0.86
	Population*Gen	6, 410	4.7	0.0001 **
	Cross*Population*Gen	12, 410	0.94	0.51
Seed germination	Cross	2, 410	5.69	0.004 **
	Population	2, 410	31.25	<.0001 ***
	Gen	3, 410	71.2	<.0001 ***
	Cross*Population	4, 410	1.43	0.22
	Cross*Gen	6, 410	0.64	0.70
	Population*Gen	6, 410	1.94	0.07
	Cross*Population*Gen	12, 410	0.47	0.93
Early survival	Cross	2, 410	4.07	0.02 *
	Population	2, 410	13.36	<.0001 ***
	Gen	3, 410	75.21	<.0001 ***
	Cross*Population	4, 410	2.43	0.05 *
	Cross*Gen	6, 410	0.38	0.89
	Population*Gen	6, 410	4.74	0.0001 **
	Cross*Population*Gen	12, 410	1.39	0.17
Survival to Flowering	Cross	2, 410	5.7	0.004 **
	Population	2, 410	34.13	<.0001 ***
	Gen	3, 410	81.71	<.0001 ***
	Cross*Population	4, 410	1.59	0.18
	Cross*Gen	6, 410	0.7	0.65
	Population*Gen	6, 410	1.5	0.18
	Cross*Population*Gen	12, 410	0.72	0.73
Flower number	Cross	2, 410	1.67	0.19
	Population	2, 410	22.62	<.0001 ***

	Gen	3, 410	88.59	<.0001 ***
	Cross*Population	4, 410	0.23	0.92
	Cross*Gen	6, 410	0.92	0.48
	Population*Gen	6, 410	3.12	0.01 **
	Cross*Population*Gen	12, 410	0.5	0.91

Table 4. 6 Analysis table for *Clarkia concinna* subsp. *concinna*. Results of mixed linear model for *Clarkia concinna*, using maternal line and experiment location as random effects. Asterisks refers to significance of the factors.

Life stage	**Source of variation**	**DF**	**F**	**P- values**
Cumulative fitness	Cross	2, 165	4.16	0.02 *
	Population	2, 165	15.05	<0.0001 ***
	Gen	1, 165	21.6	<0.0001 ***
	Cross*Population	4, 165	1.08	0.37
	Cross*Gen	2, 165	1.45	0.24
	Population*Gen	2, 165	5.59	0.004 **
	Cross*Population*Gen	4, 165	0.59	0.67
Seed viability	Cross	2, 165	2.09	0.13
	Population	2, 165	6.62	0.002 **
	Gen	1, 165	243.89	<0.0001 ***
	Cross*Population	4, 165	0.37	0.83
	Cross*Gen	2, 165	1.88	0.16
	Population*Gen	2, 165	5.79	0.004 **
	Cross*Population*Gen	4, 165	0.9	0.466
Seed germination	Cross	2, 165	4.16	0.02 *
	Population	2, 165	21.78	<0.0001 ***
	Gen	1, 165	22.37	<0.0001 ***
	Cross*Population	4, 165	1.01	0.4
	Cross*Gen	2, 165	0.24	0.78
	Population*Gen	2, 165	5.16	0.01 *
	Cross*Population*Gen	4, 165	0.2	0.94
Early survival	Cross	2, 165	2.9	0.06
	Population	2, 165	8.73	<0.0001 ***
	Gen	1, 165	4.39	0.04 *
	Cross*Population	4, 165	2.19	0.07
	Cross*Gen	2, 165	0.69	0.50
	Population*Gen	2, 165	4.66	0.01 *
	Cross*Population*Gen	4, 165	0.81	0.52
Survival to flowering	Cross	2, 165	3.65	0.03 *
	Population	2, 165	8.57	0.0002 **
	Gen	1, 165	0.3	0.59
	Cross*Population	4, 165	1.08	0.37
	Cross*Gen	2, 165	0.57	0.57
	Population*Gen	2, 165	1.35	0.26
	Cross*Population*Gen	4, 165	0.11	0.98
Flower number	Cross	2, 165	3.6	0.03 *
	Population	2, 165	11.99	<0.0001 ***

	Gen	1, 165	24.85	<0.0001 ***
	Cross*Population	4, 165	1.36	0.25
	Cross*Gen	2, 165	1.45	0.24
	Population*Gen	2, 165	3.91	0.02 *
	Cross*Population*Gen	4, 165	0.49	0.74

Table 4. 7 Model comparison using ASTER modeling for *Clarkia breweri* in the growth chamber experiment and in the greenhouse experiment. I tested the influence of cross-type, population and generation on final fitness.

Growth chamber experiment					
Models					
	df	Model deviance	Test df	Test deviance	Test P-value
Model 1: varb + fn + (cross): fn Model 2: varb + fn + (pop + cross): fn Model 3: varb + fn + (pop + cross + generation): fn Model 4: varb + fn + (pop * cross * generation): fn					
1	7	-20559			
2	9	-20356	2	203.4	<0.001
3	10	-20190	1	165.5	<0.001
4	22	-20107	12	82.73	<0.001
Greenhouse experiment					
Model 1: varb + fn + (cross): fn Model 2: varb + fn + (pop + cross): fn Model 3: varb + fn + (pop + cross + generation): fn Model 4: varb + fn + (pop * cross * generation): fn					
1	7	20622			
2	9	20776	2	154.6	<0.001
3	12	21138	3	361.6	<0.001
4	38	21346	26	208.2	<0.001

Table 4. 8 Model comparison using ASTER modeling for *Clarkia concinna* subsp. *concinna* in the growth chamber experiment and in the greenhouse experiment. I tested the influence of cross-type, population and generation on final fitness.

Growth chamber experiment					
Models					
	df	Model deviance	Test df	Test deviance	Test P-value
Model 1: varb + fn + (cross): fn Model 2: varb + fn + (pop + cross): fn Model 2: varb + fn + (pop * cross): fn					
1	6	-4705.5			
2	8	-4628.1	2	77.4	<0.001
3	10	-5625.3	2	2.8	0.245
Greenhouse experiment					
Model 1: varb + fn + (cross): fn Model 2: varb + fn + (pop + cross): fn Model 3: varb + fn + (pop + cross + generation): fn Model 4: varb + fn + (pop * cross * generation): fn					
1	7	22838			
2	9	22940	2	102.71	<0.001
3	11	23428	2	487.3	<0.001
4	31	23455	20	27.58	0.12

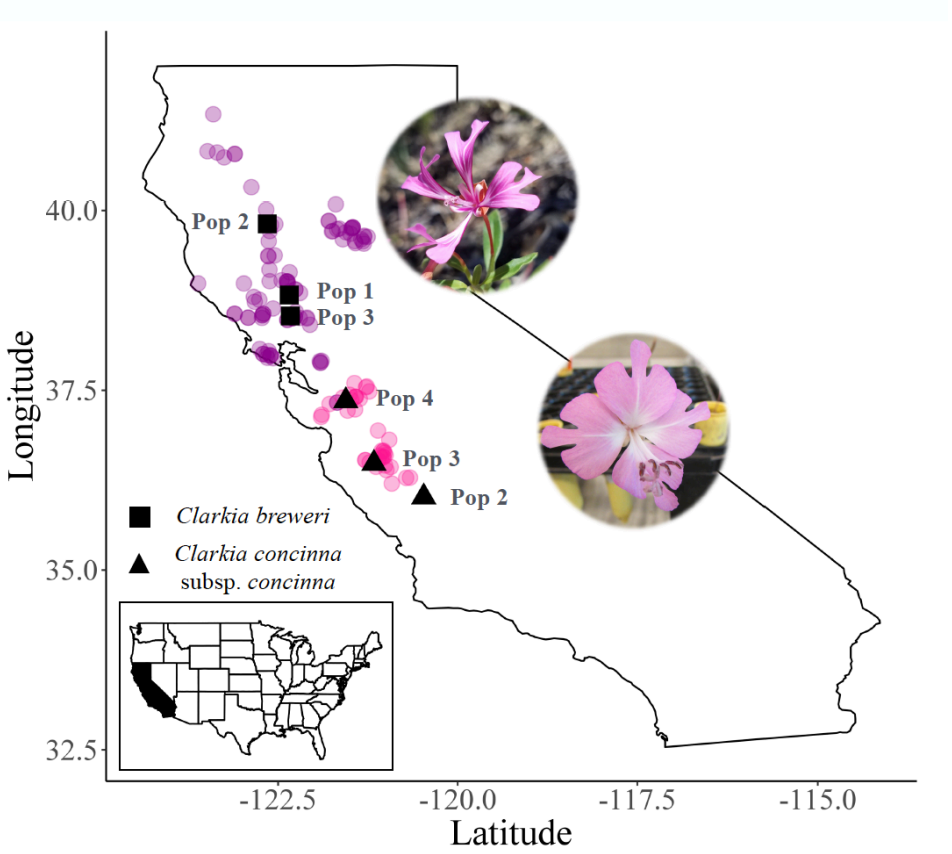

Figure 4. 1 Distribution map for *Clarkia concinna* subsp. *concinna* (purple circles) and *Clarkia breweri* (pink circles) along with the state of California. Dark squares represent the population included in the study for *Clarkia concinna* and Dark triangles represent the populations of *Clarkia breweri* included in this study.

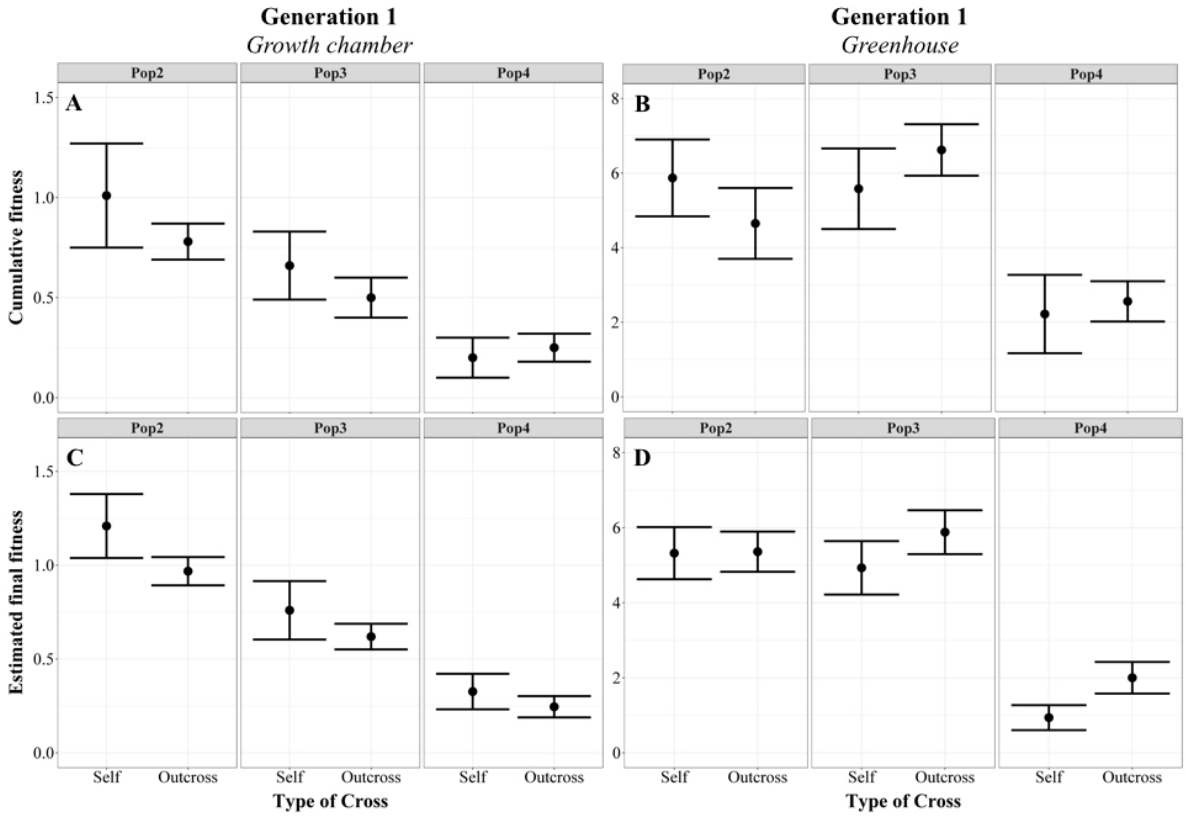

Figure 4. 2 Fitness comparisons across populations of *Clarkia breweri* in generation 1. Fitness estimated through cumulative fitness (A and B) and through ASTER (C and D) when evaluated in the growth chamber (A and C) and greenhouse experiment (B and D).

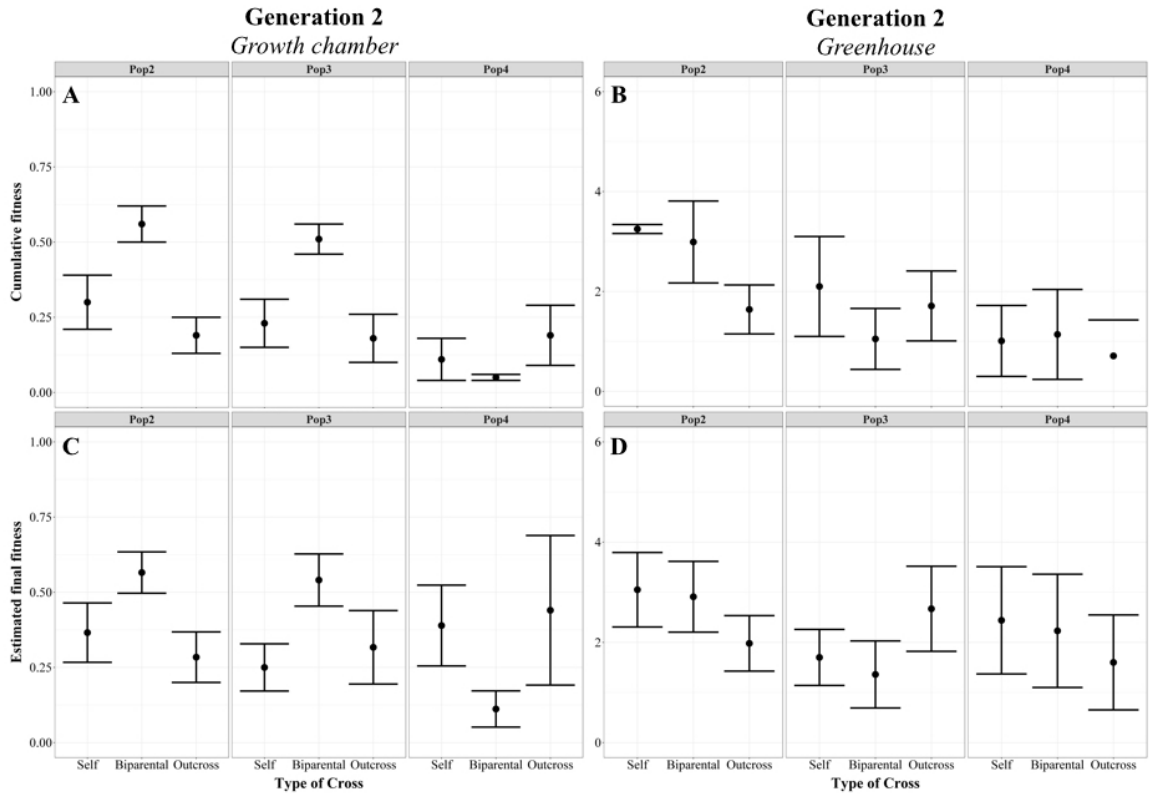

Figure 4. 3 Fitness comparisons across populations of *Clarkia breweri* in generation 2. Fitness estimated through cumulative fitness (A and B) and through ASTER (C and D) when evaluated in the growth chamber (A and C) and greenhouse experiment (B and D).

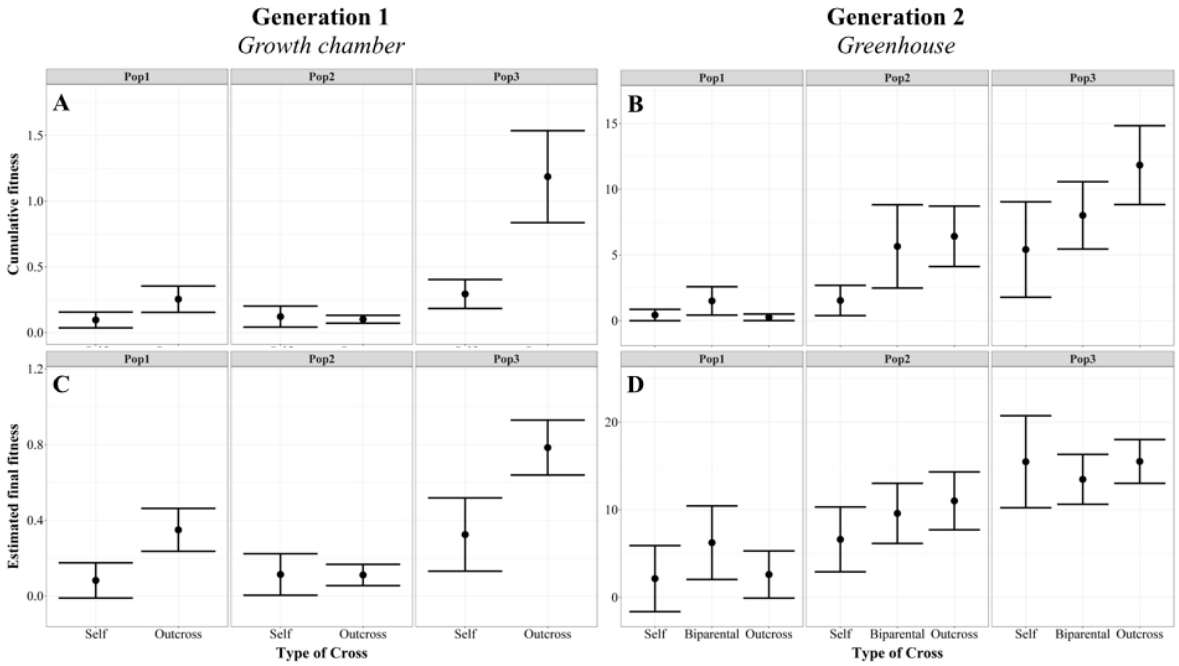

Figure 4. 4 Fitness comparisons across populations of *Clarkia concinna* subsp. *concinna* for generation 1 (A and C) grown in growth chamber and generation 2 (B and D) grown in greenhouse. Estimated shown are cumulative fitness (A and B) and using ASTER (C and D).

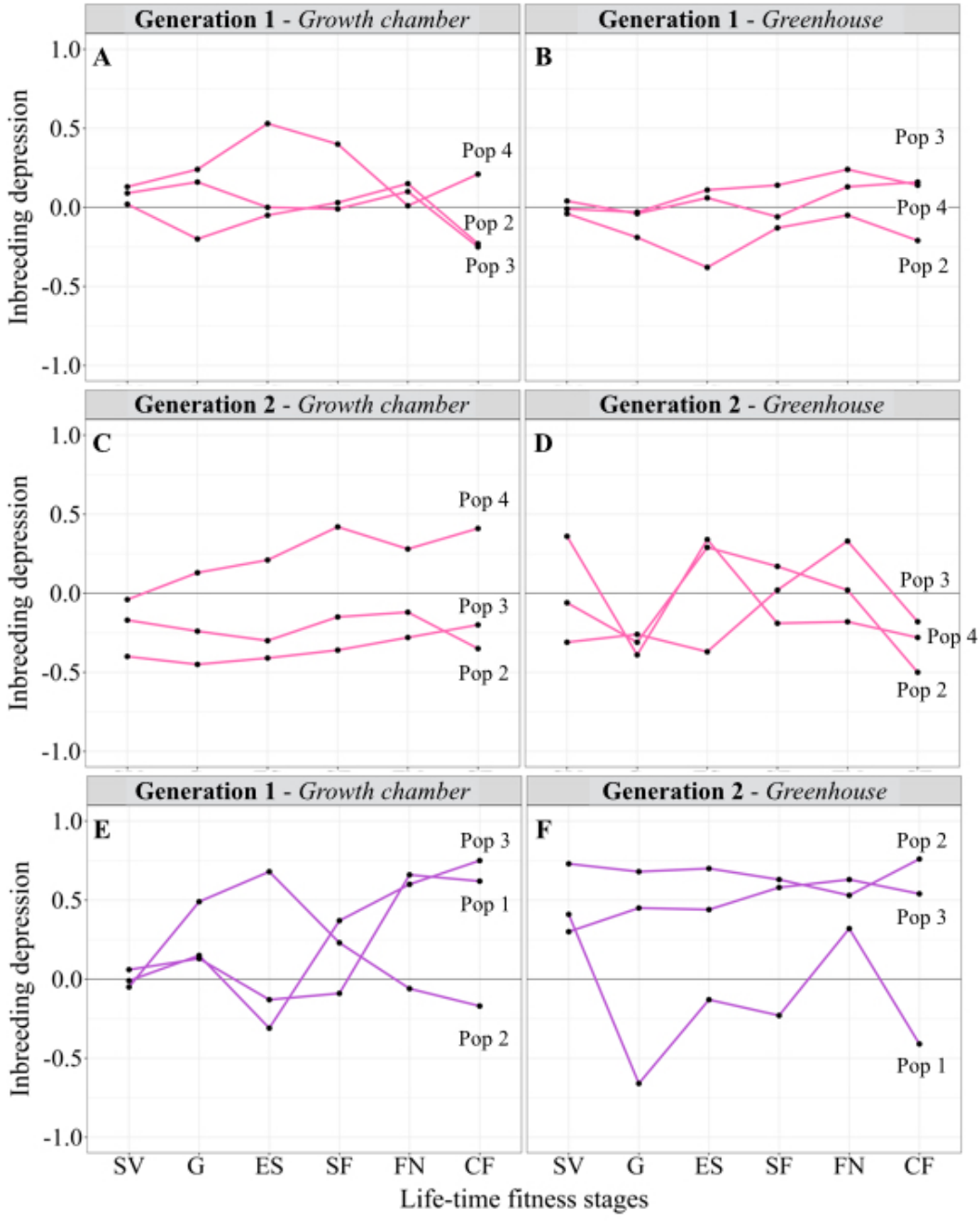

Figure 4. 5 Comparison of inbreeding depression across traits in generation 1 of *Clarkia concinna* and *Clarkia breweri.* V: viability, G: germination, ES: early survival, SF: survival to flowering, FN: flower number and CF: cumulative fitness.

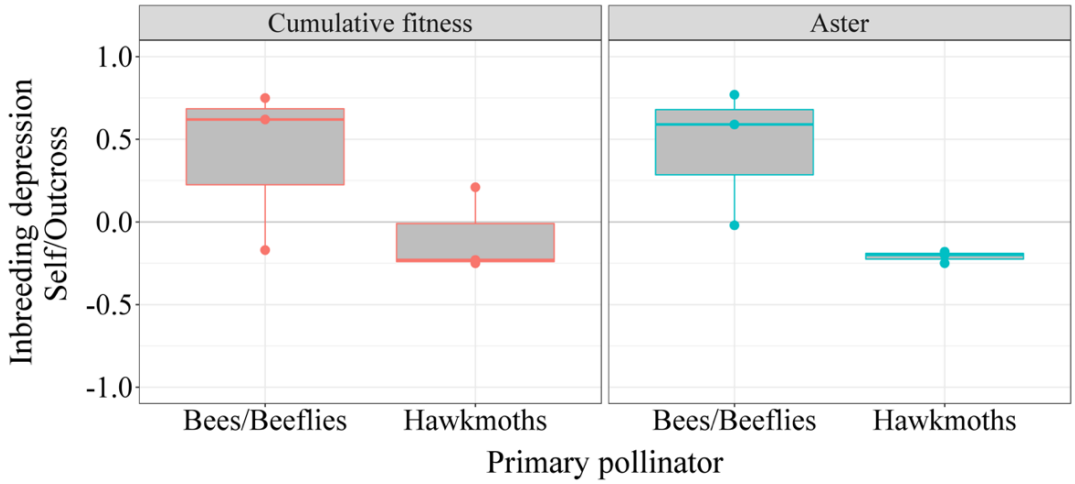

Figure 4. 6 Comparison of inbreeding depression using cumulative fitness and results from ASTER modeling. *Clarkia concinna* populations represented by their main pollinator bees and bee flies, *Clarkia breweri* populations represented by their main pollinator hawkmoths

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APPENDIX

CHAPTER 1

Table S1.1. Inbreeding coefficient dataset, information for each species and factors incorporated in the analysis. Growth form types (GF): SLP: short-lived perennial, P: perennial, A: annual. Breeding system (BS): SI: self-incompatible, SC: self-compatible. Mating system (MS): O: outcrossing, M: mixed, S: selfing. Pollinators by body size (PBS): XS: extra small, S: small, M: medium, L: large, XS: extra small. Reproductive strategy (R): S: sexual, AS: asexual.

Species	Publication	Location	Family	GF	Pollinator	BS	MS	Pop N	Pop size	Allele N	Fis	Marker	Variance	PBS	R
Sagittaria isoetiformis	Edwards et al. 2000	US	Alismataceae	SLP		SC		11	47.9	1.9	0.05	Allozyme	4.90E-05		AS
Sagittaria teres	Edwards et al. 2000	US	Alismataceae	SLP		SC		7	52.43	1.3	0.23	Allozyme	1.00E-03		AS
Cryptotaenia canadensis	Williams 1994	US	Apiaceae	SLP	Generalist	SC	M	1	120.4	2	0.66	Allozyme	3.70E-03		AS
Cryptotaenia canadensis	Williams 1994	US	Apiaceae	SLP	Generalist	SC	M	1	400	2.4	0.28	Allozyme	2.00E-04		AS
Dieffenbachia seguine	Cuartas-Hernandez 2006	Mexico	Araceae	SLP	Beetles			10	33.9	2.01	0.28	Allozyme	2.30E-03	S	AS
Chamaedorea ernesti-augusti	Cibrian-Jaramillo et al. 2009	Belize	Arecaceae	P	Thrips	SI	O	8	17	6.9	0.38	Codominant	9.20E-03	XS	AS
Agave delamateri	Parker et al. 2007	US	Asparagaceae	P	Bats	SI		11	33	2.18	-0.5	Allozyme	1.70E-02	XL	AS
Agave murpheyi	Parker et al. 2007	US	Asparagaceae	P	Bats	SI		21	24.19	2.1	-0.5	Allozyme	1.60E-02	XL	AS
Arnica montana	Luijten et al. 2000	Netherlands	Asteraceae	SLP	Flies	SI		26	28.19	1.17	0.1	Allozyme	4.00E-04	M	AS

Aster furcatus	Les et al. 1991	US	Asteraceae	SLP		SI		23	37.9	2	-0.2	Allozyme	1.40E-03		AS
Cirsium hillii	Freeland et al. 2010	Canada	Asteraceae	SLP	Bees	SI		11	30.36	4.3	-0.1	Codominant	1.50E-02	S	AS
Lophocereous schotti	Nason et al. 2002	US	Cactaceae	P	Moths	SI		21	48	2.78	0.01	Allozyme	4.20E-06	M	AS
Calystegia collina	Wolf et al. 2000	US	Convolvulacea e	P	Bees	SI	O	32	30	1.19	-0.6	Allozyme	1.30E-02	S	AS
Cladium jamaicense	Ivey and Richards 2001	US	Cyperaceae	SLP	Abiotic			18	45.44	1.22	-0.1	Allozyme	1.30E-01		AS
Lepidosperma Mt Caudan	Binks et al. 2015	Australia	Cyperaceae	SLP	Abiotic			11	24	3.97	0.16	Codominant	5.60E-03		AS
Lepidosperma Parker Range	Binks et al. 2015	Australia	Cyperaceae	SLP	Abiotic			6	24	3.48	0.38	Codominant	2.90E-03		AS
Arbutus menziesii	Beland et al. 2005	Canada	Ericaceae	P	Bees	SI	O	11	24	44	0.05	Dominant	9.60E-05	S	AS
Rhododendron championiae	Sai-Chit and Corlett 2000	China	Ericaceae	P	Bees	SI		4	31.75	2.1	-0	Allozyme	4.70E-06	S	AS
Rhododendron farrerae	Sai-Chit and Corlett 2000	China	Ericaceae	P	Bees	SI		16	30.4	2.2	0.05	Allozyme	9.20E-05	S	AS
Rhododendron ferrugineum	Cherrier et al. 2014	France	Ericaceae	P	Bumblebees	SC		33	19.55	2.71	-0	Codominant	1.50E-02	L	AS
Rhododendron hongkongense	Sai-Chit and Corlett 2000	China	Ericaceae	P	Bees	SI		8	37	2.6	0.05	Allozyme	6.90E-05	S	AS
Rhododendron moulmainense	Sai-Chit and Corlett 2000	China	Ericaceae	P	Bees	SI		9	30.4	2.4	0.05	Allozyme	6.90E-05	S	AS

Rhododendron simiarum	Sai-Chit and Corlett 2000	China	Ericaceae	P	Generalist	SI		6	44.8	1.8	0.06	Allozyme	9.40E-05		AS
Rhododendron simsii	Sai-Chit and Corlett 2000	China	Ericaceae	SLP	Bees	SI		15	32.7	2.2	0.07	Allozyme	1.40E-04	S	AS
Lathyrus sylvestris	Hossaert-McKey et al. 1996		Fabaceae	SLP	Generalist	SC	O	1	139.4	2.2	0.21	Allozyme	3.20E-04		AS
Iris ensata	Xiao et al 2015	China	Iridaceae	SLP	Bumblebees	SC	O	6	31.67	5.38	-0.3	Codominant	7.10E-02	L	AS
Origanum vulgare	Helsen et al. 2013	Belgium	Lamiaceae	SLP	Bees	SC	O	25	20	3.63	0.05	Codominant	1.00E-02	S	AS
Hemerocallis thunbergii	Chung et al. 2007	Korea	Liliaceae	SLP	Moths	SC		9	78.56	2.78	0.33	Allozyme	6.90E-04	L	AS
Mimulus gutattus	Lowry et al. 2008	US	Phrymaceae	SLP	Bumblebees	SC		28	16.5	3.68	0.22	Codominant	2.80E-02	L	AS
Mimulus guttatus	Awadalla et al. 1997	US	Phrymaceae	SLP	Bumblebees	SC	M	3	42.67	3.91	0.33	Codominant	9.10E-05	L	AS
Mimulus guttatus var. depauperatus	Awadalla et al. 1997	US	Phrymaceae	SLP	Bumblebees	SC	M	1	36	2.67	0.67	Codominant	1.30E-02	L	AS
Calibrachoa pygmaea	Mader et al. 2019	Brazil	Solanaceae	SLP	Moths	SI		3	24.33	10.73	0.37	Codominant	2.90E-04	L	AS
Zelkova carpinfolia	Maharramova 2015	Caucasus	Ulmaceae	P	Abiotic			30	16.6	3.41	-0.1	Codominant	9.70E-02		AS
Narcissus longispathus	Medrano 2008	Iberian penninsula	Amaryllidaceae	SLP	Bees	SC		27	31.78	1.4	0.05	Allozyme	1.50E-02	S	S
Pistacia lentiscus	Aparicio et al. 2012	Spain	Anacardiaceae	SLP	Abiotic	SI		23	27.8	1.69	0.71	Allozyme	1.90E-02		S

Annona crassiflora	Collevatti et al. 2014	Brazil	Annonaceae	SLP	Beetles			2	102	15.1	0.12	Codominant	6.70E-05	S	S
Osmorhiza claytonii	Williams 1994	US	Apiaceae	SLP	Generalist	SC	S	1	262	2	0.92	Allozyme	3.20E-03		S
Sanicula gregaria	Williams 1994	US	Apiaceae	SLP	Generalist	SC	M	1	163.3	2	0.25	Allozyme	3.70E-04		S
Sanicula gregaria	Williams 1994	US	Apiaceae	SLP	Generalist	SC	M	1	321.1	2	0.23	Allozyme	1.70E-04		S
Euterpe edulis	Gaiotto et al. 2003	Brazil	Arecaceae	P	Bees	SC	O	2	72	9.85	0.09	Codominant	1.80E-05	S	S
Agave cocui	Figueredo et al. 2011	Venezuela	Asparagaceae	P	Bats	SI		7	32.7	1.53	0.09	Allozyme	2.30E-04	XL	S
Ainsliaea faurieana	Mitsui et al. 2010		Asteraceae	SLP	Flies	SC		18	16.5	2.74	0.77	Codominant	2.40E-02	M	S
Argyroxiphium sandwicense	Friar et al. 2000	US	Asteraceae	SLP	Bees	SI	O	1	20	2.88	0.4	Codominant	8.30E-03	S	S
Argyroxiphium sandwicense subsp.	Friar et al. 2000	US	Asteraceae	SLP	Bees	SI	O	1	20	2.75	0.2	Codominant	2.20E-03	S	S
Cirsium pitcheri	Fant et al. 2014	US	Asteraceae	SLP	Generalist	SI		24	33.79	2.86	0.12	Codominant	8.20E-03		S
Cirsium pitcheri	Gauthier et al. 2010	Canada	Asteraceae	SLP	Generalist	SI		17	16.82	2.65	0.37	Codominant	1.90E-02		S
Erigeron arisolius	Edwards et al. 2014	US	Asteraceae	SLP		SI	O	7	24.5	8.89	0.22	Codominant	1.00E-03		S
Erigeron lemmonii	Edwards et al. 2014	US	Asteraceae	SLP		SI	O	1	121	7.1	0.19	Codominant	3.00E-04		S

Senecio squalidus	Brennan et al. 2003	United Kindom	Asteraceae	A		SI	O	1	24	0.27	0.03	Allozyme	3.90E-05		S
Avicennia marina	Maguire et al. 2000	South Africa	Avicenniaceae	P		SC	S	2	10	12	0.53	Codominant	1.90E-03		S
Avicennia marina	Maguire et al. 2000	Australia	Avicenniaceae	P		SC	O	7	14.29	18	0.02	Codominant	3.60E-03		S
Avicennia marina	Maguire et al. 2000	Australia	Avicenniaceae	P		SC	M	4	17.5	11.5	0.29	Codominant	8.00E-04		S
Avicennia marina	Arnaud-Haond et al. 2006	Vietnam	Avicenniaceae	P		SC	S	9	23.22	2.16	0.55	Codominant	1.10E-02		S
Avicennia marina	Arnaud-Haond et al. 2006	Australia	Avicenniaceae	P		SC	O	3	31.33	6.23	0.06	Codominant	2.80E-04		S
Begonia socotrana	Huges et al 2002	Yemen	Begoniaceae	P	Bees	SC	O	10	13.04	5.38	0.04	Codominant	1.40E-02	S	S
Alnus maritima	Gibson et al. 2008	US	Betulaceae	P	Abiotic	SC		7	26.1	1.55	0.48	Allozyme	9.30E-03		S
Alnus serrulata	Gibson et al. 2008	US	Betulaceae	P	Abiotic	SC		8	13.25	1.71	0.27	Allozyme	5.90E-03		S
Brassica oleraceae	Raybould 1999	UK	Brassicaceae	SLP	Bumblebees	SI		7	50	3.14	-0	Codominant	9.90E-06	L	S
Crambe pritzelii	Soto et al. 2016	Canaria islands	Brassicaceae	SLP		SI		8	66.88	2.74	0.19	Allozyme	1.60E-03		S
Crambe tamadabensis	Soto et al. 2016	Canaria islands	Brassicaceae	SLP		SI		4	46.5	2.93	0.24	Allozyme	1.10E-03		S
Lavenworthia alabamica	Koelling et al. 2011	US	Brassicaceae	A	Bees	SI		6	57.33	2.68	0.32	Allozyme	1.80E-03	S	S

Lavenworthia alabamica	Koelling et al. 2011	US	Brassicaceae	A	Bees	SC		4	89.75	2.24	0.29	Allozyme	9.20E-04	S	S
Lavenworthia crassa	Koelling et al. 2011	US	Brassicaceae	A	Bees	SC		3	94.3	2.45	0.41	Allozyme	1.80E-03	S	S
Lavenworthia crassa	Koelling et al. 2011	US	Brassicaceae	A	Bees	SI		2	156	2.7	0.34	Allozyme	7.50E-04	S	S
Sibara filifolia	McGlaughlin et al. 2015	US	Brassicaceae	A		SC		8	36.5	3.32	0.7	Codominant	1.50E-02		S
Guzmania monostachia	Cascante et al. 2014	Costa Rica	Bromeliaceae	P	Birds	SC		18	20.22	2.08	0.92	Codominant	4.10E-03	XL	S
Tillandsia achyrostachys var.	Gonzalez et al. 2004	Mexico	Bromeliaceae	P	Birds			6	16.1	1.86	0.43	Allozyme	1.20E-02	XL	S
Vriesea gigantea	Palma-Silva et al 2009	Brazil	Bromeliaceae	P	Bats			13	33.15	2.83	0.27	Codominant	4.20E-03	XL	S
Vriesea minarum	Lavor et al. 2014	Brazil	Bromeliaceae	P	Bats	SC	M	12	17.17	5.5	0.34	Codominant	7.10E-03	XL	S
Protium subserratum	Misiewicz et al. 2014	Peru	Burseraceae	P	Bees			8	25.13	4.81	0.07	Codominant	7.70E-04	S	S
Cereus repandus	Nassar et al. 2003	Venezuela a	Cactaceae	P	Bats	SI		7	41.5	2.05	0.18	Allozyme	8.20E-04	XL	S
Melocactus curvispinus	Nassar et al. 2001	Venezuela	Cactaceae	P	Birds	SC		9	48	1.54	0.35	Allozyme	2.60E-03	XL	S
Pereskia guamacho	Nassar et al. 2002	Venezuela	Cactaceae	P	Bees	SI		17	48	1.9	0.18	Allozyme	6.90E-04	S	S
Pilosocerus lanuginosus	Nassar et al. 2003	Venezuela and Caribbean	Cactaceae	P	Bats	SI		7	36.5	2.29	0.18	Allozyme	8.70E-04	XL	S

Stenocerus griseus	Nassar et al. 2003	Venezuela and Caribbean	Cactaceae	P	Bats	SI		8	47.5	1.78	0.15	Allozyme	4.50E-04	XL	S
Campanula thyrsoides	Frei et al. 2012	Switzerland	Campanulacea e	P	Bumblebees	SI		24	12	5.03	-0	Codominant	1.80E-02	L	S
Hanabusaya asiatica	Chung et al. 2001	Korea	Campanulacea e	SLP	Bees	SC		5	69.6	2.06	0.21	Allozyme	6.40E-04	S	S
Weigela coraeensis var. fragrans	Yamada 2012	Japan	Caprifoliaceae	SLP	Bumblebees	SI		17	29.65	4.54	0.12	Codominant	1.50E-02	L	S
Caryocar brasiliense	Collevatti et al. 2010	Brasil	Caryocaraceae	SLP	Bats	SC	O	1	101	16.1	0.13	Codominant	1.70E-04	XL	S
Lichnis floss-cuculi	Aavik et al. 2012	Switzerland	Caryophyllace ae	SLP	Generalist	SC	O	15	26.27	5	0.05	Codominant	2.80E-02		S
Lychnis flos-cuculi	Galeuchet et al. 2005	Switzerland	Caryophyllace ae	SLP	Generalist	SC		28	16.68	8.43	0.45	Codominant	9.40E-03		S
Silene ciliata	Garcia-Fernandez et el. 2012	Spain	Caryophyllace ae	SLP	Moths	SC		6	30	76.33	0.4	Codominant	2.60E-04	M	S
Silene latifolia	Barluenga et al. 2001	Netherlands	Caryophyllace ae	SLP	Moths	SI		15	17.2	10.49	0.35	Codominant	4.30E-03	M	S
Cercidiphyllum japonicum	Sato et al. 2005	Japan	Cerdiphyllacea e	P	Abiotic		O	6	55.5	14.5	0.02	Codominant	5.50E-05		S
Beta vulgaris ssp. maritima	Cauwer et al. 2012	Europe	Chenopodiacea e	SLP	Abiotic	SI		2	843	10.89	0.04	Codominant	6.50E-07		S
Cistus salvifolius	Aparicio et al. 2012	Spain	Cistaceae	SLP	Generalist	SI		23	27.8	1.39	0.02	Allozyme	9.60E-06		S
Ipomea microdactyla	Geiger et al. 2014	Cuba, Bahamas and southeastern	Convolvulacea e	SLP	Birds	SI		12	17.67	4.58	0.08	Codominant	6.50E-03	XL	S

Cycas miltipinnata	Gong et al. 2014	China	Cycadaceae	P	Beetles			5	80.6	4.74	0.25	Codominant	1.10E-04	S	S
Erica coccinea	Segarra-Moragues and Ojeda 2010	South Africa	Ericaceae	P	Birds			22	24.5	8.29	0.07	Codominant	1.30E-03	XL	S
Albizia lebbeck	Dunphy et al. 2005	Puerto Rico	Fabaceae	P	Generalist	SI	O	10	21.9	1.68	0.21	Allozyme	2.00E-03		S
Anadenanthera colubrina var. cebil	Barrandeguy et al. 2014	Argentina	Fabaceae	P	Bees		O	4	17.25	9.72	0.02	Codominant	3.50E-05	S	S
Brongniartia vazquezii	Gonzalez et al. 2001	Mexico	Fabaceae	P	Bumblebees	SC		4	40	2.03	0.53	Allozyme	7.30E-03	L	S
Dalbergia nigra	Ribeiro et al. 2005	Brazil	Fabaceae	P	Bees			3	21	1.93	0.13	Allozyme	8.70E-04	S	S
Dicornya guianensis	Latouche-Halle et al 2003	French Guiana	Fabaceae	P	Bees	SC	O	6	170.67	7.33	0.01	Codominant	7.40E-05	S	S
Dillwynia tenuifolia	Rymer et al. 2002	Australia	Fabaceae	P	Bees	SC	O	8	37.13	1.84	0.31	Allozyme	1.40E-03	S	S
Intsia palembanica	Lee et al. 2002	Malaysia	Fabaceae	P	Bees	SC			40	2.4	0.04	Allozyme	1.00E-03	S	S
Lupinus aridorum	Bupp et al. 2017	US	Fabaceae	SLP	Bees	SC		7	26.57	4.58	0.13	Codominant	5.10E-04	S	S
Lupinus westianus	Bupp et al. 2017	US	Fabaceae	SLP	Bees	SC		3	25	4.45	0.08	Codominant	7.50E-05	S	S
Castaneae sativa	Luisini et al. 2014	Bulgaria	Fagaceae	P	Bees			6	56	7.07	0.08	Codominant	2.20E-04	S	S
Cyclobalanopsis championii	Cheng et al. 2001	Taiwan	Fagaceae	P	Abiotic		O	5	44	1.9	0.21	Codominant	1.00E-03		S

Fagus lucida	Ying 2016	China	Fagaceae	P	Abiotic			14	26.79	8.65	0.41	Codominant	2.50E-02		S
Quercus acutissima	Chun et al. 2004	Korea	Fagaceae	P	Abiotic	SI		3	156	2	0.09	Allozyme	5.20E-05		S
Quercus coccifera	Aparicio et al. 2012	Spain	Fagaceae	P	Abiotic	SI		23	27.8	2.24	0.01	Allozyme	4.50E-06		S
Quercus laevis	Berg et al. 1995	US	Fagaceae	P	Abiotic			1	768	21	0.05	Allozyme	3.50E-06		S
Quercus serrata	Ohsawa et al. 2008	Japan	Fagaceae	P	Abiotic			15	21.93	8.34	0.06	Codominant	1.90E-03		S
Gentiana pneumonanthe	Raijmann et al.1994	Netherlands	Gentianaceae	SLP	Bumblebees	SC		25	33.8	1.16	0.1	Allozyme	3.20E-04	L	S
Streptocarpus primulifolius	Hughes et al. 2007	South Africa	Gesneriaceae	P	Flies	SI	O	10	24.2	4.95	0.19	Codominant	3.50E-03	M	S
Kirengeshoma palmata	Yuan et al. 2014	China and Japan	Hydrangeaceae	SLP	Bees		O	6	54.17	8.2	0.29	Codominant	4.20E-04	S	S
Phlomis crinita ssp crinita	Albaladejo et al. 2007	Spain	Lamiaceae	SLP	Bumblebees	SC		10	31.2	1.25	0.04	Allozyme	2.00E-02	L	S
Phlomis crinita ssp malacitana	Albaladejo et al. 2007	Spain	Lamiaceae	SLP	Bumblebees	SC		16	31.31	1.29	0.03	Allozyme	2.40E-02	L	S
Thymus algeriensis	Ben El Hadj Ali et al. 2012	Tunisia	Lamiaceae	SLP	Bees	SC		21	20	19.24	0.5	Allozyme	7.60E-03	S	S
Thymus capitatus	Ben El Hadj Ali et al. 2012	Tunisia	Lamiaceae	SLP	Bees	SI		25	20	19.44	0.47	Allozyme	2.20E-02	S	S
Ocotea catharinensis	Martins et al. 2015	Brazil	Lauraceae	P	Thrips	SC		6	28.17	8.42	0.21	Codominant	1.30E-03	XS	S

Ocotea catharinensis	Tarzai et al. 2010	Brazil	Lauraceae	P	Generalist			4	47	2.08	-0	Allozyme	1.60E-04		S
Ocotea odorifera	Martins et al. 2015	Brazil	Lauraceae	P	Thrips	SC		9	31	8.94	0.16	Codominant	2.00E-03	XS	S
Ocotea porosa	Martins et al. 2015	Brazil	Lauraceae	P	Thrips	SC		7	26.14	7.29	0.12	Codominant	3.70E-03	XS	S
Bertholletia excelsa	Sujii et al. 2015	Brazil	Lecythidaceae	SLP	Bees		O	9	42.11	5.09	-0.2	Codominant	6.30E-03	S	S
Trillium erectum	Irwin 2001	US	Liliaceae	SLP	Flies	SC	M	1	74	2.57	-0	Allozyme	2.00E-05	S	S
Trillium grandiflorum	Irwin 2001	US	Liliaceae	SLP	Flies	SC	O	1	94	2	-0.3	Allozyme	7.60E-04	S	S
Magnolia stellata	Setsuko et al. 2007	Japan	Magnoliaceae	P	Beetles	SC	O	8	38.5	7.65	0.04	Codominant	2.50E-04	S	S
Helicteres brevispira	Franceschinelli et al. 1999	Brazil	Malvaceae	SLP	Birds	SC		1	298	2.5	0.05	Allozyme	8.40E-06	XL	S
Swietenia macrophylla	Lemes et al. 2003	Brazil	Meliaceae	P	Generalist	SC		7	27.31	9.49	0.04	Codominant	3.30E-04		S
Tibouchina hatschbachii	Maia et al. 2017	Brazil	Melostomataceae	SLP	Bees	SC			22.78	3.33	0.14	Codominant	3.90E-03	S	S
Acacia dealbata	Broadhurst et al. 2008	Australia	Mimosaceae	P	Bees	SC	O	12	26.48	2.46	0.13	Allozyme	5.80E-03	S	S
Musa ornata	Burgos-Hernandez et al. 2013	Mexico	Musaceae	P	Bats	SC		6	18	4.31	0.91	Codominant	7.30E-04	XL	S
Calothamnus quadrifius ssp. teretifolius	Sampson et al 2014	Australia	Myrtaceae	P	Birds	SC		8	20	4.03	0.03	Codominant	9.40E-05	L	S

Eucalyptus benthamii	Butcher et al. 2005	Australia	Myrtaceae	P	Generalist	SC	M	4	18.5	6.66	0.05	Codominant	1.80E-03		S
Myrtus communis	Aparicio et al. 2012	Spain	Myrtaceae	P	Generalist	SC		23	27.8	1.44	0.15	Allozyme	8.80E-04		S
Nothofagus alpina	Vergara et al. 2014	Chile	Nothofagus	P	Abiotic		O	12	15.75	5.47	-0	Codominant	1.80E-02		S
Nothofagus glauca	Vergara et al. 2014	Chile	Nothofagus	P	Abiotic		O	8	15.41	4.54	0.08	Codominant	6.10E-03		S
Nothofagus obliqua	Vergara et al. 2014	Chile	Nothofagus	P	Abiotic		O	20	15.26	6.37	0.09	Codominant	9.30E-03		S
Fraxinus excelsior	Bacles et al. 2005	UK	Oleaceae	P	Abiotic	SC	O	5	17.6	10.23	0.18	Codominant	2.00E-03		S
Fraxinus excelsior	Heuertz et al. 2003	Romania	Oleaceae	P	Abiotic	SI		5	30	14.64	0.02	Codominant	1.90E-04		S
Fraxinus mandshurica	Hu et al 2008	China	Oleaceae	P	Abiotic	SI	O	30	47.83	11.06	0.04	Codominant	4.90E-04		S
Picconia azorica	Martins et al. 2013	Brazil	Oleaceae	P	Abiotic			31	14.29	4.72	-0.1	Codominant	1.60E-01		S
Clarkia temblorensis	Holtsford et al. 1990		Onagraceae	A	Bees	SC		4	30.25	3.25	0.53	Allozyme	9.70E-03	S	S
Oenothera harringtonii	Skogen et al. 2019	US	Onagraceae	A	Moths	SI		27	26.11	9.39	0.09	Codominant	3.00E-03	L	S
Oenothera harringtonii	Rhodes et al. 2014	US	Onagraceae	A	Moths	SI		1	323	17.9	0.04	Codominant	6.00E-06	L	S
Oenothera primiveris	Cisternas	US	Onagraceae	A	Moths	SI	O	1	20	1.62	0.03	Codominant	4.70E-05	M	S

Oenothera primiveris	Cisternas	US	Onagraceae	A	Moths	SC		2	20	1.5	0.13	Codominant	2.60E-06	M	S
Oenothera primiveris	Cisternas	US	Onagraceae	A	Moths	SC	S	3	20	1.19	0.33	Codominant	1.80E-02	M	S
Caladenia ampla	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	2	20	4.97	0.05	Codominant	8.50E-06	XS	S
Caladenia clavigera	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	1	20	4.62	0.05	Codominant	1.10E-04	XS	S
Caladenia cruciformis	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	4	27.25	5.86	-0	Codominant	9.70E-04	XS	S
Caladenia douglassiorum	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	1	20	5.43	-0	Codominant	6.80E-05	XS	S
Caladenia hastata	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	2	20	5.02	0.16	Codominant	3.40E-03	XS	S
Caladenia lowanensis	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	3	24.33	6.38	-0	Codominant	8.70E-06	XS	S
Caladenia reticulata	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	6	22.67	4.92	0.2	Codominant	2.60E-03	XS	S
Caladenia rigida	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	1	18	4.8	0.14	Codominant	1.10E-03	XS	S
Caladenia sp. Raymond Island	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	1	23	5.52	0.2	Codominant	1.80E-03	XS	S
Caladenia valida	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	1	25	4.38	0.33	Codominant	4.50E-03	XS	S
Caladenia xanthochila	Swarts et al 2014	Australia	Orchidaceae	SLP	Wasp		O	1	20	5	0.01	Codominant	1.30E-06	XS	S

Cephalanthera longibracteata	Chung et al. 2004	South Korea	Orchidaceae	SLP	Bees	SC	M	2	150.5	1.4	0.32	Allozyme	2.70E-07	S	S
Jumellea rossii	Mallet et al. 2014	Mascarene archipielago	Orchidaceae	SLP	Moths	SC		10	41	9.12	0.39	Codominant	2.90E-03	M	S
Laelia rubescens	Trapnell and Hamrick 2004	Costa Rica	Orchidaceae	SLP	Birds	SC	O	16	50	2.23	0.09	Allozyme	1.50E-04	XL	S
Oncidium hookeri	Alcantara et al. 2006	Brazil	Orchidaceae	SLP	Bees	SI		6	20.65	1.75	0.65	Allozyme	2.10E-02	S	S
Plathanthera praeclara	Ross et al. 2016	US	Orchidaceae	SLP	Moths	SC	S	8	29.75	36.75	0.23	Codominant	1.30E-03	L	S
Spiranthes sprialis	Machon et al. 2003	France	Orchidaceae	SLP	Bumblebees	SC		7	122.43	0.55	-0.2	Allozyme	9.50E-04	L	S
Castilleja affinis subsp. affinis	Widener et al. 2018	US	Orobanchaceae	SLP	Birds	SI		6	23.5	6.53	-0.1	Codominant	5.10E-04	XL	S
Castilleja affinis subsp. neglecta	Widener et al. 2018	US	Orobanchaceae	SLP	Bees	SI		12	28.67	8.55	0.05	Codominant	8.10E-04	S	S
Castilleja levisecta	Godt 2005	US	Orobanchaceae	SLP	Bumblebees	SI		11	44.7	2.94	0.07	Allozyme	1.20E-04	L	S
Parnassia palustris	Borgen and Hultgard 2003	Scandinavia	Parnassiaceae	SLP	Generalist	SC	O	64	11.5	1.54	-0.1	Allozyme	1.30E-03		S
Passiflora contracta	Turchetto et al. 2018	Brazil	Passifloraceae	SLP	Bats	SC		11	18	5.18	0.27	Codominant	8.90E-03	XL	S
Mimulus lacinatus	Sexton et al. 2016	US	Phrymaceae	A	Bees	SC	S	23	41.3	9.18	0.93	Codominant	5.30E-04	S	S
Mimulus laciniatus	Awadalla et al. 1997	US	Phrymaceae	A	Bees	SC	S	2	34.5	2	0.92	Codominant	3.80E-04	S	S

Mimulus laciniatus	Awadalla et al. 1997	US	Phrymaceae	A	Bees	SC	M	1	38	3.33	0.56	Codominant	8.50E-03	S	S
Mimulus nasutus	Awadalla et al. 1997	US	Phrymaceae	SLP	Bees	SC	M	1	28	4	0.28	Codominant	2.90E-03	S	S
Mimulus platycalyx	Awadalla et al. 1997	US	Phrymaceae	SLP	Bees	SC	M	1	31	3.5	0.36	Codominant	4.30E-03	S	S
Nuttallanthus canadensis	Crawford 2006	US	Plantaginaceae	A	Generalist	SC	S	22	14.8	1.25	0.93	Allozyme	6.20E-02		S
Nuttallanthus floridanus	Crawford 2006	US	Plantaginaceae	A	Generalist	SC	S	8	13.8	1.15	0.99	Allozyme	7.60E-02		S
Nuttallanthus texanus	Crawford 2006	US	Plantaginaceae	A	Generalist	SC	S	20	10.7	1.21	0.96	Allozyme	9.40E-02		S
Penstemon debilis	Wolf et al 2014	US	Plantaginaceae	SLP	Bees	SC	O	5	19.2	4.15	0.23	Codominant	7.30E-03	S	S
Penstemon destusus	Kramer et al. 2011	US	Plantaginaceae	SLP	Bees	SC		8	32	11.54	0.06	Codominant	4.70E-04	S	S
Penstemon pachyllus	Kramer et al. 2011	US	Plantaginaceae	SLP	Bees	SI		10	31.9	8.33	0.03	Codominant	7.40E-04	S	S
Penstemon rostriflorus	Kramer et al. 2011	US	Plantaginaceae	SLP	Birds	SI		10	31.7	13.57	0.02	Codominant	1.60E-04	XL	S
Plantago lanceolata	Tonsol et al. 1993	US	Plantaginaceae	SLP	Abiotic	SI	O	1	214	4	-0.1	Allozyme	7.20E-05		S
Primula elatior	Rossum et al. 2002	Belgium	Primulaceae	SLP	Bumblebees	SI		9	63.56	1.27	0.11	Allozyme	3.10E-03	L	S
Primula sieboldii	Honjo et al. 2009	Japan	Primulaceae	SLP	Bumblebees	NA		32	28.75	5.52	-0	Codominant	4.40E-03	L	S

Primula vulgaris	Barmentlo et al. 2018	Netherlands	Primulaceae	SLP	Bumblebees	SI	O	3	12.67	1.42	-0.4	Codominant	1.20E-02	L	S
Primula vulgaris	Barmentlo et al. 2018	Netherlands	Primulaceae	SLP	Bumblebees	SI	O	3	12.67	1.42	-0.4	Codominant	3.40E-02	L	S
Primula vulgaris	Van Geert et al. 2008		Primulaceae	SLP	Bumblebees	SI	O	7	18.14	3.9	0.06	Codominant	2.90E-03	L	S
Primula vulgaris	Van Rossum and Triest 2003		Primulaceae	SLP	Bumblebees	SI	O	41	60.37	1.18	0.1	Allozyme	2.10E-02	L	S
Grevillea mucronulata	Forrest et al. 2011	Australia	Proteaceae	P	Birds	SI		15	17.2	5	-0	Codominant	1.60E-02	XL	S
Grevillea robusta	de Sousa et al 2018	Brazil/Australia	Proteaceae	P	Birds	SI	O	6	78.83	2.93	0.23	Allozyme	1.20E-03	XL	S
Aquilegia coerulea	Brunet et al. 2012	US	Ranunculaceae	SLP	Bumblebees	SC	M	6	45	8.75	0	Codominant	3.60E-04	L	S
Aquilegia coerulea	Brunet et al. 2012	US	Ranunculaceae	SLP	Moths	SC	O	13	45	9.21	0.02	Codominant	2.40E-04	L	S
Berchemiella wilsonii	Kang et al. 2008	China	Rhamnaceae	SLP	Birds			4	22.25	2.78	0.05	Codominant	1.30E-03	XL	S
Prunus africana	Farwig et al. 2008	Kenya	Rosaceae	P	Bees	SI	O	8	13.75	6.84	0.14	Codominant	7.10E-03	S	S
Pyrus pyraster	Holderegger et al. 2008	Switzerland	Rosaceae	P	Generalist	SI		15	21.53	14.73	0.11	Dominant	1.40E-03		S
Bathysa australis	Reis et al. 2015	Brazil	Rubiaceae	SLP	Bees	SC		6	44.83	8.52	0.18	Codominant	2.30E-04	S	S
Glionnetia sericea	Finger et al. 2014	Seychelles	Rubiaceae	P	Moths	SC	O	4	35.5	3.07	0.16	Codominant	3.50E-04	L	S

Baillonella toxisperma	Ndiade-Bourobou et al. 2010	Gabon	Sapotaceae	P	Bats			3	82.33	7.37	0.14	Codominant	7.60E-05	XL	S
Sarracenia alata	Koopman et al. 2010	US	Sarraceniaceae	SLP	Bees		O	5	17.2	3.65	0.15	Codominant	5.70E-04	S	S
Sarracenia jonesii	Furches et al. 2013		Sarraceniaceae	SLP	Bumblebees	SC	O	6	10	2.28	0.33	Codominant	1.40E-02	L	S
Sarracenia jonesii	Gost 1996	US	Sarraceniaceae	SLP	Bees	SC	O	8	48	1.33	0.04	Allozyme	3.40E-05	S	S
Sarracenia oreophila	Furches et al. 2013		Sarraceniaceae	SLP	Bees	SC	O	7	13	2.25	0.4	Codominant	5.40E-03	S	S
Sarracenia oreophila	Gost 1996	US	Sarraceniaceae	SLP	Bumblebees	SC	O	14	48	1.34	-0	Allozyme	3.40E-05	L	S
Antirrhinum microphyllum	Torres et al. 2003	Spain	Scrophulariaceae	SLP	Bees	SI		4	46	2.1	0.17	Allozyme	1.50E-04	S	S
Euphrasia officinalis subsp. anglica	French et al. 2005	England	Scrophulariaceae	SLP	Generalist	SC	M	2	15	1.63	0.74	Codominant	3.90E-02		S
Euphrasia officinalis subsp.Rostkovia	French et al. 2005	England	Scrophulariaceae	SLP	Generalist	SC	M	3	28	4.08	0.22	Codominant	1.90E-03		S
Euphrasia rivularis	French et al. 2005	England	Scrophulariaceae	SLP	Generalist	SC	M	1	30	3.75	0.77	Codominant	2.00E-02		S
Euphrasia vigursii	French et al. 2005	England	Scrophulariaceae	SLP	Generalist	SC	M	2	28	2.63	0.55	Codominant	1.10E-02		S
Viola pubescens	Culley et al. 2003	US	Violaceae	SLP	Generalist	SC	M	3	36.27	2.29	0.23	Allozyme	1.20E-04		S
Viola pubescens	Culley et al. 2003	US	Violaceae	SLP	Generalist	SC	O	6	37.82	2.15	-0.2	Allozyme	5.40E-03		S

Dioon caputoi	Cabrero-Toledo et al. 2010	Mexico	Zamiaceae	P	Beetles	SI		4	45.5	1.98	-0.5	Allozyme	4.60E-03	S	S
Dioon merolae	Cabrero-Toledo et al. 2010	Mexico	Zamiaceae	P	Beetles	SI		7	29.5	2	-0.6	Allozyme	1.20E-02	S	S

Table S1.2. Inbreeding depression dataset, information for each species and factors incorporated in the analysis. Growth form types (GF): SLP: short-lived perennial, P: perennial, A: annual. Breeding system (BS): SI: self-incompatible, SC: self-compatible. Mating system (MS): O: outcrossing, M: mixed, S: selfing. Inbreeding depression (ID). Pollinators by body size (PBS): XS: extra small, S: small, M: medium, L: large, XS: extra small. Reproductive strategy (R): S: sexual, AS: asexual.

Species	Family	Publication	Location	GF	BS	Pollinator	MS	ID	Pop	N traits	ID provided?	PBS	R
Silene vulgaris	Caryophyllaceae	Bailey, 2006	United states	SLP	SI	Bumblebees		0.4	1	4	Yes	L	S
Silene vulgaris	Caryophyllaceae	Bailey, 2006	United states	SLP	SI	Bumblebees		0.15	1	4	Yes	L	S
Silene vulgaris	Caryophyllaceae	Bailey, 2006	United states	SLP	SI	Bumblebees		0.29	1	4	Yes	L	S
Scabiosoa columbaria	Caprifoliaceae	Angeloni, 2014	Netherlands	SLP	SC	Butterflies		0.37	1	7	Yes	M	S
Scabiosoa columbaria	Caprifoliaceae	Angeloni, 2014	Netherlands	SLP	SC	Butterflies		0.39	1	7	Yes	M	S
Scabiosoa columbaria	Caprifoliaceae	Angeloni, 2014	Netherlands	SLP	SC	Butterflies		0.33	1	7	Yes	M	S
Scabiosoa columbaria	Caprifoliaceae	Angeloni, 2014	Netherlands	SLP	SC	Butterflies		0.11	1	7	Yes	M	S
Scabiosoa columbaria	Caprifoliaceae	Angeloni, 2014	Netherlands	SLP	SC	Butterflies		0.48	1	7	Yes	M	S
Begonia hirsuta	Begoniaceae	Agren and Schemske, 1993	Costa Rica	A	SC	Bees	S	0.22	1	5	Yes	S	AS
Begonia semiovata	Begoniaceae	Agren and Schemske, 1993	Costa Rica	A	SC	Bees	S	0.42	1	5	Yes	S	AS
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	S	-0.2	1	8	No	S	S
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	S	0.14	1	8	No	S	S
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	S	-0.1	1	8	No	S	S
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	S	-0.1	1	8	No	S	S
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	S	-0.1	1	8	No	S	S

Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	M	-0.3	1	8	No	S	S
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	M	0.25	1	8	No	S	S
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	M	0.01	1	8	No	S	S
Turnera ulmifolia	Passifloraceae	Belaoussoff and Shore 1995	Jamaica	SLP	SC	Bees	O	-0.4	1	8	No	S	S
Mimulus guttatus	Schrophulariaceae	Carr and Dudash1996	United states	SLP	SC	Bees	O	0.82	3	5	Yes	S	AS
Mimulus micranthus	Schrophulariaceae	Carr and Dudash 1996	United states	SLP	SC		S	0.62	2	5	Yes		AS
Viola canadensis	Violaceae	Culley 2000	United states	SLP	SC	Generalist	S	-0.8	1	8	No		AS
Clarkia davyi	Onagraceae	Barringer 2008	United states	A	SC	Bees	S	0.27	1	4	Yes	S	S
Clarkia gracilis ssp. Sonomensis	Onagraceae	Barringer 2008	United states	A	SC	Bees	O	0.66	1	4	Yes	S	S
Clarkia lassenensis	Onagraceae	Barringer 2008	United states	A	SC	Bees	S	0.44	1	4	Yes	S	S
Clarkia williamsonii	Onagraceae	Barringer 2008	United states	A	SC	Bees	O	0.51	1	4	Yes	S	S
Sabatia angularis	Gentianaceae	Dudash 1990	United states	A	SC	Bees		0.53	1	3	Yes	S	S
Silene virginia	Caryophyllaceae	Dudash 2001	United states	SLP	SC	Birds		0.49	2	8	Yes	XL	S
Silene nutants	Caryophyllaceae	Dufay 2010	France and Belgium	SLP	SI	Generalist		0.31	2	3	Yes		S
Silene vulgaris	Caryophyllaceae	Glaettli 2006	Switzerland	SLP	SC	Moths		0.5	3	4	Yes	M	S
Sidalceae oregana subsp. spicata	Malvaceae	Ashman, 1992	United states	SLP	SI	Bumblebees		0.72	1	4	No	L	S
Leptosiphon jepsonii	Polemoniaceae	Goodwillie 2006	United states	A	SC	Flies	S	0.3	1	5	Yes	S	S
Leptosiphon jepsonii	Polemoniaceae	Goodwillie 2006	United states	A	SC	Flies	M	0.5	1	5	Yes	S	S
Leptosiphon jepsonii	Polemoniaceae	Goodwillie 2006	United states	A	SC	Flies	O	0.5	1	5	Yes	S	S
Salvia elegans	Lamiaceae	Guerrero 2016	Mexico	SLP	SC	Birds		-0.9	1	4	No	XL	S
Clarkia concinna	Onagraceae	Groom 2000	United states	A	SC	Bees		0.04	4	5	Yes	S	S
Clarkia concinna	Onagraceae	Groom 2000	United states	A	SC	Bees		0.2	4	5	Yes	S	S
Campanula americana	Campanulaceae	Galloway et al. 2003	United states	SLP	SC	Bumblebees	O	0.23	1	3	No	L	S

Campanula americana	Campanulaceae	Galloway et al. 2003	United states	SLP	SC	Bumblebees	O	0.14	1	3	No	L	S
Campanula americana	Campanulaceae	Galloway et al. 2003	United states	SLP	SC	Bumblebees	O	0.41	1	3	No	L	S
Scabiosa canescens	Dispasaceae	Andersson 2002	Sweden	SLP	SC			0.79	1	6	No		S
Gaillardia pulchella	Asteraceae	Heywood 1993	United states	SLP	SI	Bees	O	0.22	1	3	No	S	S
Magnolia stellata	Magnoliaceae	Hirayama 2007	Japan	P	SC	Beetles	O	0.59	1	1	Yes	S	AS
Magnolia stellata	Magnoliaceae	Hirayama 2007	Japan	P	SC	Beetles	O	0.7	1	1	Yes	S	AS
Vaccinium angustifolium	Ericaceae	Hokanson 2000	United states	P	SI	Bees	O	0.98	3	3	No	S	AS
Vaccinium corymbosum	Ericaceae	Hokanson 2000	United states	P	SI	Bees	O	0.9	3	3	No	S	AS
Vaccinium myrtilloides	Ericaceae	Hokanson 2000	United states	P	SI	Bees	O	0.99	3	3	No	S	AS
Clarkia tembloriensis	Onagraceae	Holtsford and Ellstrand 1990	United states	A	SC	Bees	S	0.81	1	7	No	S	S
Clarkia tembloriensis	Onagraceae	Holtsford and Ellstrand 1990	United states	A	SC	Bees	M	0.86	1	7	No	S	S
Clarkia tembloriensis	Onagraceae	Holtsford and Ellstrand 1990	United states	A	SC	Bees	M	0.98	1	7	No	S	S
Ficus aurea	Moraceae	Hossaert 2001	United states	P	SC	Wasp		0.05	1	1	Yes	XS	AS
Ipomea hederacea var. integriuscula	Convolvulaceae	Hull-Sanders 2005	United states	A	SC	Generalist	M	0.49	1	3	No		S
Ipomea hederacea var. integriuscula	Convolvulaceae	Hull-Sanders 2005	United states	A	SC	Generalist	M	0.96	1	3	No		S
Epilobium angustifolium	Onagraceae	Husband 1995	United states	SLP	SC	Bees	S	0.95	1	4	Yes	S	S
Epilobium angustifolium	Onagraceae	Husband and Schemske 1997	United states	SLP	SC	Bees	M	0.95	1	4	Yes	S	S
Epilobium angustifolium	Onagraceae	Husband and Schemske 1997	United states	SLP	SC	Bees	M	0.94	1	4	Yes	S	S
Epilobium angustifolium	Onagraceae	Husband and Schemske 1997	United states	SLP	SC	Bees	M	0.73	1	4	Yes	S	S
Epilobium angustifolium	Onagraceae	Husband and Schemske 1997	United states	SLP	SC	Bees	M	0.77	1	4	Yes	S	S

Epilobium angustifolium	Onagraceae	Husband and Schemske 1997	United states	SLP	SC	Bees	M	0.52	1	4	Yes	S	S
Fragaria vesca subsp. bracteata	Rosaceae	Dalton 2013	United states	SLP	SC		M	0.21	1	3	Yes		AS
Lychnis flos-cuculi	Caryophyllaceae	Hauser 1994	Denmark	SLP	SC	Generalist		0.56	4	10	No		S
Magnolia obovata	Magnoliaceae	Ishida 2008	Japan	P	SC	Beetles	S	0.21	1	3	No	S	AS
Magnolia obovata	Magnoliaceae	Ishida 2008	Japan	P	SC	Beetles	M	0.97	1	3	No	S	AS
Limnanthes alba	Limnanthaceae	Jain 1978	United states	SLP	SC	Bees	M	-0.3	1	3	No	S	S
Limnanthes alba	Limnanthaceae	Jain 1978	United states	SLP	SC	Bees	M	-0.2	1	3	No	S	S
Limnanthes alba	Limnanthaceae	Jain 1978	United states	SLP	SC	Bees	O	0.11	1	3	No	S	S
Limnanthes alba	Limnanthaceae	Jain 1978	United states	SLP	SC	Bees	O	0.67	1	3	No	S	S
Limnanthes alba	Limnanthaceae	Jain 1978	United states	SLP	SC	Bees	M	0.54	1	3	No	S	S
Limnanthes alba	Limnanthaceae	Jain 1978	United states	SLP	SC	Bees	O	0.39	1	3	No	S	S
Limnanthes alba	Limnanthaceae	Jain 1978	United states	SLP	SC	Bees	NA	0.17	1	3	No	S	S
Collinsia verna	Plantaginaceae	Kalisz 1989	United states	A	SC	Generalist	NA	0.26	1	4	No		S
Lobelia cardinalis	Lobeliaceae	Johnston 1992	United states	SLP	SC	Birds	O	0.57	1	6	No	XL	S
Lobelia cardinalis	Lobeliaceae	Johnston 1992	United states	SLP	SC	Birds	O	0.51	1	6	No	XL	S
Lobelia siphilitica	Lobeliaceae	Johnston 1992	United states	SLP	SC	Bumblebees	O	0.49	1	6	No	L	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	-0.1	1	4	Yes	S	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	0.04	1	4	Yes	S	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	0.08	1	4	Yes	S	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	0.08	1	4	Yes	S	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	0.09	1	4	Yes	S	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	0.22	1	4	Yes	S	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	0.51	1	4	Yes	S	S
Collinsia parviflora	Plantaginaceae	Kennedy and Elle 2008	Vancouver	A	SC	Flies	NA	0.18	1	4	Yes	S	S
Lupinus arboreus	Fabaceae	Kittelson and Maron 2000	United states	SLP	SC	Bumblebees	O	0.59	1	4	Yes	L	S

Plantago coronopus	Plantaginaceae	Koelewijn and Van Damme 2005	Netherlands	SLP	SC	Wind	NA	0.37	1	3	Yes		S
Saintpaulia ionantha ssp. Grotei	Gesneriaceae	Kolehmainen et al. 2009	Tanzania	SLP	SC	Bees	NA	0.64	1	5	No	S	AS
Saintpaulia ionantha ssp. Grotei	Gesneriaceae	Kolehmainen et al. 2009	Tanzania	SLP	SC	Bees	NA	0.71	1	5	No	S	AS
Phlox drummondii	Polemoniaceae	Levin and Bulinska-Radomska 1988	United states	A	SI	Butterflies	NA	0.06	1	3	No	M	S
Phlox drummondii	Polemoniaceae	Levin and Bulinska-Radomska 1988	United states	A	SI	Butterflies	NA	0.17	1	3	No	M	S
Phlox drummondii	Polemoniaceae	Levin and Bulinska-Radomska 1988	United states	A	SI	Butterflies	NA	0.23	1	3	No	M	S
Swertia perennis	Gentianaceae	Lienert and Fischer 2004	Switzerland	SLP	SC	Generalist	NA	0.64	6	5	No		S
Leavenworthia alabamica	Brassicaceae	Busch 2005	United states	A	SI	Bees	NA	0.45	5	2	No	S	S
Leavenworthia alabamica	Brassicaceae	Busch 2005	United states	A	SC	Bees	NA	-0	5	2	No	S	S
Arenaria uniflora	Caryophyllaceae	Fishman 2001	United states	A	SC	Flies	S	0.05	1	NA	Yes	S	S
Arenaria uniflora	Caryophyllaceae	Fishman 2001	United states	A	SC	Flies	O	0.19	1	NA	Yes	S	S
Calluna vulgaris	Ericaceae	Mahy 1999	Belgium	P	SI	Generalist	NA	0.76	1	2	No		S
Triodanis perfoliata	Campanulaceae	Ansaldi et al. 2019	United states	A	SC	Generalist	S	0.23	1	3	Yes		S
Triodanis perfoliata	Campanulaceae	Ansaldi et al. 2019	United states	A	SC	Generalist	S	0.23	1	3	Yes		S
Triodanis perfoliata	Campanulaceae	Ansaldi et al. 2019	United states	A	SC	Generalist	S	0.1	1	3	Yes		S
Leavenworthia alabamica	Brassicaceae	Baldwin and Schoen 2019	United states	A	SI	Bees	NA	0.72	6	4	Yes	S	S
Conradina glabra	Lamiaceae	Bladow et al. 2017	United states	SLP	SC	Flies	NA	0.65	3	2	Yes	S	S
Datura stramonium	Solanaceae	Jimenez-Lobato 2018	Mexico	A	SC	Moths	NA	0.37	1	1	Yes	M	S

Chionographis japonica var. kurohimensis	Melanthiaceae	Maki 1993	Japan	SLP	SC	Generalist	S	0.32	1	3	Yes/No		AS
Chionographis japonica var. kurohimensis	Melanthiaceae	Maki 1993	Japan	SLP	SC	Generalist	S	0.24	1	3	Yes/No		AS
Crinum erubescens	Amaryllidaceae	Manasse and Stanton 1991	Panama	SLP	SC	Moths	NA	0.96	1	4	No	M	AS
Opuntia rastrera	Cactaceae	Mandujano et al. 1996	Mexico	P	SC	Bees	NA	0.98	1	5	No	S	AS
Solanum carolinense	Solanaceae	Mena-Ali et al. 2008 (and 2007)	United states	SLP	SI	Bees	O	0.83	1	4	Yes	S	S
Lupinus perennis	Fabaceae	Michaels et al. 2008	United states	SLP	SC	Bumblebees	O	0.4	6	4	Yes	L	S
Aquilsegia caerulea	Ranunculaceae	Montalvo 1994	United states	SLP	SI	Moths	NA	0.52		4	Yes	M	S
Lobelia siphilitica	Lobeliaceae	Mutikainen 1998	United states	SLP	SI	Bumblebees	NA	0.25	1	4	Yes	L	S
Collinsia heterophylla	Schrophulariaceae	Mayer et al. 1996	United states	A	SC	Bees	M	0.29	1	6	No	S	S
Collinsia heterophylla	Schrophulariaceae	Mayer et al. 1996	United states	A	SC	Bees	M	0.24	1	6	No	S	S
Collinsia heterophylla	Schrophulariaceae	Mayer et al. 1996	United states	A	SC	Bees	M	0.33	1	6	No	S	S
Collinsia heterophylla	Schrophulariaceae	Mayer et al. 1996	United states	A	SC	Bees	M	0.1	1	6	No	S	S
Clarkia breweri	Onagraceae	Cisternas	United states	A	SC	Moths	NA	-0.2	1	4		M	S
Clarkia breweri	Onagraceae	Cisternas	United states	A	SC	Moths	NA	-0.3	1	4		M	S
Clarkia breweri	Onagraceae	Cisternas	United states	A	SC	Moths	NA	0.21	1	4		M	S
Clarkia concinna	Onagraceae	Cisternas	United states	A	SC	Bees	NA	0.62	1	4		S	S
Clarkia concinna	Onagraceae	Cisternas	United states	A	SC	Bees	NA	-0.2	1	4		S	S
Clarkia concinna	Onagraceae	Cisternas	United states	A	SC	Bees	NA	0.75	1	4		S	S
Oenothera primiveris	Onagraceae	Cisternas	United states	A	SI	Moths	NA	0.86	1	3		M	S
Oenothera primiveris	Onagraceae	Cisternas	United states	A	SI	Moths	NA	-0.4	1	3		M	S
Oenothera primiveris	Onagraceae	Cisternas	United states	A	SI	Moths	NA	-0.8	1	3		M	S

Oenothera primiveris	Onagraceae	Cisternas	United states	A	SC	Moths	NA	0.4	1	3		M	S
Oenothera primiveris	Onagraceae	Cisternas	United states	A	SC	Moths	NA	0.94	1	3		M	S
Oenothera primiveris	Onagraceae	Cisternas	United states	A	SC	Moths	NA	0.54	1	3		M	S
Oenothera primiveris	Onagraceae	Cisternas	United states	A	SC	Moths	NA	0.42	1	3		M	S
Nigella degenii	Ranunculaceae	Elmer and Andersson 2004	Greece	SLP	SC	NA	NA	0.11	1	2	No		S
Viola septemloba	Violaceae	Oakley	United states	SLP	SC	NA	S	0.43	1	NA	Yes		AS
Salvia pratensis	Lamiaceae	Ouborg 1994	Netherlands	SLP	SC	Bees	O	0.37	6	2	No	S	S
Scabiosoa columbaria	Dispasaceae	Pico 2004	Netherlands	SLP	SC	Butterflies	O	-0	1	6	Yes	M	S
Hypochaeris radicata	Asteraceae	Pico 2004	Netherlands	SLP	SI	Bees	O	0.72	1	9	No	S	S
Vaccinium myrtillus	Ericaceae	Raspe et al. 2004	Belgium	P	SC	Bumblebees	M	0.67	1	7	Yes	L	AS
Kalmia latifolia	Ericaceae	Rathcke 1993	United states	P	SC	Bumblebees	S	0.78	1	1	Yes	M	AS
Kalmia latifolia	Ericaceae	Rathcke 1993	United states	P	SC	Bumblebees	NA	0.77	1	1	Yes	L	AS
Yucca whipplei	Agavaceae	Richter 1998	United states	P	SC	Moths	NA	0.92	1	5	Yes	M	AS
Sophora microphylla	Fabaceae	Robertson 2011	New Zeland	P	SC	Birds	NA	0.94	1	9	Yes	XL	S
Sophora microphylla	Fabaceae	Robertson 2011	New Zeland	P	SC	Birds	NA	0.99	1	9	Yes	XL	S
Fuchsia excorticata	Onagraceae	Robertson 2011	New Zeland	P	SI	Birds	NA	0.74	1	6	Yes	XL	S
Fuchsia excorticata	Onagraceae	Robertson 2011	New Zeland	P	SI	Birds	NA	0.84	1	4	Yes	XL	S
Kosteletzkya virginica	Malvaceae	Ruan 2009	United states	SLP	SI	Birds	M	0.62	1	5	Yes	XL	AS
Kosteletzkya virginica	Malvaceae	Ruan 2009	United states	SLP	SI	Birds	M	0.66	1	5	Yes	XL	AS
Geum rivale	Rosaceae	Rusham 2010	Scotland	P	SC	Bumblebees	O	-0.4	1	5	No	L	AS
Geum rivale	Rosaceae	Rusham 2010	Scotland	P	SC	Bumblebees	O	-0.1	1	5	No	L	AS
Geum urbanum	Rosaceae	Rusham 2010	Scotland	P	SC	Flies	S	0.07	1	5	No	S	AS
Geum urbanum	Rosaceae	Rusham 2010	Scotland	P	SC	Flies	S	0.1	1	5	No	S	AS

Schiedea globosa	Caryophyllaceae	Sakai 1998	United states	SLP	SI	Wind	NA	0.26	1	4	No		S
Schiedea salicaria	Caryophyllaceae	Sakai 1998	United states	SLP	SI	NA	NA	0.82	1	4	No		S
Schiedea adamantis	Caryophyllaceae	Sakai 1997	United states	SLP	SI	Flies	NA	0.6	1	6	Yes	S	S
Costus allenii	Zingiberaceae	Schemske 1983	Panama	SLP	SC	Bees	NA	0.49	1	4	Yes	S	S
Costus guanaiensis	Zingiberaceae	Schemske 1983	Panama	SLP	SC	Bees	NA	0.29	1	4	Yes	S	S
Costus laevis	Zingiberaceae	Schemske 1983	Panama	SLP	SC	Bees	NA	0.3	1	4	Yes	S	S
Sarrecenia flava	Sarraceniaceae	Sheridan 2000	United states	SLP	SC	Bumblebees	NA	0.93	1	4	Yes	L	S
Witheringia solanaceae	Solanaceae	Stone 2010	Mexico and Caribean	SLP	SI	Bees	S	0.28	1	1	Yes	S	S
Witheringia solanaceae	Solanaceae	Stone 2010	Mexico and Caribean	SLP	SI	Bees	M	0.7	1	1	Yes	S	S
Campanula rapunculoides	Campanulaceae	Vogler et al. 1999	United states	SLP	SI	Bees	M	0.98	1	5	Yes	S	S
Campanula rapunculoides	Campanulaceae	Vogler et al. 1999	United states	SLP	SI	Bees	M	0.9	1	5	Yes	S	S
Knautia arvensis	Dispasaceae	Vange 2002	Norway	SLP	SI	Generalist	M	0.42	1	3	No		S
Grevillea barklyana	Protoceae	Vaughton 1995	Australia	P	SC	Birds	NA	-0.1	1	4	No	XL	S
Grevillea barklyana	Protoceae	Vaughton 1995	Australia	P	SC	Birds	NA	0.17	1	4	No	XL	S
Bulbine vagans	Asphodelaceae	Vaughton 2008	Australia	SLP	SC	Bees	M	0.41	1	6	Yes	S	S
Bulbine vagans	Asphodelaceae	Vaughton 2008	Australia	SLP	SC	Bees	M	0.49	1	6	Yes	S	S
Linaria cavanilesii	Plantaginaceae	Voilemont 2017	Spain	SLP	SC	Bees	O	0	1	11	Yes	S	S
Linaria cavanilesii	Plantaginaceae	Voilemont 2017	Spain	SLP	SI	Bees	M	0.49	1	11	Yes	S	S
Platanthera leucophaea	Orchidaceae	Wallace 2003	United states	SLP	SI	Moths	NA	0.77	1	2	No	M	S
Hebe subalpina	Schrophulariaceae	Delph 1996	United states	SLP	SC	NA	O	0.45	1	1	Yes		S
Crepis sancta	Asteraceae	Cheptou 2001	France	SLP	SI	Bees	O	0.27	1	3	Yes	S	AS
Gentianella germanica	Gentianaceae	Fischer 1997	Switzerland	P	SC	Flies	M	0.54	1	3	No	S	S
Dactylorhiza praetermissa	Orchidaceae	Ferdy 2001	France	SLP	SC	Bumblebees	NA	0.12	1	2	Yes	L	S
Dactylorhiza praetermissa	Orchidaceae	Ferdy 2001	France	SLP	SC	Bumblebees	NA	0.39	1	2	Yes	L	S

Dactylorhiza praetermissa	Orchidaceae	Ferdy 2001	France	SLP	SC	Bumblebees	NA	-0.8	1	2	Yes	L	S
Vaccinium myrtillus	Ericaceae	Guillaumea 1999	Belgium	P	SI	Bumblebees	NA	0.55	1	4	No	L	AS
Vaccinium vitis-ideae	Ericaceae	Guillaumea 1999	Belgium	P	SC	Bumblebees	NA	0.76	1	3	No	L	AS
Eucalyptus globulus subsp. globolus	Myrtaceae	Hardener 1995	Australia	P	SI	Bees	O	0.98	1	5	No	S	S
Cyclamen creticum	Primulaceae	Affre and Thompson 1997	Crete	SLP	SC	Flies	M	0.38	3	3	Yes	S	S
Pleurothallis ochreata	Orchidaceae	Borba et al. 2001	Brazil	SLP	SC	Flies	NA	0.69	4	1	No	S	S
Pleurothallis teres	Orchidaceae	Borba et al. 2001	Brazil	SLP	SC	Flies	NA	0.72	6	1	No	S	S
Pleurothallis adamantinensis	Orchidaceae	Borba et al. 2001	Brazil	SLP	SI	Flies	O	0.93	1	1	No	S	S
Pleurothallis fabiobarrosii	Orchidaceae	Borba et al. 2001	Brazil	SLP	SI	Flies	O	0.95	2	1	No	S	S
Pleurothallis johannensis	Orchidaceae	Borba et al. 2001	Brazil	SLP	SC	Flies	NA	0.79	7	1	No	S	S
Eupatorium perfoliatum	Asteraceae	Byers 1998	US	SLP	SI	Generalist	NA	0.04	1	3	No		AS
Eupatorium resinosum	Asteraceae	Byers 1998	United states	SLP	SI	Generalist	NA	0.29	1	3	No		AS
Mimulus guttatus	Schrophularaceae	Carr 1995	United states	SLP	SC	Bees	NA	0.43	2	3	No	S	AS
Mertensia ciliata	Boraginaceae	Geber 1985	United states	SLP	SC	Bumblebees	NA	0.46	1	1	No	L	S
Diodia teres	Rubiaceae	Hereford 2014	United states	A	SC	Bees	NA	0.22	1	1	No	S	S
Amsinckia douglasiana	Boraginaceae	Johnston and Schoen 1996	United states	A	SI	Bees	O	0.09	1	3	Yes	S	S
Amsinckia douglasiana	Boraginaceae	Johnston and Schoen 1996	United states	A	SI	Bees	O	0.18	1	3	Yes	S	S
Amsinckia gloriosa	Boraginaceae	Johnston and Schoen 1996	United states	A	SC	Bees	S	0.09	1	3	Yes	S	S
Amsinckia gloriosa	Boraginaceae	Johnston and Schoen 1996	United states	A	SC	Bees	S	0.02	1	3	Yes	S	S
Amsinckia spectabilis	Boraginaceae	Johnston and Schoen 1996	United states	A	SI	Bees	M	0.08	1	3	Yes	S	S

Amsinckia spectabilis	Boraginaceae	Johnston and Schoen 1996	United states	A	SI	Bees	M	0.17	1	3	Yes	S	S
Amsinckia spectabilis	Boraginaceae	Johnston and Schoen 1996	United states	A	SC	Bees	M	0.25	1	3	Yes	S	S
Amsinckia spectabilis	Boraginaceae	Johnston and Schoen 1996	United states	A	SC	Bees	S	-0	1	3	Yes	S	S
Amsinckia spectabilis	Boraginaceae	Johnston and Schoen 1996	United states	A	SC	Bees	NA	-0	1	3	Yes	S	S
Schidea lydgatei	Caryophyllaceae	Norman et al. 1995	United states	SLP	SC	Moths	O	0.61	1	4	Yes	M	S

CHAPTER 2

Table S2.1. Number of controlled pollinated flowers to evaluate self-incompatibility, including number of maternal lines evaluated by population, total number of individuals evaluated, number of crosses performed and number of those that produced seeds (>0). Proportion of flowers that produce seeds for each cross type and Bawa index to determinate the nature of the breeding system.

Population ID	Number of unique maternal lines	Total number of different indviduals	Number of pollinated flowers		Number of pollinated flowers with seeds (>0)		Proportion of flowers setting fruit		Bawa index
			Self	*Outcross*	*Self*	*Outcross*	*Self*	*Outcross*	
Pop 1	10	20	18	8	2	4	0.11	0.5	0.22
Pop 2	12	34	27	21	12	12	0.44	0.57	0.77
Pop 3	7	11	6	6	2	4	0.33	0.67	0.49
Pop 4	13	46	37	33	30	26	0.81	0.79	1.02
Pop 6	5	25	30	8	22	8	0.73	1	0.73
Pop 7	5	29	32	21	26	18	0.81	0.86	0.94
Pop 8	13	45	42	31	40	24	0.95	0.77	1.24

Table S2.2. Number of maternal lines used to evaluate their self-incompatibility index (SCI), including number of flowers pollinated, the average number of seed produced by crosses made in the population (without considering maternal line variation), and average SCI across maternal lines and in parenthesis the variation observed across maternal lines.

Population ID	Number of unique maternal lines	Number of pollinated flowers		Average number of seeds per flower crossed		Average SCI across maternal lines and their variation
		Self	*Outcross*	*Self*	*Outcross*	
Pop 1	10	18	8	2.7 (SE= 3)	20.6 (SE= 8.5)	0.13 (0 – 1)
Pop 2	12	27	21	10.6 (SE=3.4)	16.1 (SE= 4.2)	0.39 (0 – 1)
Pop 3	7	6	6	23.8 (SE= 15.5)	40 (SE= 13.8)	0.29 (0 – 1)
Pop 4	13	37	33	24.2 (SE= 3.54)	31.6 (SE= 3.5)	0.67 (0 – 1)
Pop 6	5	30	8	22.1 (SE= 3.9)	31.1 (SE= 6.5)	0.69 (0.28 – 1)
Pop 7	5	32	21	20.2 (SE= 4.1)	25.6 (SE= 5.6)	0.81 (0.35 – 1)
Pop 8	13	42	31	26.2 (SE=)	21.5 (SE= 3.4)	0.77 (0 – 1)

Table S2.3. Number seeds produced through autogamous self-pollination (in the absence of pollination) measured for each population, mean flower diameter and mean herkogamy for the population. Number of maternal lines evaluated, sample size and standard errors reported for each evaluated trait.

Population ID	Number of maternal lines evaluated	Number of fruits measured	Average number of autogamous seeds	SE1	Number of maternal lines evaluated	Number of flowers measured	Flower diameter	SE2	Herkogamy	SE3
Pop 1	7	17	0.00	4.28	8	24	57.47	1.36	7.60	0.69
Pop 2	11	42	13.6	2.72	12	51	59.58	1.8	5.51	0.47
Pop 3	4	13	2.08	4.89	7	10	59.88	3.27	9.04	1.07
Pop 4	16	241	20.3	1.14	13	82	30.85	1.02	0.59	0.38
Pop 6	5	75	22.5	2.04	5	41	38.47	1.23	0.85	0.53
Pop 7	5	85	24.8	1.91	5	43	38.84	1.41	-0.31	0.52
Pop 8	15	189	26.4	1.29	14	77	34.38	1.12	-0.28	0.39

Table S2.4. Summary of the evaluated traits for each population used to test the role of breeding system and flower size on genetic diversity. Bawa index of self-incompatibility (Bawa ISI), self-incompatibility index, mean flower diameter, mean herkogamy measured in the growth-chamber. Genetic parameters estimated using RADseq. Flower size is either large (LF) o small (SF).

Population ID	Bawa SI index	Self-incompatibility index (SCI)	Mean flower diameter (mm)	Mean herkogamy (mm)	%P	N_A	H_E	F_{IS}	Estimated N_E	Flower size
Pop 1	0.22	0.13	57.47	7.6	61.73	1.21	0.13	0.03	47.4	LF
Pop 2	0.77	0.39	59.58	5.51	45.92	1.28	0.11	0.13	32.3	LF
Pop 3	0.49	0.29	59.88	9.04	54.24	1.19	0.12	0.12	28.2	LF
Pop 4	1.02	0.67	30.47	0.6	16.64	1.11	0.06	-0.15	11.8	SF
Pop 6	0.73	0.69	38.47	0.85	18.3	1.08	0.05	0.58	3.8	SF
Pop 7	0.94	0.81	38.84	-0.31	21.46	1.08	0.05	0.55	16.1	SF
Pop 8	1.24	0.77	34.38	-0.28	-	-	-	-	-	SF

CHAPTER 3

Table S3.1 Self-compatibility index (SCI) for each maternal line grown of *Oenothera primiveris*. Average SCI index was calculated and reported in the chapter.

Population ID	Total maternal lines used		Maternal lines	SCI
Pop 1	**10**			**0.13**
		1	OP_8_POP1	0
		2	OP_9_POP1	0
		3	OP_11_POP1	0
		4	OP_13_POP1	0.27
		5	OP_17_POP1	0
		6	OP_18_POP1	0
		7	OP_19_POP1	1
		8	OP_23_POP1	0
		9	OP_24_POP1	0
		10	OP_26_POP1	0
Pop 2	**12**			**0.39**
		1	OP_1_POP2	0.67
		2	OP_3_POP2	0
		3	OP_5_POP2	0
		4	OP_6_POP2	0.34
		5	OP_8_POP2	0.66
		6	OP_9_POP2	1
		7	OP_12_POP2	0
		8	OP_13_POP2	0.89
		9	OP_14_POP2	0.03
		10	OP_18_POP2	0
		11	OP_20_POP2	0.06
		12	OP_21_POP2	1
Pop 3	**7**			**0.29**
		1	OP_1_POP3	0
		2	OP_7_POP3	0
		3	OP_17_POP3	1
		4	OP_18_POP3	0
		5	OP_20_POP3	0
		6	OP_24_POP3	0
		7	OP_25_POP3	1
Pop 4	**13**			**0.67**
		1	OP_1_POP4	1
		2	OP_2_POP4	0
		3	OP_4_POP4	0.55
		4	OP_5_POP4	0.74
		5	OP_12_POP4	0.65

		6	OP_14_POP4	0
		7	OP_16_POP4	1
		8	OP_18_POP4	1
		9	OP_19_POP4	1
		10	OP_20_POP4	1
		11	OP_21_POP4	0
		12	OP_22_POP4	1
		13	OP_23_POP4	0.74
Pop 6	**5**			**0.69**
		1	OP_2_POP6	1
		2	OP_4_POP6	1
		3	OP_6_POP6	0.63
		4	OP_7_POP6	0.55
		5	OP_8_POP6	0.28
Pop 7	**5**			**0.81**
		1	OP_1_POP7	0.35
		2	OP_3_POP7	0.99
		3	OP_4_POP7	1
		4	OP_5_POP7	0.84
		5	OP_6_POP7	0.86
Pop 8	**13**			**0.77**
		1	OP_1_POP8	0.78
		2	OP_2_POP8	1
		3	OP_3_POP8	0.18
		4	OP_5_POP8	0.62
		5	OP_6_POP8	1
		6	OP_7_POP8	1.00
		7	OP_8_POP8	1
		8	OP_9_POP8	1
		9	OP_10_POP8	1
		10	OP_11_POP8	0
		11	OP_13_POP8	0.42
		12	OP_14_POP8	1
		13	OP_15_POP8	1