HABITAT FRAGMENTATION AND MATING SYSTEM EVOLUTION OF A NATIVE WILDFLOWER

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Abstract

Depending on the magnitude of inbreeding depression (IBD), autonomous selfing may provide reproductive assurance (RA) for flowering plants in pollen-limited environments. Pollen limitation may result from the breakdown of once-continuous habitat into smaller, more isolated patches (habitat fragmentation) if fragmentation reduces plant and/or pollinator populations. Furthermore, theory predicts that IBD may evolve in concert with selfing rate, such that selection may reduce genetic load after multiple generations of inbreeding. Here I quantify the levels of RA and IBD among different population sizes of *Collinsia parviflora*, a wildflower with inter-population variation in flower size. I found that RA was greatest in small populations of small-flowered plants (where pollinator visitation was rare) and lowest in large populations of large-flowered plants (where visitation was abundant). Moreover, I found low levels of IBD in presumably selfing populations (i.e. small populations of small-flowered plants), suggesting that autonomous selfing is adaptive in fragmented habitats.

Keywords: autonomous selfing; habitat fragmentation; inbreeding depression; pollen limitation; reproductive assurance

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CHAPTER 1
General Introduction

The adaptive value of self-fertilization has been of great interest to evolutionary biologists for over 130 years (reviewed in Holsinger 1996). Darwin (1876) was the first to experimentally demonstrate a cost to selfing (inbreeding depression), while Fisher (1941) showed that selfing plants gain an automatic selection advantage because they contribute two gametes to offspring, whereas outcrossers contribute only one. More recently, it was proposed that complete outcrossing is favoured when inbreeding depression is greater than one-half, whereas complete selfing is favoured when inbreeding depression is less than one-half (Lande and Schemske 1985). In an effort to test this theory, Schemske and Lande (1985) collected estimates of outcrossing rates for 55 species and demonstrated a relatively bimodal frequency distribution (i.e. the majority of species were either predominantly selfers or outcrossers). Species that had outcrossing rates between 0.2-0.8 (i.e. mixed mating systems) were suggested to represent transitional states towards two stable endpoints of mating system evolution (Schemske and Lande 1985). However, at present, the stability of mixed mating systems remains contentious. Lande and Schemske's (1985) groundbreaking model spurred on a wealth studies, which twenty years later, enabled Goodwillie et al. (2005) to conduct a more comprehensive survey of outcrossing rates; this time expanding the sample size to include 345 species in 78 families. The bimodality in the updated analysis was substantially less apparent and the frequency of species demonstrating a mixed mating system increased by slightly more than 10% (Goodwillie et al. 2005). While the updated sample may not be completely representative of all mating systems (Igic and Kohn 2006), it still suggests that mixed mating systems are common in plants (Goodwillie et al. 2005), and further questions their stability.

Models that integrate ecological factors with the automatic selection advantage and inbreeding depression have predicted stable mixed mating systems. These ecological factors include pollen discounting (Holsinger et al. 1984), biparental inbreeding (Uyenoyama 1986), and reproductive assurance (Lloyd 1979, 1992). Reproductive assurance, the production of seeds via autonomous selfing when the opportunity to outcross is reduced, demonstrates a benefit to autonomous selfing when pollinators are rare or unpredictable (Stebbins 1957). Direct empirical tests of this benefit, however, are few and have yielded inconsistent results (Culley 2002, Herlihy and Eckert 2002, Elle and Carney 2003, Kalisz and Vogler 2003, Moeller 2006).

Reproductive assurance should be important when seed production is limited by vector-assisted pollen delivery (pollen limitation). Pollen limitation may be especially common when human-induced fragmentation results in isolated patches of habitat and smaller plant populations (Wilcock and Neiland 2002, Ashman et al. 2004, Aguilar et al. 2006). If small plant populations provide inadequate resources to support pollinator populations, then pollen delivery to plants may be severely limited in fragmented habitats (Steffan-Dewenter et al. 2006). Taken together, autonomous selfing should be adaptive if it provides reproductive assurance in highly fragmented habitats.

If habitat fragmentation leads to reduced plant population sizes, then the frequency of biparental inbreeding (vector-assisted pollen delivery between closely related individuals) may also increase (Ellstrand and Elam 1993, Dudash and Fenster 2000, Tomimatsu and Ohara 2006). Therefore, even if pollinators are abundant and are frequently visiting plants, individuals may still incur inbreeding depression in small populations. The magnitude of inbreeding depression, however, may be reduced after multiple generations of selfing if selection purges deleterious recessive alleles when expressed in the homozygous state (Lande and Schemske 1985). Accordingly, if population size is an indicator of mating history, then the level of inbreeding depression within a population may depend largely on its size (Byers and Waller, 1999).

In this thesis, I measure reproductive assurance and inbreeding depression in a wildflower that occurs within highly fragmented Garry Oak (*Quercus garryana*) and associated ecosystems. Garry Oak and associated ecosystems have been reduced by urban development over the last 150 years to less than five percent of their estimated historical extent (Fuchs 2001, Lea 2002). Here I focus on the native wildflower, *Collinsia parviflora*, which demonstrates continuous inter-population variation in flower size (Ganders and Krause 1986, Elle and Carney 2003). Flower size can affect both a plant's attractiveness to pollinators (Elle and Carney 2003) and its ability to self (Eckhart and Geber 1999, Elle 2004), both of which can influence the frequency of inbreeding. My primary focus is to test how population size and flower size affect the adaptive value of autonomous selfing (selfing without the aid of a pollinator). I achieve this by measuring the effects of flower size and population size nested within flower size on reproductive assurance (an ecological benefit of selfing, Chapter 2) and inbreeding depression (a genetic cost of selfing, Chapter 3); I conclude by speculating whether continuous inter-population variation in population size and flower size can maintain mixed mating in *C. parviflora* (Chapter 4).

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CHAPTER 2
The Reproductive Assurance Benefit of Selfing: Importance of Flower Size and Population Size

Abstract

Autonomous selfing can provide reproductive assurance (RA) for flowering plants that are unattractive to pollinators or in environments that are pollen limited. Pollen limitation may result from the breakdown of once-continuous habitat into smaller, more isolated patches (habitat fragmentation) if fragmentation negatively impacts pollinator populations. Here I quantify the levels of pollen limitation and RA among large and small populations of *Collinsia parviflora*, a wildflower with inter-population variation in flower size. I found that regardless of population or flower size, none of the populations were pollen limited, as pollen-supplemented and intact flowers did not differ in seed production. There were, however, significant flower size and population nested within flower size effects on RA; intact flowers (can self) produced significantly more seeds than emasculated flowers (require pollen delivery) in all but the large populations of large-flowered plants. Levels of RA were strongly negatively correlated with rates of pollinator visitation, whereby infrequent visitation by pollinators yielded high levels of RA via autonomous selfing. That is, RA was greatest in small populations of small-flowered plants (where visitation was rare), lowest in large populations of large-flowered plants (where visitation was abundant), and intermediate in both small populations of large-flowered plants and large populations of small-flowered plants. These results provide evidence that population size nested within flower size affects RA, and that autonomous selfing is adaptive in fragmented habitats.

Key words: autonomous selfing; Collinsia parviflora; *floral morphology; habitat fragmentation; mating system evolution; pollen limitation; reproductive assurance*

Introduction

Mixed mating systems, where reproduction occurs by both selfing and outcrossing, may be extremely common in plants (Vogler and Kalisz 2001, Goodwillie et al. 2005), although it remains unclear whether species with mixed mating systems represent fixed or transitional reproductive strategies (Goodwillie et al. 2005). The transition of mixed mating to either an obligate selfing or outcrossing mating system is customarily explained by the interplay of two genetic forces: inbreeding depression (Darwin 1876) and the automatic selection advantage (Fisher 1941), where complete outcrossing is favoured when inbreeding depression is greater than one-half (Kimura 1959, Lande and Schemske 1985). More recently, stable intermediate selfing rates have been predicted by models that integrate ecological factors with the genetic forces listed above. For example, if seed production is limited by vector-assisted pollen delivery, it is advantageous for outcrossing phenotypes to self autonomously at the end of floral life, as it provides some reproductive assurance (RA; Lloyd 1992, Schoen et al. 1996, Morgan and Wilson 2005). However, direct empirical tests of the RA benefit of autonomous selfing are few and have yielded inconsistent results (Culley 2002, Herlihy and Eckert 2002, Elle and Carney

2003, Kalisz and Vogler 2003, Moeller 2006), suggesting further empirical tests are needed to determine the importance of RA for selection of a mixed mating strategy.

The RA benefit of autonomous selfing may be especially high in habitats where pollen limitation occurs. Pollen delivery is known to be highly variable among flowers, plants, populations, and years (Knight et al. 2005), and pollen limitation may be especially common in disturbed environments such as those fragmented through anthropogenic development (Wilcock and Neiland 2002, Ashman et al. 2004, Aguilar et al. 2006). In fragmented habitats, pollinator population sizes may be smaller because small fragments have inadequate resources to support them, leading to a reduction in pollen delivery to remaining plants (Steffan-Dewenter et al. 2006). Moreover, small, isolated patches of flowering plants within fragments are less attractive to pollinators, and may be bypassed altogether (Sih and Baltus 1987, Jennersten 1988). Taken together, small plant populations in fragmented habitats reflect a specific ecological circumstance where increased selfing (assuming it leads to RA) ought to be beneficial.

Selfing rates, however, are under complex selection and determined by more than the pollination environment. For example, morphological plant attributes will affect the selfing rate because specific traits like flower size affect both a plant's attractiveness to pollinators and its ability to self autonomously (Elle and Carney 2003, Eckhart and Geber 1999). Elle and Carney (2003) found that small-flowered *Collinsia parviflora* plants had a higher RA benefit from autonomous selfing than large-flowered plants, suggesting that smaller flower size may be adaptive in pollen limited environments; this however, remains untested for this species. Most importantly, no previous study has attempted to test whether population size and flower size jointly affect RA.

In this study, I examined differences in the potential for RA through autonomous selfing in different sized populations of *C. parviflora*, and whether or not these differences depend on flower size. Specifically, I asked: 1) does pollinator visitation rate differ with flower size and/or population size nested within flower size?; 2) does the magnitude of RA differ with flower size and/or population size nested within flower size?; and 3) does pollinator visitation rate predict the magnitude of RA? I predict that the greatest benefit through autonomous selfing should be accrued in small populations of small-flowered plants (where pollinators are predicted to be most rare), and the lowest RA benefit should be in large populations of large-flowered plants (where pollinators should be most abundant).

Methods

Study organism

Collinsia parviflora (Scrophulariaceae *s.l.*) is a winter annual common in various habitats including grassy slopes, mossy rock outcrops, and beaches in western North America (Douglas et al. 1998). On Vancouver Island, British Columbia, *C. parviflora* is found in highly fragmented Garry Oak (*Quercus garryana*) and associated ecosystems, which have been reduced by urban development over the last 150 years to less than five percent of their estimated historical extent (Fuchs 2001, Lea 2002). At my study sites, *C. parviflora* flowers bloom from March through June, depending on location. Flowers consist of two upper banner petals, two lower wing petals, and a folded keel petal encasing the sexual parts; flower sizes as measured by the width of the two attached banner petals vary continuously among natural populations in B.C. (Ganders and Krause 1986, Elle and Carney 2003), and these differences have a genetic basis (Elle 2004). The fruit is a capsule, typically encasing 4-8 seeds that drop when ripe (Elle and Carney 2003). Although flower size varies continuously in this species in British Columbia, for the purposes of the current experiment I focused on the extremes of the flower size distribution, ignoring intermediate phenotypes (see below).

Study sites

I classified eight *C. parviflora* populations on Vancouver Island, B.C., as either small or large, and either small-flowered or large-flowered. I defined a population as a group of plants at least 50m apart from conspecific groups of plants (as in Kéry and Matthies 2004, Wagenius 2006). Population size classification was based on the estimated total number of flowering plants per population (calculated by multiplying area [m^2] by density [number of flowering plants/m^2]). Population area was quantified using a Garmin 76S GPS unit, and density was estimated by counting the number of *C. parviflora* plants present in $0.5m^2$ quadrats laid along 15m transects at 1m intervals. In an effort to reflect the entire population density, the number of transects depended on the population area (number of transects ranged from 3–7). The estimated number of flowering plants per population, area, and density varied across fragments (for a complete overview of population characteristics see Table 2-1).

Based on previous studies (Elle and Carney 2003, Elle 2004), my populations were classified a priori as either large- or small-flowered according to where they fell along the flower size continuum for this species. I confirmed my initial flower size classifications by measuring the corolla width across the two attached banner petals of each flower used in my manipulation experiment (see Floral manipulations below), calculating per-plant means, and using these means to determine population means. Flower size data were analyzed using univariate analysis of variance (ANOVA, SAS 1996), with population as the main effect, and the Ryan-Einot-Gabriel multiple range test (Day and Quinn 1989) was used to reveal which populations differed significantly. Mean flower size ranged from 3.88 ± 0.07mm to 7.92 ± 0.08mm and demonstrated significant among-population variation ($F_{7,358} = 441$, $P < 0.0001$), thereby justifying my initial population classification.

Pollinator visitation

At each population, I surveyed floral visitors (and thus potential pollinators) on clear, sunny days in 0.25m^2 quadrats placed haphazardly in areas of high *C. parviflora* density. Quadrats could not be positioned randomly due to the patchy distribution of this species. Approximately six different quadrat locations were used at each site and the number of flowering *C. parviflora* stems were counted within each quadrat. Survey periods were 15 minutes and conducted over 3 days (half between 1000-1300 hours and half between 1300-1600 hours) for a total of

3 h of observation at each population. The number of stems with flowers clearly probed by each visitor during each observation period was recorded. A mean population visitation rate (number of visits / number of flowering stems) was calculated using 12 observation periods per population. To determine whether pollinator visitation rate differed with flower size and population size, I performed a two-factor nested ANOVA with flower size and population size nested within flower size as fixed effects. Population size was treated as categorical and nested in flower size because small-flowered *C. parviflora* populations have intrinsically more plants relative to large-flowered populations (Table 2-1), possibly because of environmental differences among populations differing in flower size (Elle 2004). In other words, population size can only be defined relative to flower size. Flower size was treated categorically (large or small) for simplicity in the model, as there was no overlap in flower sizes among small-flowered populations and large-flowered populations (Table 2-1).

Visitors were identified to species when possible, and genus or family when not. A chi-square contingency test was performed to determine whether the distribution of visits among insect taxa was independent of flower size.

Floral manipulations

I performed an experiment to estimate whether seed production was limited by pollen delivery and if so, whether a potential RA gain was accrued through autonomous selfing. During the peak flowering period of each population, I haphazardly placed twelve $0.05 m^2$ quadrats in each population. Quadrats could not be positioned at random due to the patchy distribution of the plants. To reduce bias in plant selection, I selected the 4 plants closest to each quadrat corner to be used in my manipulation experiment. A total of 48 plants per population received four floral treatments, one treatment on each of four flowers: (1) emasculated, in which the bud was opened and the anthers were removed; (2) intact, in which the bud was opened but the anthers were not removed; (3) supplemented, in which the bud was opened and the anthers were removed before receiving a saturating amount of pollen from conspecific individuals several days later; and (4) unmanipulated, in which the flower was unhandled. Treatments were randomly assigned to flowers for each plant, and each calyx was marked with a dot of non-toxic correction fluid for identification purposes. Correction fluid colours were alternated among treatment types in the event that colour affected pollinator visitation. Fruits were collected and seed set compared among floral treatments. Pollen limitation was examined by calculating the difference of seed production between flowers given supplemental pollen and intact flowers receiving ambient pollen loads. If pollen-supplemented flowers produce more seeds, then fecundity is limited by pollen. The magnitude of RA through autonomous selfing was measured by finding the difference in seed production between intact flowers (which can produce seeds from either autonomous selfing or vector-assisted pollen movement) and emasculated flowers (which can only produce seeds via vector-assisted pollen movement). For simplicity, vector-assisted pollen movement was considered to be the result of outcrossing, as I was interested in levels of autonomous autogamy and not geitonogamy. Furthermore, it is unlikely that geitonogamy is prevalent in this species due to the low number of simultaneously open flowers per stem (mean = 2.4 flowers per stem across nine populations; Elle and Carney 2003). Finally, to test for a handling effect on seed production, I compared the difference in seed set between unmanipulated flowers and intact (handled) flowers. Seed production by floral manipulation type was compared using paired t-tests to determine if the average difference between floral treatments differed significantly from zero; tests were Bonferroni adjusted to account for multiple tests (three) within each population.

To determine whether RA differed with flower size and population size, I performed a two-factor nested ANOVA with flower size and population size nested within flower size as fixed effects (see ANOVA model details above). I also conducted a univariate regression across all populations to determine whether RA was affected by pollinator visitation rate.

Results

Pollinator visitation

A total of 188 potential pollinators were observed visiting *C. parviflora* plants. These included bee flies (*Bombylius major*), two morphospecies of *Osmia* (i.e. small and large, which may actually include more than two species; Megachilidae), bumble bees (*Bombus bifarius, B. flavifrons* and *B. melanopygus,* Apidae), Halictidae (small bees < 0.5 cm, *Halictus* spp. and *Lasioglossum* spp), as well as an “other” category in which I lumped honey bees (*Apis mellifera*), several species of Andrenidae, and hover flies (Syrphidae), as none of these groupings reached high frequency. The distribution of visits to large- and small-flowered plants by the five insect groupings differed significantly ($X^2 = 15.6$, $P < 0.01$). Overall, *Osmia spp.* were the most abundant visitor, comprising 43.6% of the total visits observed; *Bombylius major* and *Bombus* spp. constituted 17% and 14.4% of visits, respectively. Of these, 64.6% of the total *Osmia* visits were in large-flowered populations; 68.8% of the total *Bombylius major* visits were in large-flowered populations; and all *Bombus* spp visits were in large-flowered populations (Fig. 2-1).

Mean visitation rate per 15-minute observation period ranged from 0.01 at HC and KP to 0.18 at SM. Both flower size and population size nested within flower size significantly affected visitation rate (flower size, $F_{1,92} = 8.7$, $P < 0.004$; population size(flower size), $F_{2,92} = 12.2$, $P < 0.0001$). Mean visitation rate was lowest at small populations of small-flowered plants; greatest at large populations of large-flowered plants; and intermediate at the other two population size/flower size categories (Fig. 2-2).

Floral manipulations

Supplemental hand-pollination did not significantly increase seed production at any of my eight sites (Table 2-2), indicating adequate pollen is delivered either by pollinators or autonomously in small and large populations of both flower types. There was, however, a benefit of pollen delivery through autonomous selfing at six of my eight sites. This benefit was absent only in large populations of large-flowered plants (EF and SM, Table 2-2). Unhandled and handled control flowers showed no difference in seed production in any population (Table 2-2), confirming that my floral manipulations did not adversely affect seed set.

Both flower size and population size nested within flower size significantly affected the magnitude of RA in *C. parviflora* (flower size, $F_{1,347} = 77.5$, $P < 0.0001$; population size (flower size), $F_{2,347} = 10.2$, $P < 0.0001$). Reproductive assurance was greatest in small populations of small-flowered plants; lowest in large populations of large-flowered plants; and intermediate in the other two mixed population size/flower size categories (Fig. 2-3). Variation in RA between populations was greatest in the two mixed population size/flower size categories. Population RA means differed by 1.54 between GO and TL (large population/small-flowered) and 0.83 between SP and CR (small population/large-flowered), whereas population means differed by only 0.07 and 0.06 for the small/small and large/large population size/flower size categories, respectively (Fig. 2-3). A regression analysis showed that pollinator visitation rate explained a significant portion of the variation in RA and that the two variables were negatively correlated (Fig. 2-4).

Discussion

In accordance with my predictions, I found that visits to *C. parviflora* were abundant in large populations of large-flowered plants and uncommon in small populations of small-flowered plants. This finding is understandable considering that large populations of large-flowered plants are expected to provide a more attractive resource for pollinators. Indeed, pollinator abundance and composition have been shown to be reduced in fragmented habitats (Sih and Baltus 1987, Jennersten 1988). Pollinators are also typically more attracted to dense patches of plants (reviewed in Ghazoul 2005, Cheptou and Avendaño V 2006), but I found a marginally nonsignificant correlation ($R^2 = 0.88$, $P > 0.06$) and no correlation ($R^2 = 0.14$, $P > 0.62$) between population density and per-stem visitation rates within small- and large-flowered populations, respectively. Still, differences in density may help explain the variation in visitation rates among some populations; for example, among the two large populations of small-flowered plants, *C. parviflora* was almost 2.5x more dense at GO than TL which may partially account for the high frequency of visits at GO.

Visitation rate was also influenced by flower size as plants in large-flowered populations were visited more often than small-flowered populations (excluding GO). My results are in accordance with a previous study by Elle and Carney (2003) which demonstrated that pollinators preferred large-flowered *C. parviflora* plants over small-flowered plants in experimental choice arrays. It is possible that pollinators prefer larger flowers because corolla width advertises floral reward; a study of plants from ten *C. parviflora* populations raised in a growth chamber showed a strong positive correlation between flower width and nectar volume ($R^2 = 0.91$, $P < 0.0001$; E. Elle, *unpublished data*).

In addition to the frequency of visits, insect visitor identity also differed significantly among populations with different flower size. This appeared to be driven primarily by an absence of visits by *Bombus* spp. to small-flowered *C. parviflora.* Interestingly, I observed *Bombus* spp. at all four small-flowered populations, but the bees foraged exclusively on other flowering species (particularly *Camassia quamash* and *Plectritis congesta*). It is possible that discrimination against small-flowered *C. parviflora* has evolved because larger-bodied *Bombus* spp. are better able to remove pollen and nectar from large-flowers; however, the effectiveness of floral visitors as pollinators of different flower sizes is unknown.

Even though pollinator visitation rate varies significantly with both flower size and population size nested within flower size, the amount of pollen limitation does not. For example, the two small populations of small-flowered plants had on average only 1% of their total stems with flowers visited per 15-minute observation period, and yet seed production in these populations was not limited by pollen. This lack of pollen limitation despite low visitation is aptly explained by the RA hypothesis if pollinator failure in the evolutionary past has selected for increased autonomous selfing ability (Stebbins 1957). Thus, through autonomous selfing, *C. parviflora* demonstrates an ability to compensate for reduced pollinator visitation in fragmented habitats, and this compensatory ability covaries with the pollination environment.

As I predicted, population size significantly affected the magnitude of RA within each flower size class. The RA benefit was pronounced in small populations, similar to findings from research performed on *Clarkia xantiana*; pollen limitation was higher in small natural populations (Moeller 2004), and in artificially constructed small populations, there was selection for reduced herkogamy and protandry (which would consequently promote autonomous selfing) (Moeller and Geber 2005). Furthermore, similar to Elle and Carney

(2003), I showed that flower size also significantly affects RA in *C. parviflora*. Importantly, the present study found that population size within flower size affects RA, such that small populations of small-flowered plants have the greatest RA benefit; large populations of large-flowered plants the lowest; while mixed population size/flower size categories have intermediate levels of RA. The combined effect that population size and flower size have on RA reflects the influence these ecological factors have on pollinator visitation. This is supported by the strong negative relationship between visitation rate and RA. That is, infrequent pollinator visitation in small populations of small-flowered plants has resulted in selection for high levels of adaptive RA, with a greatly reduced RA potential in large populations of large-flowered plants where pollinator visitation rates are higher.

If population size and flower size similarly influence the frequency of pollinator visitation (i.e. bigger populations and flowers are visited more often), then it follows that both large populations of small-flowered plants and small populations of large-flowered plants benefited from intermediate levels of RA. It is probable, though, that population- and flower-size do not have equal effects on pollinator visitation and RA. For example, autonomous selfing is likely more directly influenced by flower size than population size because morphology determines the proximity of sexual parts at anthesis (Eckhart and Geber 1999, Kalisz et al. 1999). Hence, it could be that small-flowered *C. parviflora* plants are putative selfers irrespective of population size, whereas selfing rates in large-flowered populations are more dependent on actual population size. My results support this because with the exception of GO, small-flowered populations demonstrated high levels of RA, while the magnitudes of RA accrued in large-flowered populations appeared more dependent on population size (Fig. 2-3). Unfortunately, estimates of the realized selfing rate using neutral genetic markers have yet to be made.

The variability in pollinator visitation and RA (Figs. 2-2, 2-3) between populations within mixed population- and flower-size categories (i.e. small populations of large-flowered plants and large populations of small-flowered plants) suggests that pollination environments may be influenced by ecological factors beyond population- and flower-size. Ideally, experiments that manipulate additional ecological factors and measure RA and pollen limitation are needed, but at present, we can speculate that variation in the co-flowering plant community may be important if it affects the degree of interspecific competition for shared pollinators (Palmer et al. 2003, Knight et al 2005). For the purposes of a different study, we measured the density of forbs with open flowers using a stratified random sampling design at approximately 2-week intervals spanning the flowering season (March to July). Using the two survey dates that are closest (before and after) to my manipulative experiment in each site, the number of co-flowering forb species were: TL, 16; GO, 16; SP, 6; and CR, 16; and the sum of the number of stems of three highly attractive and potentially competitive species, the invasive shrub *Cytisus scoparius* and the native forbs *Plectritis congesta* and *Camassia quamash* were: TL, 352; GO, 18; SP, 71; and CR, 3. I highlight these forbs because I have observed high visitation rates to all three

(BFK, *personal observation*) and previous work has shown that at GO, where *P. congesta* is absent, small-flowered *C. parviflora* can be visited at high frequency by a bumble bee, *B. bifarius* (Elle and Carney 2003), which I have not observed in any other small-flowered population. I believe it is likely that visitation was higher and RA was lower than expected at GO and CR (relative to TL and SP) due to the combination of weaker interspecific competition (with *C. scoparius*, *P. congesta* and *C. quamash*) for pollinators, and increased attraction of pollinators to CR relative to SP due to the greater diversity of co-flowering species. Testing this hypothesis with a manipulative experiment is clearly in order, and would help our understanding of the importance of the community context for the evolution of plant traits.

Reproductive assurance, the crux of the often cited "best of both worlds" hypothesis (Cruden and Lyon 1989, Becerra and Lloyd 1992), enables mixed mating species to overcome pollen limitation by autonomously selfing when pollinators or mates are scarce. However, in a recent review, Goodwillie et al (2005) stress that empirical work on RA still lags behind theory. More empirical work such as the present study, which directly tests the RA hypothesis by comparing seed set by emasculated and intact control flowers in variable pollination conditions, is crucial for understanding both when and where selfing is adaptive. We especially require more studies that investigate how variable ecological circumstances affect pollen limitation and RA, and the role of such ecological drivers in mating system evolution.

Even though *C. parviflora* demonstrated a robust tolerance for pollinator failure by producing seeds via autonomous selfing, the extent of this RA benefit also depends on genetic costs such as inbreeding depression (Chapter 3). If selfed progeny are actually less fit than outcrossed progeny, then my realized rates of RA are overestimated here. Furthermore, it remains unknown whether flower size and/or population size nested within flower size affect genetic load (but see Chapter 3); it is possible that increased selfing in small populations of small-flowered plants purges deleterious alleles through selection (Charlesworth and Charlesworth 1987, Barrett and Charlesworth 1991). Clearly we require more empirical studies of the ecological conditions favouring mixed mating systems if we are to ever truly resolve both if and how such mating systems are maintained in nature.

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Tables

Table 2-1: Populations used in this study, their location, mean ± SE flower size (corolla width), area, density, and the population size categories (within flower size categories) based on the number of flowering plants per population. Corolla width means sharing the same upper-case letter indicates no significant difference as determined with Ryan's Q post-hoc test. Mean corolla width for a plant was based on the average of three flower measurements; N = number of plants in population mean.

Population (Abbreviation)		Location	Corolla width (mm)	N	Area (m^2)	Density (# plants/m^2)	Estimated Total plant #	Population size category
Small-flowered:								
Konukson Park	(KP)	48° 47'15.8"N 123° 16'25.4"W	4.91 ± 0.09^A	45	2272.4	2.8	6362.7	Small
Highrock (Cairn) Park	(HC)	48° 26'08.8"N 123° 24'24.9"W	4.27 ± 0.06^B	45	3988.0	1.5	5982.0	Small
Thetis Lake Regional Park	(TL)	48° 28'00.9"N 123° 27'58.6"W	3.88 ± 0.07^C	47	8368.2	2.0	16736.4	Large
Cowichan Garry Oak Preserve	(GO)	48° 48'30.8"N 123° 37'52.5"W	3.96 ± 0.08^C	45	1707.9	5.4	9222.7	Large
Large-flowered:								
Sooke Potholes Park	(SP)	48° 25'44.3"N 123° 42'40.9"W	6.69 ± 0.06^D	48	982.5	1.6	1572.0	Small
Cowichan River Provincial Park	(CR)	48° 46'39.0"N 123° 56'30.2"W	7.08 ± 0.12^E	44	979.5	2.0	1959.0	Small
Stolz Meadows	(SM)	48° 46'54.0"N 123° 53'06.9"W	7.70 ± 0.09^F	45	1981.3	1.7	3368.2	Large
Elk Falls Provincial Park	(EF)	50° 02'34.9"N 125° 19'32.0"W	7.92 ± 0.08^F	47	1152.4	3.7	4263.9	Large

Table 2-2: Mean differences (SE) between two flower treatments per plant, and results of t-tests comparing the two flower treatments. Reproductive Assurance: compared # seeds from intact handled flowers and # seeds from emasculated flowers; Pollen Limitation: compared # seeds from pollen supplemented flowers and # seeds from open-pollinated intact flowers; Manipulation Effect: compared # seeds from unhandled flowers and # seeds from intact handled flowers. Probabilities were Bonferroni corrected within populations where * indicates $P < 0.01$ and ** $P < 0.001$. The number of paired flowers (one pair per plant) ranged from 42 – 48 per population (mean = 45 pairs).

Population	Population Size	Flower Size	Reproductive Assurance	Pollen Limitation	Manipulation Effect
KP	small	small	2.83 (0.38)**	-0.67 (0.40)	0.27 (0.28)
HC	small	small	2.91 (0.31)**	-0.40 (0.34)	0.07 (0.30)
TL	large	small	2.98 (0.29)**	-0.37 (0.33)	0.44 (0.25)
GO	large	small	1.44 (0.37)**	-0.34 (0.30)	0.09 (0.31)
SP	small	large	1.69 (0.31)**	-0.20 (0.34)	0.30 (0.23)
CR	small	large	0.86 (0.23)*	-0.05 (0.21)	0.09 (0.21)
SM	large	large	0.10 (0.21)	0.20 (0.33)	-0.15 (0.18)
EF	large	large	0.15 (0.14)	-0.19 (0.23)	0.13 (0.16)

Figures

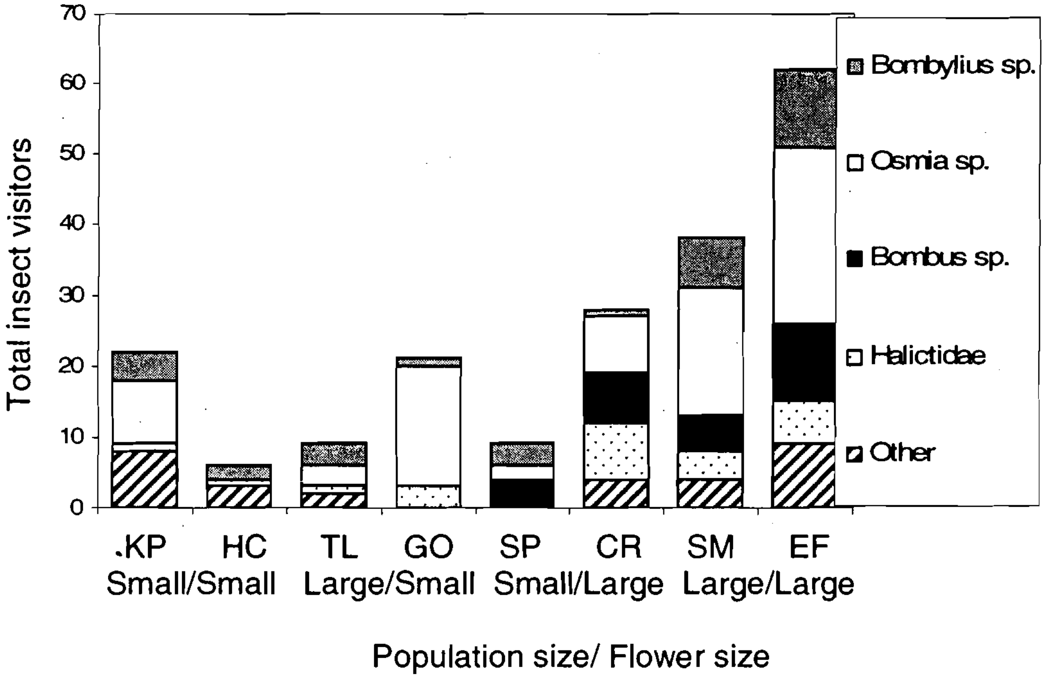

Figure 2-1: Total number of insect visitors to enter a quadrat and probe at least one flowering stem over three hours within four population size/flower size categories (two populations per category). Multiple foraging bouts by the same individual were recorded only if the insect completely exited the plot and then returned later. Contributions of insect taxa are given for each population.

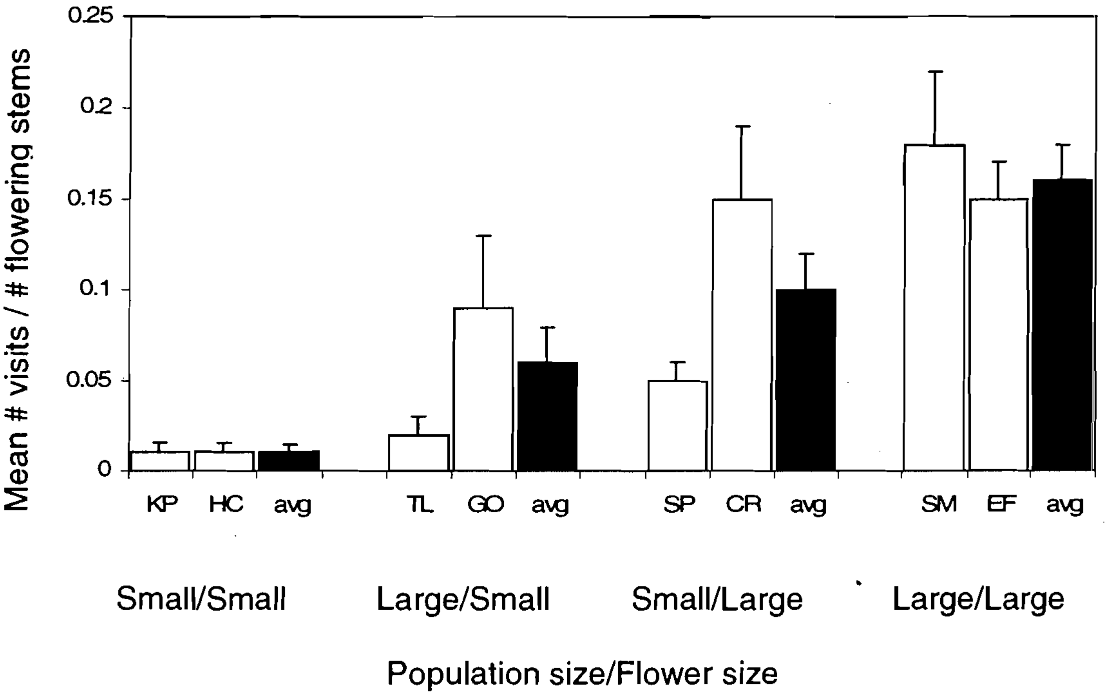

Figure 2-2: Mean visitation rate over three hours in eight populations comprising four different population size/flower size categories. Filled bars indicate average visitation rates for each population size/flower size combination.

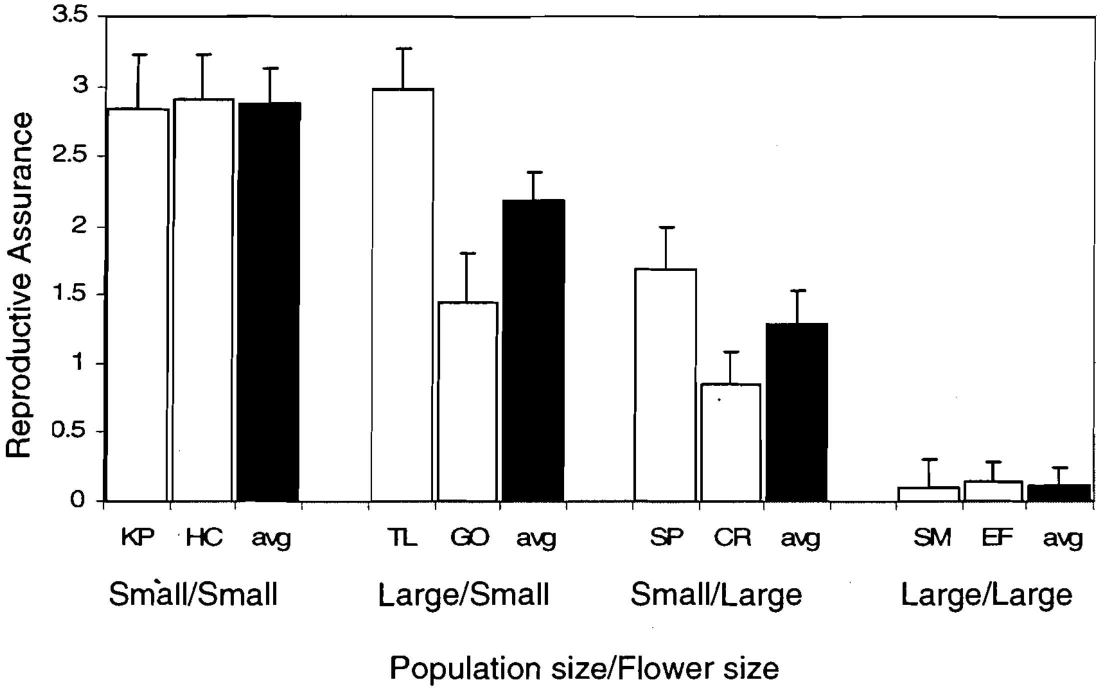

Figure 2-3: Reproductive assurance (quantified by the # of seeds from intact flowers minus the # of seeds from emasculated flowers) in eight populations comprising four different population size/flower size categories. Sample size ranges from 42 to 46 plants per population. Filled bars indicate average reproductive assurance values for each population size/flower size combination.

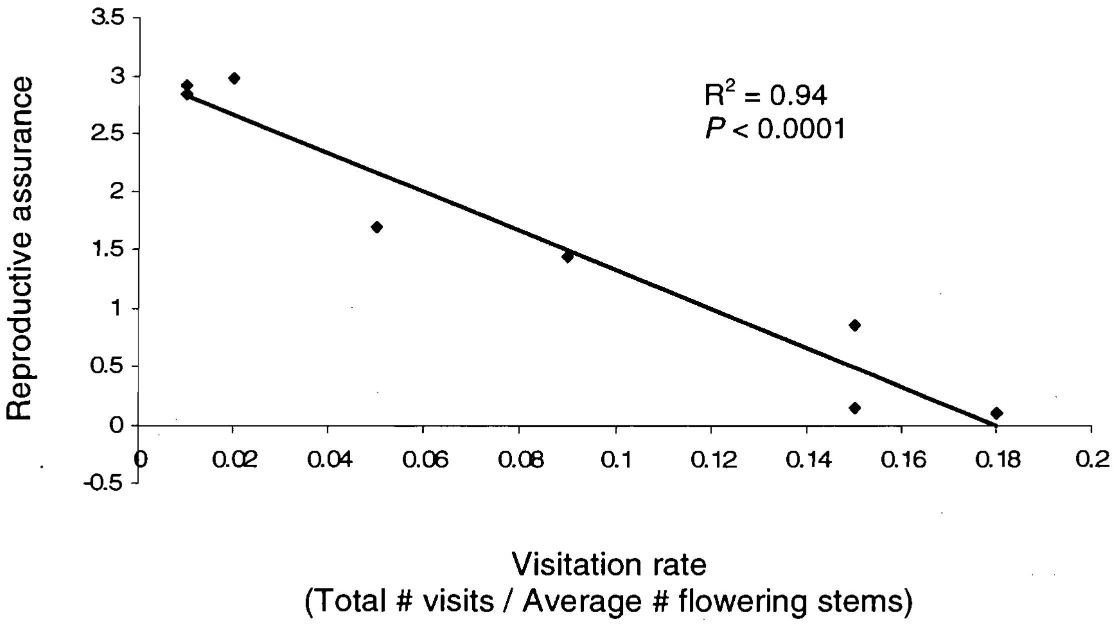

Figure 2-4: Relationship between pollinator visitation rate and reproductive assurance (quantified by the # of seeds from intact flowers minus the # of seeds from emasculated flowers) in eight natural populations of *C. parviflora*.

CHAPTER 3

The Inbreeding Depression Cost of Selfing: Importance of Flower Size and Population Size

Abstract

Inbreeding depression, the reduced fitness of inbred relative to outbred progeny, is regarded as the main genetic cost of selfing. Theory predicts that inbreeding depression may evolve in concert with selfing rate, whereby frequent inbreeding results in selection against deleterious alleles. Autonomous and/or biparental inbreeding is expected to increase following the breakdown of once-continuous habitat into smaller, more isolated patches (habitat fragmentation). Moreover, if habitat fragmentation reduces population size, then fragmentation may also affect the expression of inbreeding depression. Here I quantify the levels of inbreeding depression among different population sizes of *Collinsia parviflora*, a wildflower with inter-population variation in flower size. Specifically, I determine if pollination crosstype (self versus outcross), flower size, population size nested within flower size, and competition affect fitness traits and/or selfing correlates. When grown with a competitor, average cumulative inbreeding depression was low in all population size and flower size combinations ($\delta \leq 0.05$), except large populations of large-flowered plants ($\delta = 0.45$), however neither flower size nor population size nested within flower size significantly affected the multiplicative fitness of selfed relative to outcrossed progeny across all life stages. Significant inbreeding depression was detected for survival to flowering in large-flowered populations, suggesting that purging has not occurred in presumably highly outcrossing large-flowered populations. Furthermore, across all populations, corolla length was significantly affected by pollination crosstype, but corolla width was not. Selfed progeny had significantly shorter corolla lengths than outcrossed progeny. A shorter corolla length likely promotes autonomous selfing, and thus only represents inbreeding depression if outcrossing is more beneficial in the population. These results provide indirect evidence that deleterious recessive alleles are purged by selection in highly selfing populations, and suggest that the cost of inbreeding depression may be reduced in fragmented habitats.

Key words: Collinsia parviflora; *floral morphology; habitat fragmentation; inbreeding depression; mating system evolution; purging*

Introduction

The adaptive value of self-fertilization has been questioned since Darwin (1876) experimentally demonstrated a reduction of fitness in inbred, relative to outbred progeny. Fisher (1941), however, showed that an allele for self-fertilization should spread in an outcrossing population, because it is passed along through both pollen and ovules in selfed seeds, as well as through outcrossed pollen deposited on conspecific plants. The interplay of these opposing genetic forces (inbreeding depression and the automatic selection advantage, respectively) is customarily invoked to explain the predicted transition of mixed mating to either an obligate selfing or outcrossing mating system (Lande and Schemske 1985). However, mixed mating systems are common in plants, although the stability of these reproductive strategies remains unclear (Goodwillie et al. 2005).

The major cost to selfing, inbreeding depression, results from the expression of deleterious recessive alleles (partial dominance hypothesis) and/or overdominant loci (overdominance hypothesis), but most data support that partially recessive alleles are the primary source of the cost of selfing (Charlesworth and Charlesworth 1987, Johnston and Schoen 1995). Moreover, inbreeding depression may not be fixed, as selection should purge deleterious homozygous recessive alleles over time (Lande and Schemske 1985). Empirical support for purging remains equivocal, as comparative studies indicate that it probably occurs, but its importance is highly variable across plant taxa (Husband and Schemske 1996, Byers and Waller 1999).

If purging is in fact important for the evolution of inbreeding depression, then the magnitude of inbreeding depression within a population should depend largely on mating history. Various population and plant characteristics can affect population mating history, but perhaps none more directly than population size and flower size. Indeed, the frequency of autonomous selfing and/or biparental inbreeding is higher in small populations and fragmented habitats (Ellstrand and Elam 1993, Dudash and Fenster 2000). In addition, small flower size can reduce both a plant's attractiveness to pollinators (Elle and Carney 2003) and increase its ability to self (Eckhart and Geber 1999, Elle 2004), both of which lead to increased inbreeding. Clearly, both population size and flower size have the potential to influence the mating history of a population, and yet, no study has attempted to test within a species the effect that these two factors have on the expression of inbreeding depression.

Unlike conventional fitness traits used in inbreeding depression studies (e.g. flower production), adaptive phenotypes for selfing correlates likely vary depending on the environment. For instance, small corolla width and/or length may in fact be beneficial if they increase autonomous selfing and provides reproductive assurance when pollinators are rare (Stebbins 1957); differences among populations in pollinator visitation rates may select for differences in the benefit associated with reproductive assurance (Chapter 2). Inbreeding depression for corolla width and length can therefore not be measured across populations if optimal phenotypes are population-specific. Rather, the effects of selfing correlates on inbreeding depression should be considered separately for populations with different ecological contexts.

Expression of inbreeding depression is often higher under more stressful conditions (Dudash 1990, Wolfe 1993, Mayer et al. 1996, Cheptou et al. 2000). This can mean that the measurement of inbreeding depression under benign growth conditions, such as in growth chambers or greenhouses, might underestimate actual values. Accordingly, experiments under benign environments should benefit from incorporating a stress treatment in their design. This should reduce the risk of committing a type 2 error, and provide a more representative estimate of inbreeding depression under natural conditions.

In this study, I measured the expression of inbreeding depression across various life stages in plants from different flower-sized populations of *C.*

parviflora, and examined whether or not the magnitude of inbreeding depression varied with population size and/or competition. I predict that the greatest degree of inbreeding depression should occur in large populations of large-flowered plants (where purging is less likely to have occurred) and little to no inbreeding depression should be measured in small populations of small-flowered plants (where purging has likely occurred after numerous generations of selfing). Moreover, I predict that interspecific competition will amplify the magnitude of inbreeding depression, especially in populations where significant purging has not occurred. Finally, I examined whether selfed or outcrossed pollination treatments affected the expression of two selfing correlates (corolla width and length), and whether differences due to pollen source depended on flower size and/or population size nested within flower size.

Methods

Study organism

Collinsia parviflora (Scrophulariaceae *s.l.*) is a winter annual found in grassy slopes, mossy rock outcrops, and beaches in western North America (Douglas et al. 1998). At my study sites on Vancouver Island, British Columbia, *C. parviflora* is found in highly fragmented Garry Oak (*Quercus garryana*) and associated ecosystems. Flowers consist of two upper banner petals, two lower wing petals, and a folded keel petal containing the sexual parts; a corolla tube is formed where petals unite at the mouth of the flower.

In BC, natural populations vary significantly in flower size (Ganders and Krause 1986, Elle and Carney, 2003), and these differences have a genetic basis

(Elle 2004). Although flower size varies continuously in this species on Vancouver Island, I focused on the extreme flower size phenotypes for the purposes of this study. Outcrossing may occur via visits by bees in the genera *Osmia* and *Bombus*, as well as beeflies (Chapter 2); however, autonomous selfing rates are high when pollinators are excluded (Elle 2004), and autonomous selfing has been shown to provide reproductive assurance in some populations (Chapter 2, Elle and Carney 2003).

Measurement of inbreeding depression

Plants for this study were established from seeds collected from haphazardly selected individuals in eight populations on Vancouver Island, B.C. Based on previous studies (Elle and Carney 2003, Elle 2004, Chapter 2) we classified populations a priori as either large or small, according to the estimated number of flowering plants; and large- or small-flowered, according to the corolla width measured across the two banner petals (for a complete overview of population characteristics see Table 2-1). Field-collected seeds were raised in a growth chamber at Simon Fraser University and served as parent plants to create 24 hand-pollinated, reciprocal selfed and outcrossed maternal families for each population. Outcrossed flowers were emasculated at the bud stage; removing anthers at the bud stage does not significantly affect seed set (Chapter 2). Self-pollinations and outcrossed-pollinations (hereafter 'crosstypes') were performed on at least three flowers per maternal plant, on flowers in close proximity in order to minimize any effect of flower position on seed size.

The effect of inbreeding was estimated at four life stages: seed mass, seed germination (proportion of seeds to germinate), survival to flowering (proportion of plants to reach flowering), and fecundity (above-ground vegetative biomass and total flower production). Seed mass for crosstypes of 12 randomly chosen maternal families per population was estimated to the nearest 0.01mg by dividing the total mass of eight randomly selected seeds of each crosstype by eight. Seeds were planted in sterile potting soil in 48-cell flats (16 flats) and initially raised in a growth chamber under short days (10 h 20°C day/14 h 10°C night), and switched to long days (16 h 20°C day/8 h 10°C night) eight weeks later. Proportion to germinate was based on two seeds per cell; however, if both seeds germinated then one was randomly removed, leaving four plants of each crosstype per family.

Of the four seeds per crosstype, half were grown under a 'competitive' treatment, with *Anthoxanthum odoratum*, an invasive grass common in Garry Oak ecosystems (Pojar and MacKinnon 1994, BFK, *personal observation*), while the other half were raised alone ('noncompetitive'). A competitive stress treatment was included because other studies have demonstrated that competition can influence the magnitude of inbreeding depression (Dudash 1990, Wolfe 1993, Mayer et al. 1996, Cheptou et al. 2000). Competitive and noncompetitive treatments were raised in separate flats, as were small- and large-flowered plants, in order to avoid asymmetric competition (Fishman 2001). Families were randomly assigned positions within a flat. Selfed and outcrossed treatments of the same family were planted adjacent to one another; the crosstype positioned at the edge of the flat was alternated between families to eliminate any confound between crosstype and edge effects. Flats were watered and rotated within the growth chamber every 4 days, and fertilized once after 8 weeks (15:30:15 NPK). Plants were harvested upon completion of flowering and dried in envelopes for at least 21 days before being weighed to the nearest 0.01mg.

In total, there were 12 families per population with four seeds per crosstype grown to maturity, half of which were grown with a competitor. A "treatment" was a combination of crosstype (self or outcross), competition (competitive or noncompetitive), flower size (large or small) and population size (large or small) and was replicated twice at the family level.

To determine whether inbreeding depression differed by treatment, I performed a nested analysis of variance (ANOVA, SAS 1996) on each fitness trait individually, as well as on the multiplicative fitness of all traits (excluding biomass as it was significantly correlated with flower production, $r = 0.67$, $P < 0.001$) with crosstype, flower size, population size nested within flower size, and competition as fixed effects. A significant crosstype effect, or any significant interactions with crosstype (whereby selfed progeny performance < outcross progeny performance) indicates inbreeding depression; accordingly, these effects are the focus of this chapter. Competition was excluded from the model for seed mass, as the competitor was not introduced until the seeds were planted. Family was not included in the model because having less than five replicates biases inbreeding depression downward (Johnston and Schoen 1994).

Population size was treated as categorical and nested in flower size because population size can only be defined relative to flower size in this species (Table 2-1). Flower size was also treated categorically for simplicity in the model, as previous surveys have consistently found no overlap in flower sizes among the small-flowered and large-flowered populations used in this study (Table 2-1). To reduce heteroscedasticity, proportional variables (germination and survival to flowering) were arcsine-square root transformed, and seed mass, flower production, and multiplicative fitness were log transformed. Johnston and Schoen (1994) note that to test for variation among populations in inbreeding depression, data should be log transformed. However, log transforming vegetative biomass resulted in data that violated ANOVA assumptions of homoscedasticity and so was left untransformed; this produced similar results (see Results).

Inbreeding depression (δ) was calculated for each variable that contributed to fitness in all populations as $\delta = 1 - (w_s/w_o)$, where w_s and w_o are the mean performances of selfed and outcrossed progeny, respectively. Population means for selfed and outcrossed progeny at each life stage were estimated as the mean of all family means within the population. Cumulative inbreeding depression was calculated by applying the formula above to the product of the fitness values for each crosstype across all life stages. Vegetative biomass and flower production were strongly correlated ($r = 0.67$, $P < 0.001$), and consequently, only flower production was included as a fecundity measure in the calculation of cumulative inbreeding depression; flower production is more likely to directly influence fecundity in an annual species. Relative crosstype fitness was compared for each trait within all populations using paired t-tests, to determine if the average difference in fitness between selfed and outcrossed progeny differed significantly from zero; *P*-values from t-tests were Bonferroni adjusted to account for multiple tests (four with competition and five without) within each population.

Measurement of selfing correlates

To estimate whether crosstype, competition, flower size and/or population size nested within flower size affect selfing correlates, I scored corolla width (measured across two banner petals) and corolla length (measured from tip of lower wing petals to base of ovary). The influence of crosstype, competition, flower size and/or population size nested within flower size on corolla width and length was analyzed with an ANOVA (see ANOVA model details above). Corolla width and length were log transformed to reduce heteroscedasticity.

Inbreeding depression (δ) was not calculated using corolla width or length because adaptive phenotypes for these selfing correlates are likely population dependent; i.e. a short corolla tube may be adaptive in small (pollen limited) populations, but maladaptive in large (outcrossing) populations. Finally, I used paired t-tests to compare relative corolla length for selfed and outcrossed progeny within all populations, as the ANOVA indicated a significant crosstype effect on this selfing correlate (see Results).

Results

Interspecific competition reduced seed germination, flower production, and vegetative biomass across all populations (Tables 3-1 and 3-2). There was a significant competition x crosstype interaction ($F = 4.87$, $P < 0.03$) for vegetative biomass when data were log transformed as per Johnston and Schoen (1994), however, this interaction was marginally nonsignificant (Table 3-1) when left untransformed to satisfy ANOVA assumptions.

Flower size had a significant effect on fitness across three life stages (Table 3-1), as small-flowered populations had smaller F_1 seeds, higher survival to flowering and more flowers per plant (Table 3-2). Moreover, population size nested within flower size significantly affected seed mass, survival, vegetative biomass and flower production (Table 3-1). Within small-flowered plants, large populations had higher flower production, but lower seed mass and vegetative biomass (Table 3-2). Whereas in large-flowered plants, large populations had greater seed mass and vegetative biomass, but lower survival and flower production (Table 3-2).

Inbreeding depression

Despite a significant crosstype effect on survival to flowering and flower production (Table 3-1), there was only a consistent fitness reduction in flower production for selfed relative to outcrossed progeny (Table 3-2). Interestingly, there was a significant crosstype x flower size interaction on the rate of survival (Table 3-1), whereby the negative impact of selfing was present in large-flowered populations (Table 3-2). Moreover, the significant competition x crosstype x flower size interaction on survival (Table 3-1) was driven by a consistent expression of inbreeding depression in large- relative to small-flowered populations, both in the presence and absence of a competitor (Table 3-2). This three-way interaction was also significant for germination rate (Table 3-1), however, this cannot be interpreted as inbreeding depression because outcrossed progeny did not consistently have higher germination success than selfed progeny (Table 3-2).

There was a significant crosstype effect on the multiplicative fitness of selfed relative to outcrossed progeny across four life stages; however, none of the interactions with crosstype were significant (Table 3-1). Population cumulative inbreeding depression was generally low in all population size/flower size categories except large populations of large-flowered plants (Fig. 3-1). Population cumulative inbreeding depression ranged from -0.19 to 0.65 and -0.12 to 0.45, with and without competition, respectively (Fig. 3-1).

Selfing correlates

Across all populations, corolla length, but not width, was significantly smaller in selfed than outcrossed progeny (Table 3-3). The significant difference in floral length between crosstypes appears most pronounced in large-flowered populations under competition (Fig. 3-2); however, the competition x crosstype x flower size interaction was not significant (Table 3-3). Flower size and population size nested within flower size significantly affected both corolla length and width (Table 3-3). The population size nested within flower size effect was driven by differences in corolla length between population sizes of large-flowered plants (Fig. 3-2).

Discussion

Flower production was significantly reduced across all populations following self-pollination, thus demonstrating some, albeit minor, inbreeding depression in *C. parviflora*. Furthermore, small-flowered populations tended to have smaller seeds, higher survival to flowering, and more flowers per plant – the latter perhaps reflecting a resource trade-off (i.e. smaller but more flowers). The main effect of population size nested within flower size is more difficult to interpret biologically, as population size affected traits inconsistently between flower sizes.

Inbreeding depression

That *C. parviflora* incurred inbreeding depression is not, in itself, surprising, considering inbreeding depression has been commonly documented in the tribe Collinseae. More unexpected was the mildness of inbreeding depression, and how it was absent across most life stages of *C. parviflora*. Indeed, Mayer et al. (1996) detected inbreeding depression at all life stages in populations of *C. heterophylla*, while Kalisz (1989) observed that outcrossed seeds of *C. verna* were larger, had higher germination rates and produced more fecund plants than selfed seeds. What remains uncertain, however, is how specific population and plant characteristics influence the evolution of inbreeding depression in light of the purging hypothesis. Specific characteristics such as flower size and population size are of particular interest since they are both expected to influence selfing rate. Estimates of realized selfing rate are unknown for these populations, but pollinator visitation patterns (Fig 2-2) and variation in autonomous selfing ability (Elle 2004) are congruent with the prediction that opportunities to outcross are diminished when population size and flower size are reduced.

Flower size significantly affected the magnitude of inbreeding depression, whereby selfed progeny were less likely to survive to flower than outcrossed progeny in large-flowered populations. None of the within population paired t-tests comparing survival between selfed and outcrossed progeny were significant, indicating that the significant flower size effect on inbreeding depression was driven by the accumulation of small, yet consistent, differences between crosstypes across populations. Similarly, in a comparison between two closely related species of *Linanthus* (that differ in flower size and mating system type), Goodwillie (2000) mostly found cumulative, and not within-population, effects on inbreeding depression in the larger-flowered *L. jepsonii* species. Furthermore, the only small-flowered *L. bicolor* population to actually incur significant cumulative inbreeding depression had larger corollas than any of the other small-flowered *L. bicolor* populations (Goodwillie, 2000). A relationship between flower size and inbreeding depression may be common among taxa that exhibit intra-taxon variation in flower size, perhaps reflecting how flower morphology affects autonomous selfing rate, and in turn, the rate in which deleterious recessive alleles are culled by selection. This should be investigated.

Flower size affected the expression of inbreeding depression in both the presence and absence of competition (as indicated by a significant competition x crosstype x flower size interaction, Table 3-1). The significant three-way interaction opposes theory on the evolution of inbreeding depression, because the magnitude of inbreeding depression among the competition and flower size treatments differed significantly only in survival to flowering and not in a more early-acting life trait such as seed mass or germination success. The prediction that inbreeding depression will be expressed in early-acting traits within large-flowered populations (i.e. where early acting homozygous recessive alleles are less likely to have been exposed to selection as the selfing rate in nature is likely lower) was not supported, and is therefore inconsistent with the idea that deleterious recessive alleles are expressed and purged early in life (Husband and Schemske, 1996).

Individually, none of the response variables demonstrated a significant interaction between population size nested within flower size and crosstype. Furthermore, when taking into account the multiplicative fitness across all life stages, there was also no significant interaction between population size nested within flower size and crosstype. Taken together, population size nested within flower size appears to play an insignificant role relative to flower size in the expression of inbreeding depression. However, based on the less informative descriptive cumulative data, I can speculate that population size may in fact influence inbreeding depression within small- and large-flowered plants of this species, as cumulative inbreeding depression appeared to vary with population size nested within flower size and competition (Fig 3-1). The average cumulative inbreeding depression for noncompetitive treatments of large populations of large-flowered plants was only marginally bigger than small populations of the same flower size (Fig 3-1a). Conversely, when grown with a competitor, average cumulative inbreeding depression was substantially greater in large populations of large-flowered plants versus small populations of large-flowered plants (Fig 3-1b.). Which of these two scenarios is more likely to reflect patterns of inbreeding depression in natural populations? On Vancouver Island, *C. parviflora* populations frequently coexist with *A. odoratum* and a suite of other grass and forb species (BFK, *personal observation*), suggesting that the result under the competitive treatment (Fig. 3-1b) is more realistic.

While there was no significant population size nested within flower size x crosstype interaction effect on multiplicative fitness, I again turn to the descriptive data to posit that population size may only influence cumulative inbreeding depression in large-flowered populations. The difference in average cumulative inbreeding depression between large and small populations of small-flowered plants was relatively small, and is explained if small-flowered plants reduce their genetic load because they are putative autonomous selfers (due to morphology) regardless of population size. Moreover, biparental inbreeding is typically more common in small populations than large populations (Tomimatsu and Ohara 2006), which may account for the contrasting levels of inbreeding depression found in the different sized populations of large-flowered plants. In other words, even though small populations of large-flowered plants are visited frequently by pollinators (Chapter 2), the increased biparental inbreeding in these small populations likely leads to increased purging and a reduced genetic load relative to large populations of large-flowered plants. Interestingly, a similar trend of population size mainly affecting large-flowered populations was also found for reproductive assurance benefits (Fig 2-3). While support for the purging hypothesis remains equivocal (Byers and Waller 1999), my results provide moderate support for purging, as cumulative inbreeding depression appears lower in what are presumably more selfing populations. The presence of some, albeit low, cumulative inbreeding depression in small populations of small flowered plants may be the result of the fixation of mildly deleterious alleles due to drift.

Selfing correlates

It is curious that corolla length, but not width, was significantly affected by crosstype, considering these traits were found to be highly correlated in a separate study ($R^2 = 0.91$, $P < 0.05$, Elle 2004). Moreover, both floral traits were significantly negatively correlated with autonomous selfing rate (Elle 2004). Taken together, corolla length and width are likely selfing rate modifiers, although it is possible that they influence selfing rates differently. For example, corolla width may promote outcrossing by advertising floral reward to potential pollinators. Indeed, a study of plants from ten *C. parviflora* populations raised in a growth chamber showed a strong positive correlation between flower width and nectar volume ($R^2 = 0.91$, $P < 0.0001$; E. Elle, *unpublished data*). Moreover, Elle and Carney (2003) demonstrated that pollinators preferred large-flowered (as measured across banner petals) *C. parviflora* plants over small-flowered plants in experimental choice arrays. Conversely, corolla length may directly affect selfing ability if flowers with short corolla tubes have the sexual parts of a flower in closer proximity at anthesis (Armbruster et al. 2002). Nevertheless, corolla width and length are predictably highly correlated, and reduced phenotypes of both traits are correlated with higher autonomous selfing rates (Elle 2004). Interestingly, my data suggest that inbreeding depression may be expressed through corolla length but not width.

It is not explicitly clear whether having longer corollas is beneficial for this species, but selection may reduce corolla length if it increases autonomous selfing rates in pollen limited environments. Selfed progeny in three of the four large-flowered populations with competition had significantly shorter corollas than outcrossed progeny, while only one small-flowered population (HC) demonstrated a significant difference in length between crosstypes (Fig. 3-2b). The reduction in corolla length in selfed progeny at HC may in fact be beneficial, if it promotes selfing when pollinator visitation is rare. Indeed, a separate study revealed that reproductive assurance was high in HC (Table 2-3), further supporting that a shorter corolla length in this particular population is likely adaptive. Conversely, in the large populations of large-flowered plants (EF and SM), significantly shorter corollas in selfed, relative to outcrossed progeny, represents an indirect cost to selfing because having shorter corollas likely promotes selfing which can lead to lower survival and flower production in this species. That is, by reducing corolla length, selfing promotes the chances of selfing in subsequent generations, which may provide positive feedback for the expression of inbreeding depression in large populations of large-flowered populations, until purging eventually transpires.

Evolution of mating system in *C. parviflora*

Inbreeding depression, the main genetic cost of selfing, should evolve jointly with the degree of selfing within a population (Lande and Schemske 1985). Consequently, if deleterious recessive alleles are purged by selection, then a mixed mating system should evolve toward becoming predominately selfing (Lande and Schemske 1985). In *C. parviflora*, it is likely that flower size predicts variation in realized selfing, as flower size covaries with autonomous selfing ability when pollinators are excluded (Elle 2004). By focusing on the extreme flower sizes and population sizes on Vancouver Island, my data suggests that inbreeding depression may in fact have been purged except in large populations of large-flowered plants. The apparent contrast in purging among treatments may be paramount in understanding if and how mixed mating is maintained in *C. parviflora*.

Theory holds that if selfing can be achieved without reducing the siring of outcrossed seed on other individuals (pollen discounting), then inbreeding depression must be greater than 0.5 to counteract the 3:2 advantage of selfer alleles (Kimura 1959, Lande and Schemske 1985). In the competitive treatment (as this treatment is likely more representative of natural conditions than the noncompetitive one), the average cumulative inbreeding depression for small/small, large/small, small/ large and large/large population size/flower size categories was 0.05, 0.04, 0.02, and 0.45, respectively. In the latter category, average cumulative inbreeding depression was nearly 0.5, indicating an ecological scenario where outcrossing may be adaptive. Conversely, inbreeding depression in the first three categories was negligible, suggesting that alleles promoting selfing would be favoured in these populations. However, it should be noted that in the two mixed population size/flower size categories (large populations of small-flowered plants and small populations of large-flowered plants), the overall negligible cumulative inbreeding depression was the result of an average of two very contrasting inbreeding depression values (Fig 3-1b). Variation among population size/flower size replicates suggests that there may be other population-specific effects; however, the two extreme categories (small populations of small-flowered plants and large populations of large-flowered plants) demonstrated a relatively consistent trend (Fig 3-1b).

Inbreeding depression among different population sizes of small- and large-flowered *C. parviflora* is moderately congruent with a comparative study of 54 species that found inbreeding depression = 0.23 in primarily selfing species, and inbreeding depression = 0.53 in primarily outcrossing species (Husband and Schemske 1996). The findings in the present study offer a unique perspective on the overall trend documented by Husband and Schemske (1996), as the variation in cumulative inbreeding depression was expressed within a species. However, multiplicative fitness of inbred relative to outbred progeny across four life stages did not vary significantly with flower size or population size nested within flower size, suggesting that further work is needed to resolve which factors play a prominent role in determining the cost of selfing, and ultimately, mating system evolution.

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Tables

Table 3-1: Analysis of variance (ANOVA) testing the effects of competition (present versus absent), crosstype (self- versus cross-fertilization), flower size (small versus large) and population size (small versus large) nested within flower size on seed mass, germination, survival to flower, flower production, vegetative biomass, and the multiplicative fitness across all life stages (excluding biomass because it was significantly correlated with flower production). Competition was not included in the model for seed mass because the competitor was not introduced until after the seeds were planted. *P*-values in boldface indicate significance ($P < 0.05$). Seed mass, number of flowers, and multiplicative fitness were log transformed while rate of germination and rate of survival to flowering were arcsine- square root transformed.

Response Variable	Source of Variation	d.f.	MS	*F*	*P*
F_1 Seed Mass	Crosstype	1	0.085	1.64	0.2
	Flower size	1	11.14	214.4	**0.0001**
	Population size(Flower size)	2	0.67	12.93	**0.0001**
	Crosstype*Flower size	1	0.023	0.45	0.51
	Crosstype*Population size(Flower size)	2	0.042	0.81	0.45
Rate of germination	Competition	1	0.13	4.66	**0.03**
	Crosstype	1	0.01	0.54	0.46
	Flower size	1	0.05	2.00	0.16
	Population size(Flower size)	2	0.02	0.67	0.51
	Crosstype*Flower size	1	0.05	1.71	0.19
	Crosstype*Population size(Flower size)	2	0.02	0.64	0.53
	Competition*Crosstype	1	0.07	2.68	0.10
	Competition*Crosstype*Flower size	2	0.10	3.56	**0.03**
	Competition*Crosstype*Population size(Flower size)	4	0.02	0.63	0.64

Response Variable	Source of Variation	d.f.	MS	*F*	*P*
Rate of survival to flowering	Competition	1	0.03	0.86	0.35
	Crosstype	1	0.19	6.03	**0.01**
	Flower size	1	0.45	14.34	**0.0002**
	Population size(Flower size)	2	0.29	9.17	**0.0001**
	Crosstype*Flower size	1	0.15	4.62	**0.03**
	Crosstype*Population size(Flower size)	2	0.07	2.13	0.12
	Competition*Crosstype	1	0.0003	0.01	0.92
	Competition*Crosstype*Flower size	2	0.09	3.03	**0.04**
	Competition*Crosstype*Population size(Flower size)	4	0.04	1.14	0.34
Flower production	Competition	1	51.07	284.6	**0.0001**
	Crosstype	1	1.15	6.39	**0.01**
	Flower size	1	4.74	26.45	**0.0001**
	Population size(Flower size)	2	1.04	5.82	**0.003**
	Crosstype*Flower size	1	0.36	2.02	0.16
	Crosstype*Population size(Flower size)	2	0.38	2.10	0.12
	Competition*Crosstype	1	0.043	0.24	0.63
	Competition*Crosstype*Flower size	2	0.09	0.50	0.60
	Competition*Crosstype*Population size(Flower size)	4	0.18	0.97	0.42
Vegetative biomass	Competition	1	195109.4	381.8	**0.0001**
	Crosstype	1	426.6	0.83	0.36
	Flower size	1	25.8	0.05	0.82
	Population size(Flower size)	2	3980.5	7.79	**0.0005**
	Crosstype*Flower size	1	809.3	1.58	0.21
	Crosstype*Population size(Flower size)	2	115.9	0.23	0.80
	Competition*Crosstype	1	1700.8	3.33	0.07
	Competition*Crosstype*Flower size	2	483.2	0.95	0.39
	Competition*Crosstype*Population size(Flower size)	4	318.1	0.62	0.65

Response Variable	Source of Variation	d.f.	MS	*F*	*P*
Multiplicative fitness	Competition	1	58.92	148.01	**0.0001**
	Crosstype	1	2.80	7.03	**0.008**
	Flower size	1	6.12	15.37	**0.0001**
	Population size(Flower size)	2	0.21	0.53	0.59
	Crosstype*Flower size	1	0.88	2.21	0.14
	Crosstype*Population size(Flower size)	2	0.35	0.89	0.41
	Competition*Crosstype	1	0.05	0.11	0.73
	Competition*Crosstype*Flower size	2	0.02	0.04	0.96
	Competition*Crosstype*Population size(Flower size)	4	033	0.84	0.50

Table 3-2: Mean (S.E.) performance of selfed and outcrossed progeny in the absence (a) and presence (b) of a competitor (*A. odoratum*). An asterisk indicates that selfed progeny (S) performance was significantly lower than outcross progeny (O) performance (*P* < 0.05), as determined by Bonferroni corrected paired t-tests. *N* = 12 families per population.

a) Competitor absent

	Seed mass (mg)		Germination rate		Survival rate		Flower production		Vegetative biomass (mg)	
Population	S	O	S	O	S	O	S	O	S	O
Small-flowered										
KP	0.74	0.83	0.98	0.94	0.96	0.96	16.13	13.54	117.25	109.71
	(0.04)	(0.07)	(0.02)	(0.03)	(0.42)	(0.42)	(2.76)	(1.02)	(9.57)	(4.55)
HC	0.64*	0.75	0.98	0.96	1.0	0.96	14.79	13.83	97.09	92.42
	(0.03)	(0.04)	(0.02)	(0.04)	(0.00)	(0.04)	(0.88)	(0.84)	(6.19)	(5.98)
GO	0.71	0.69	0.90	0.96	0.92	1.0	15.25	16.46	91.83	81.57
	(0.05)	(0.05)	(0.06)	(0.03)	(0.06)	(0.00)	(1.67)	(1.41)	(4.96)	(4.04)
TL	0.63	0.65	1.0	1.0	1.0	1.0	19.17	19.83	91.18	88.10
	(0.05)	(0.05)	(0.00)	(0.00)	(0.00)	(0.00)	(2.60)	(2.62)	(6.45)	(5.02)
Large-flowered										
SP	0.91	0.91	0.85	0.96	0.96	1.0	10.25	11.92	64.10	66.71
	(0.03)	(0.04)	(0.06)	(0.03)	(0.04)	(0.00)	(0.54)	(1.05)	(2.52)	(3.01)
CR	1.09	1.17	0.96	0.96	0.96	1.0	14.63	16.38	112.26	112.25
	(0.08)	(0.07)	(0.28)	(0.28)	(0.04)	(0.00)	(1.64)	(1.39)	(7.56)	(7.72)
SM	1.10	1.13	0.94	0.94	0.75	0.96	9.58	13.33	93.09	95.20
	(0.05)	(0.04)	(0.04)	(0.03)	(0.10)	(0.04)	(1.77)	(1.53)	(6.57)	(5.09)
EF	1.42	1.39	0.98	1.0	1.0	1.0	14.38	16.58	97.26	101.28
	(0.08)	(0.07)	(0.02)	(0.00)	(0.00)	(0.00)	(1.45)	(1.93)	(6.90)	(10.35)

b) Competitor present

	Germination rate		Survival rate		Flower production		Vegetative biomass (mg)	
Population	S	O	S	O	S	O	S	O
Small-flowered								
KP	0.94	0.90	1.0	1.0	8.04	7.71	60.99	70.60
	(0.05)	(0.07)	(0.00)	(0.00)	(1.36)	(0.51)	(5.61)	(4.69)
HC	0.96	0.83	1.0	1.0	6.29	6.71	36.14	40.55
	(0.04)	(0.90)	(0.00)	(0.00)	(0.42)	(0.29)	(2.97)	(2.02)
GO	0.85	0.83	1.0	1.0	9.83	9.88	48.67	43.29
	(0.08)	(0.06)	(0.00)	(0.00)	(1.77)	(1.86)	(7.47)	(5.07)
TL	0.93	0.88	1.0	1.0	8.04	9.50	37.44*	47.98
	(0.04)	(0.04)	(0.00)	(0.00)	(1.00)	(1.27)	(3.79)	(5.03)
Large-flowered								
SP	1.0	0.92	1.0	1.0	6.95	6.42	38.47	36.45
	(0.00)	(0.04)	(0.00)	(0.00)	(0.57)	(0.91)	(2.21)	(3.57)
CR	0.92	1.0	0.92	0.96	7.33	7.75	55.23	67.48
	(0.47)	(0.00)	(0.06)	(0.04)	(0.91)	(1.29)	(4.88)	(6.61)
SM	1.0	0.92	0.58	0.83	2.87*	6.04	45.88	58.14
	(0.00)	(0.05)	(0.12)	(0.09)	(0.81)	(1.07)	(6.41)	(7.62)
EF	0.92	0.96	0.96	0.96	5.54	7.33	46.93	55.90
	(0.47)	(0.42)	(0.04)	(0.04)	(0.26)	(0.78)	(4.05)	(4.15)

Table 3-3: Analysis of variance (ANOVA) testing the effects of competition (competitive versus noncompetitive), crosstype (self- versus cross-fertilization), flower size (small versus large) and population size (small versus large) nested within flower size on corolla length and width. *P*-values in boldface indicate significance ($P < 0.05$). Corolla length and width were log transformed.

Response Variable	Source of Variation	d.f.	MS	*F*	*P*
Corolla length	Competition	1	0.11	13.3	**0.0003**
	Crosstype	1	0.13	14.9	**0.0001**
	Flower size	1	14.3	1647.5	**0.0001**
	Population size(Flower size)	2	0.23	26.9	**0.0001**
	Crosstype*Flower size	1	0.001	0.15	0.70
	Crosstype*Population size(Flower size)	2	0.0009	0.10	0.90
	Competition*Crosstype	1	0.002	0.25	0.62
	Competition*Crosstype*Flower size	2	0.002	0.25	0.78
	Competition*Crosstype*Population size(Flower size)	4	0.0009	0.11	0.98
Corolla width	Competition	1	0.11	5.63	**0.02**
	Crosstype	1	0.04	2.00	0.16
	Flower size	1	24.07	1249.7	**0.0001**
	Population size(Flower size)	2	0.25	13.22	**0.0001**
	Crosstype*Flower size	1	0.18	0.92	0.34
	Crosstype*Population size(Flower size)	2	0.004	0.19	0.83
	Competition*Crosstype	1	0.001	0.06	0.81
	Competition*Crosstype*Flower size	2	0.002	0.08	0.92
	Competition*Crosstype*Population size(Flower size)	4	0.0009	0.11	0.98

Figures

Figure 3-1: Cumulative inbreeding depression within four population size/flower size categories (two populations per category). Filled bars indicate average cumulative inbreeding depression for each population size/flower size category. Individual plants were grown alone (a) or in the presence of a competitor (b). Dashed line indicates the theoretical threshold whereby populations with inbreeding depression values below the line gain an automatic transmission advantage.

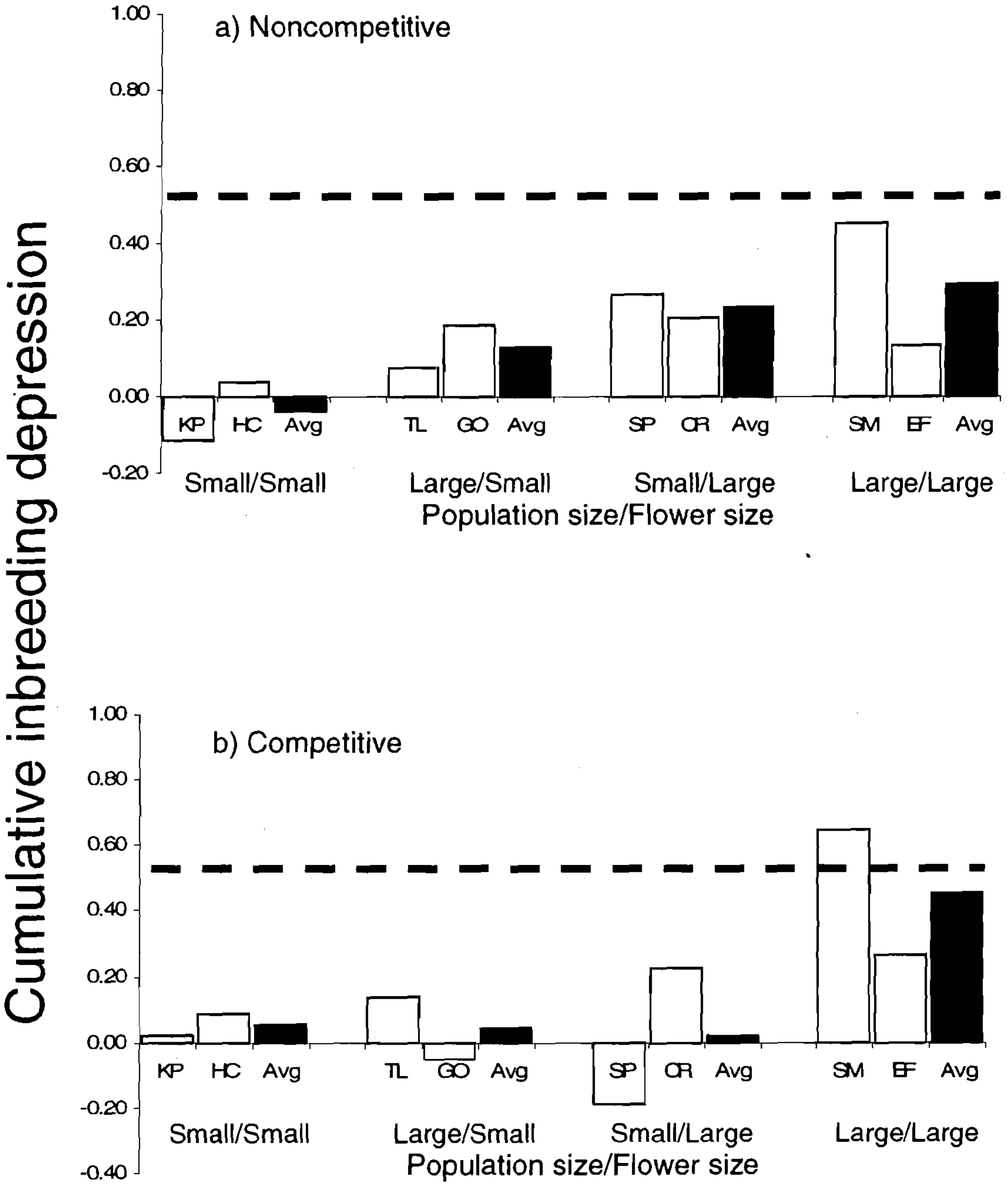
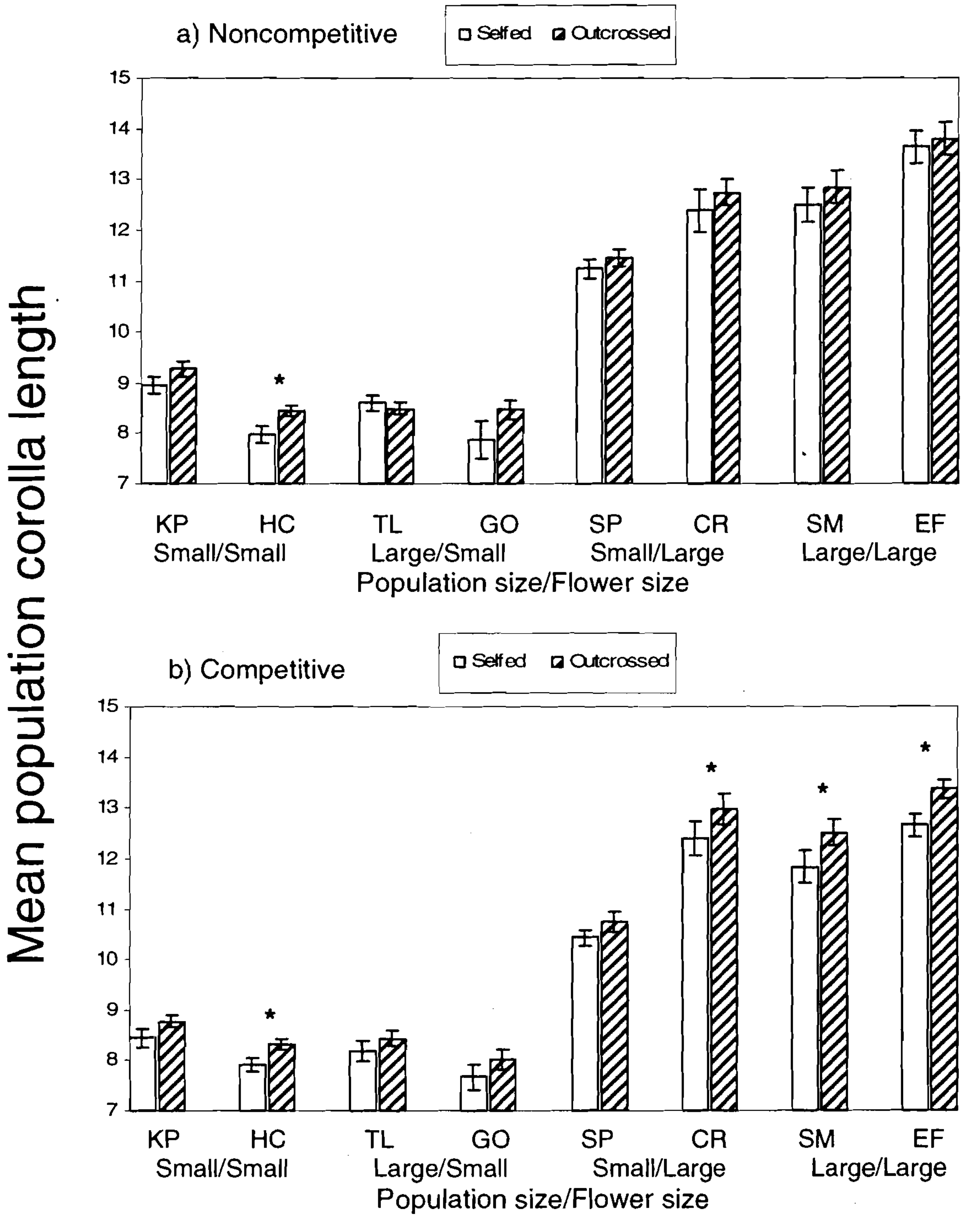

Cumulative inbreeding depression
a) Noncompetitive
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
KP
HC
Avg
TL
GO
Avg
SP
CR
Avg
SM
EF
Avg
Small/Small
Large/Small
Small/Large
Large/Large
Population size/Flower size
b) Competitive
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
KP
HC
Avg
TL
GO
Avg
SP
CR
Avg
SM
EF
Avg
Small/Small
Large/Small
Small/Large
Large/Large
Population size/Flower size

Figure 3-2: Mean population ± SE corolla length for selfed and outcrossed progeny within four population size/flower size categories (two populations per category). Plants were grown alone (a) or in the presence of a competitor (b). Asterisks above bars indicate a significant difference ($P < 0.05$) between selfed and outcrossed progeny.



Mean population corolla length
a) Noncompetitive
□ Selfed ▨ Outcrossed
15
14
13
12
11
10
9
8
7
*
KP
HC
TL
GO
SP
CR
SM
EF
Small/Small
Large/Small
Small/Large
Large/Large
Population size/Flower size
b) Competitive
□ Selfed ▨ Outcrossed
15
14
13
12
11
10
9
8
7
*
*
*
*
KP
HC
TL
GO
SP
CR
SM
EF
Small/Small
Large/Small
Small/Large
Large/Large
Population size/Flower size

CHAPTER 4
General Conclusions

Over the last century, evolutionary biologists have attempted to measure the costs and benefits associated with self-fertilization (reviewed in Holsinger 1996). It is apparent that both genetic and ecological factors are important for mating system evolution (Goodwillie et al. 2005). Here, I investigated how a population trait (population size) and floral trait (flower size) in *Collinsia parviflora*, influenced both an ecological benefit (Reproductive assurance, Chapter 2) and a genetic cost (Inbreeding depression, Chapter 3) of selfing.

The reproductive assurance benefit of autonomous selfing

Autonomous selfing is beneficial if it provides reproductive assurance when pollinators are rare or absent (Stebbins 1957, Lloyd 1992). My data in Chapter 2 suggest that reproductive assurance is especially important in small populations of small-flowered *C. parviflora* plants (as plants were likely less attractive to pollinators, and pollinators were less common). Indeed, levels of reproductive assurance were strongly negatively correlated with rates of pollinator visitation. Moreover, the negative relationship between pollinator visitation and reproductive assurance accounts for why none of the populations were pollen limited. Overall, Chapter 2 presents one of the most comprehensive examples to date of how autonomous selfing can be adaptive in certain ecological circumstances. Furthermore, because inbreeding depression was low in all small-flowered populations (Chapter 3), any benefit accrued by these plants through autonomous selfing will not be diminished by reduced fitness in their offspring. Hence, autonomous selfing appears adaptive for *C. parviflora* plants because it provides reproductive assurance in fragmented (and thus potentially pollen-limited) habitats.

The inbreeding depression cost of selfing

Models predict that recessive deleterious alleles in inbred lines should be eliminated by selection (Lande and Schemske 1985, Charlesworth et al. 1990). Indeed, this has been supported by many empirical studies (Barrett and Charlesworth 1991, Dole and Ritland 1993, Holtsford and Ellstrand 1990); although the overall evidence remains equivocal (Byers and Waller 1999). My data in Chapter 3 indirectly supports that purging is important for the evolution of inbreeding depression because the magnitude of inbreeding depression was lower in presumably more selfing populations. Specifically, small-flowered plants demonstrated a small genetic load regardless of population size, but large populations of large-flowered plants incurred substantial cumulative fitness costs when inbred. Past inbreeding events in small-flowered populations likely reflect autonomous selfing (due to morphology), and thus it is understandable why inbreeding depression was low in all small-flowered populations (because of similar morphology). On the other hand, the substantially smaller magnitude of inbreeding depression detected in small populations of large-flowered plants (relative to large populations), likely reflects greater purging due to more frequent biparental inbreeding in small populations. Biparental inbreeding does not provide reproductive assurance because it involves vector-assisted pollen delivery.

Across all populations, corolla length was significantly reduced in inbred progeny and might therefore perpetuate selfing because shorter corolla tubes reduce the distance between the sexual parts of a flower (Armbruster et al. 2002). Thus, until purging occurs, shorter corolla length may provide positive feedback for the expression of inbreeding depression in outcrossing populations.

Is mixed mating stable in *Collinsia parviflora*?

If inbreeding depression is purged with self-fertilization, then theory suggests that plant taxa should demonstrate either pure outcrossing or selfing strategies (Lande and Schemske 1985). However, the high frequency of species with intermediate levels of outcrossing (Goodwillie 2005) suggests that mixed mating systems may in fact be stable. Nevertheless, Goodwillie et al. (2005) warn of creating a potential false dichotomy; one in which we presume mixed mating is either stable or unstable. It may be more realistic to predict that some species have fixed mixed mating systems, while others are in transition towards obligate selfing or outcrossing. Whether or not mixed mating is stable in *C. parviflora* remains unknown; however, I believe the results presented throughout my thesis support a case for stability.

By measuring reproductive assurance and inbreeding depression costs in populations of the phenotypic flower size extremes, I showed that the smallest-flowered plants likely self-fertilize while the largest-flowered plants likely cross-fertilize. However, if flower size is a selfing rate modifier (and because it varies continuously among populations), then selfing rate also probably demonstrates continuous inter-population variation in this species. In other words, mixed mating may not be maintained within all B.C. populations, but overall as a species (i.e. across populations), *C. parviflora* may well represent a case where mixed mating is stable. Furthermore, habitat fragmentation, or perhaps more specifically the effect of fragmentation on population size, likely contributes to inter-population variation in selfing rates. I showed that the effect of population size on reproductive assurance (Chapter 2) and inbreeding depression (Chapter 3) was especially pronounced in large-flowered populations, implying that population size nested within flower size influences the benefit and frequency of selfing. Taken together, it is reasonable to assume that ecological circumstances help maintain intermediate levels of outcrossing among populations of *C. parviflora*, and hence, an overall mixed mating system in this species. However, whether or not mixed mating is maintained within populations is another question. In order to address this uncertainty, I consider the reproductive assurance benefit and inbreeding depression cost within each population, and predict the current mating system under some basic assumptions.

Assumptions

I considered the mean population reproductive assurance "high" if it was greater than 1.0. This assumes that if a flower gains an additional seed via autonomous selfing then it can potentially produce an additional offspring in the next generation. A mean reproductive assurance value of one or more should be considered high in *C. parviflora* because seed set typically ranges from 4-8 seeds per fruit in this species (Elle and Carney 2003). Conversely, I considered mean reproductive assurance "low" if it was less than 1.0. Based on the conventional threshold, I considered cumulative inbreeding depression "high" if it was greater than 0.5 and "low" if it was below 0.5. I used the competitive cumulative inbreeding depression measure, as this treatment is likely more reflective of natural conditions.

Mating system prediction model

Table 4-1: Mating system prediction model

Reproductive assurance	Inbreeding depression	Mating system
High	Low	Selfing
High	High	Mixed*
Low	Low	Mixed**
Low	High	Outcrossing

* Over time, this scenario may lead to a predominately selfing mating system if the genetic load is purged

* *Over time, this scenario may lead to a predominately selfing mating system because of the automatic selection advantage

Predictions

Based on the reproductive assurance and inbreeding depression found in these populations, I predict five of the eight populations to be predominately selfing, SM to be predominately outcrossing, and CR and EF to be both selfing and outcrossing (Table 4-2). Whether or not mixed mating is stable within CR and/or EF remains uncertain; however, because inbreeding depression was low in both populations, it is probable that the automatic selection benefit will act as a destabilizing force that will eventually lead to a selfing mating system. This begs the question: is any mixed mating population of *C. parviflora* actually stable? When considering these specific eight populations and only reproductive assurance and inbreeding depression, theory suggests not; however it is possible that mixed mating may be stabilized by other factors even in the face of low inbreeding depression. For instance, pollen discounting may provide negative feedback for selfing. Indeed, Johnston (1998) used a model that incorporated inbreeding depression and reproductive assurance to show that intermediate outcrossing can be evolutionarily stable if pollen discounting increases with the selfing rate. Unfortunately, empirical work on pollen discounting, and other potential stabilizing factors, still lags behind theory (Goodwillie et al. 2005). Clearly, our understanding of mating system evolution will improve with more empirical work on the genetic and ecological circumstances that maintain mixed mating.

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Tables

Table 4-2: Predicted mating system for each population based on the population means for reproductive assurance and cumulative inbreeding depression. Mean cumulative inbreeding depression was assessed using the competitive treatment data.

Population	Flower size	Population size	Reproductive assurance	Cumulative Inbreeding depression	Predicted Mating system
KP	Small	Small	High (2.83)	Low (0.02)	Selfing
HC	Small	Small	High (2.91)	Low (0.09)	Selfing
TL	Small	Large	High (2.98)	Low (0.14)	Selfing
GO	Small	Large	High (1.44)	Low (-0.05)	Selfing
SP	Large	Small	High (1.69)	Low (-0.19)	Selfing
CR	Large	Small	Low (0.86)	Low (0.23)	Mixed
SM	Large	Large	Low (0.10)	High (0.65)	Outcrossing
EF	Large	Large	Low (0.15)	Low (0.26)	Mixed