

VARIATION IN THE TIMING OF AUTONOMOUS SELFING  
AMONG POPULATIONS THAT DIFFER IN FLOWER SIZE, TIME TO  
REPRODUCTIVE MATURITY, AND CLIMATE<sup>1</sup>

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- *Premise of the study:* Early reproductive maturity is common in dry and ephemeral habitats and often associated with smaller flowers with increased potential for within-flower (autonomous) self-pollination. We investigated whether populations from locations that differ in moisture availability, known to vary for whole-plant development rate, also varied in the timing of autonomous selfing. This timing is of interest because the modes of selfing (prior, competing, and delayed) have different fitness consequences.
- *Methods:* We measured timing of anther dehiscence, stigma receptivity, and herkogamy under pollinator-free conditions for plants from three populations of *Collinsia parviflora* that differed in annual precipitation, flower size, and time to sexual maturity. Using a manipulative experiment, we determined potential seed production via prior, competing, and delayed autonomous selfing for each population.
- *Key results:* Stigma receptivity, anther dehiscence, and selfing ability covaried with whole-plant development and climate. Plants from the driest site, which reached sexual maturity earliest, had receptive stigmas and dehiscent anthers in bud. Most seeds were produced via prior selfing. The population from the wettest site with slowest development was not receptive until after flowers opened. Although competing selfing was possible, all selfing was delayed. The intermediate population was between these extremes, with significant contributions from both competing and delayed selfing.
- *Conclusions:* Our results demonstrate that within-species variation in the timing of selfing occurs and is related to both environmental conditions and whole-plant development rates. We suggest that, if these results can be generalized to other species, mating systems may evolve in response to ongoing climatic change.

**Key words:** climate; *Collinsia parviflora*; delayed selfing; flower morphology; phenology; Plantaginaceae; prior selfing.

Understanding the factors that select for self- vs. cross-fertilization has been a major aim of botany since the time of Darwin. Much recent attention has been paid to the study of intermediate outcrossing rates and especially to the ecological factors that can counter genetic costs of selfing such as inbreeding depression (Lloyd, 1992; Vogler and Kalisz, 2001; Goodwillie et al., 2005). A knowledge gap remains, however, regarding the functional aspects of self-pollination (Schoen and Lloyd, 1992; Barrett, 2003; Eckert et al., 2010). Specifically, very little has been documented on when and how autonomous selfing (within-flower self-fertilization without the assistance of a pollinator) occurs (Lloyd and Schoen, 1992), and few studies have examined how between-population variation in the mechanisms and

timing of autonomous selfing may be related to ecological factors that select for such strategies.

The three modes of autonomous selfing, prior, competing, and delayed, differ in timing: whether they occur before, during, or after opportunities for outcrossing. These differences in timing lead to different conditions under which each is favored (Lloyd, 1992). Autonomous selfing will provide the most benefit when it is delayed until after opportunities for outcrossing have passed (Lloyd, 1992). This benefit accrues because costs of self-pollination such as pollen or seed discounting (where pollen or ovules that participate in selfing are unavailable for outcrossing) should be lower for delayed selfing compared to selfing prior to or contingent with opportunities for outcrossing. These low costs suggest that delayed selfing should be strongly favored when it provides reproductive assurance (Stebbins, 1957), defined as seed production via selfing when pollinators or mates are absent (e.g., Fausto et al., 2001; Elle and Carney, 2003; Kalisz et al., 2004; Kennedy and Elle, 2008b). Delayed selfing is predicted to be most advantageous for annual plants experiencing pollinator failure, as their single opportunity for fitness accrual eliminates survival costs paid by perennials from resource allocation to inbred seeds (Lloyd, 1992; Morgan et al., 1997).

Ecological factors other than pollinator failure may also select for autonomous selfing ability, but these are less often studied. Self-pollination may allow for fitness gain in ephemeral habitats when there is simply not enough time for the processes necessary for outcrossing (Moore and Lewis, 1965; Arroyo, 1973; Guerrant, 1989). These processes may include building

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a larger, more attractive flower than a selfing counterpart or waiting for pollinator visits before seed development can commence. Even when pollinators are present, the additional time necessary for outcrossing may mean the difference between some seed production and death before seeds are mature, with profound consequences for semelparous individuals. Populations, species, or races from extreme or more ephemeral habitats often grow to a smaller final size and produce smaller flowers due to developmental constraints associated with shortened growing seasons (Diggle, 1992; Aarssen, 2000; Li and Johnston, 2000; Kudo, 2006). Because flower size usually indicates breeding system, with small flowers associated with higher self-pollination rates (reviewed in Goodwillie et al., 2010), ephemeral habitats may select indirectly for self-pollination (Stebbins, 1957). Indirect selection for selfing may occur more broadly under climate change; warming temperatures and increased drought frequency have resulted in earlier flowering in many species (Fitter and Fitter, 2002; Franks et al., 2007; Bertin, 2008). Here, we propose that if climatic factors select for earlier sexual maturity of individuals, then within-flower selfing may also evolve to occur earlier. Autogamy could then be favored even prior to opportunities for outcrossing, despite associated costs such as pollen or seed discounting.

To date, research on the question of the relationship between development time and selfing has focused on comparisons of species or subspecies (e.g., Moore and Lewis, 1965; Arroyo, 1973; Guerrant, 1989; Hill et al., 1992; Runions and Geber, 2000). Research comparing populations within species, however, has the benefit of results that are less confounded by differences in evolutionary history. Here, we focus on among-population variation in the timing of selfing in *Collinsia parviflora* L., an annual plant with among-population differentiation in development time and flower size. As flower size decreases, autonomous selfing ability increases, and the time required for plants to reach sexual maturity decreases (Elle, 2004). More rapidly developing populations occur in drier, more ephemeral environments in coastal British Columbia (Elle, 2004). Summer drought is predicted to become more intense with climate change in this region (Spittlehouse, 2008), and so studying populations that differ in current climatic conditions may provide insight into future mating system responses to climate.

We first investigated the sequence of development within individual flowers from populations that differ in flower size and time to sexual maturity, including the timing of anther dehiscence, stigma receptivity, and herkogamy. We expected that,

under controlled conditions, within-flower developmental timing will be most rapid in the population from the driest site, with the shortest time to whole-plant sexual maturity, because we expected this population is under strong selection for rapid reproduction. We then used the flower development data to predict when, relative to opportunities for outcrossing, self-pollination may occur for each population. Finally, we measured the relative contributions of prior, competing, and delayed autonomous selfing to seed production under pollinator-free conditions, comparing the realized autonomous selfing for each mode to that predicted based on the sequence of floral development. We expected earlier selfing in the population from the driest site, but later (delayed) selfing in the population from the wetter site, where selection for rapid development should be weak, and costs of pollen or seed discounting should select against prior and competing selfing.

## MATERIALS AND METHODS

**Study species, focal populations, and rearing conditions**—*Collinsia parviflora* L. (Plantaginaceae) is a winter annual common throughout western North America. As is true for all species of *Collinsia*, flowers consist of two upper banner petals, two lower wing petals, and a keel petal that is folded around the sexual parts. The four epipetalous anthers dehisce sequentially and are shed with the corolla at flower senescence.

In coastal British Columbia, *Collinsia parviflora* is tetraploid (N. D. Tunbridge, Simon Fraser University; C. Sears, University of British Columbia; and E. Elle, unpublished manuscript), and flower size exhibits continuous, genetically based among-population variation (Elle and Carney, 2003; Elle, 2004). Previous research has demonstrated that relatively smaller-flowered populations have greater autonomous selfing ability and shorter times between germination and flowering than do relatively larger-flowered populations (Elle, 2004; Table 1). Differences in flower size and time to sexual maturity in the growth chamber are highly correlated with annual precipitation in source populations (Elle, 2004), and differences in the magnitude of summer drought appear especially important. Under natural pollination conditions, autonomous selfing provides substantial reproductive assurance for small-, but not large-flowered populations (Elle and Carney, 2003; Kennedy and Elle, 2008b). Inbreeding depression is generally low, but is higher in large-flowered populations than in small-flowered populations (Kennedy and Elle, 2008a).

For this research, we used three populations that represent the range of flower size found on Vancouver Island, B.C.; small-flowered Thetis Lake (TL-S), mid-flowered Jack Point (JP-M), and large-flowered Elk Falls (EF-L). Flower size, autonomous selfing ability, and development time for these populations have been previously reported (Elle, 2004) and are summarized in Table 1 along with several climatic variables.

Rearing conditions were identical for plants in all parts of this research. Seeds were germinated in standard potting soil in multiwell flats under short days (20°C/10-h day, 10°C/14-h night) in an environmental chamber. Eight

TABLE 1. Means  $\pm$  SE of phenotypic traits measured in a pollinator-free growth chamber on up to 24 maternal sibships from three populations of *Collinsia parviflora* from British Columbia (Elle, 2004) and weather data for the population locations. Autonomous selfing rate was calculated at the plant level as (fruit set/flower production)  $\times$  100. Weather data were calculated based on population location and elevation using the program Climate BC v. 2 (Wang et al., 2006), using weather station data for the period 1961–1990. Summer includes June through August.

Phenotypic trait or climatic variable	Elk Falls Provincial Park (EF-L)	Jack Point Park (JP-M)	Thetis Lake Regional Park (TL-S)
Corolla width (mm)	9.47 $\pm$ 0.12	7.08 $\pm$ 0.14	3.64 $\pm$ 0.04
No. days, germination to first flower	92.74 $\pm$ 1.05	64.90 $\pm$ 1.34	56.84 $\pm$ 0.69
Autonomous selfing rate (%)	63.97 $\pm$ 2.42	74.50 $\pm$ 2.81	79.94 $\pm$ 1.51
Annual: Precipitation (mm)	1599	1028	980
Mean temperature (°C)	8.7	9.9	9.8
Heat: moisture index	5.4	9.6	10.0
Summer: Precipitation (mm)	168	95	70
Mean temperature (°C)	15.9	16.4	15.8
Heat: moisture index	94.6	172.6	225.7

Note: Heat: moisture index = temperature/(precipitation/1000); L = large-flowered; M = mid-flowered; S = small-flowered.

weeks later, the chamber was switched to long days (20°C/16-h day, 10°C/8-h night). Seeds for use in experiments on floral development were collected in 2002 from plants >1 m apart to reduce relatedness. Seeds for estimation of the timing and amount of autonomous selfing were collected similarly in 2008, but stored as a single bulk collection rather than by maternal family.

**Floral development—Anther dehiscence and floral lifetime**—We marked a single flower on 25 plants per population, one from each of 25 maternal families for TL-S and JP-M, but from 19 families for EF-L, due to uneven across-family germination in this population. Flowers were checked every 8 h, beginning when the flower was in bud and ending when the corolla was shed. We noted the floral stage at each time period as in Kalisz et al. (1999) with the stage (0–4) representing the number of anthers that had dehisced. We noted when the corolla opened, when it was shed, and the position of the stigma relative to the anthers. We defined total floral lifetime as extending from either when the flower opened or when anthers began to dehisce (whichever was first) until the corolla was shed.

**Stigma receptivity and herkogamy**—We collected 20 flowers at each stage of anther dehiscence (0–4) from each population. Flowers were haphazardly collected from between 15 and 24 plants (one plant per sibship) within each population until 20 flowers per stage were collected. For TL-S, many “flowers” at stages 0 and 1 were unopened buds. Stigma receptivity was determined by excising the stigma and placing it in a drop of hydrogen peroxide. Receptive stigmas have bubbling on their surface when placed in this solution (Kearns and Inouye, 1993, p. 68). The torn end of the stigma was used as a reference for bubbling, and only vigorous bubbling was counted as receptive, whereas weak bubbling (e.g., similar to the torn end) was assumed to be a false positive (not receptive).

In *Collinsia* species, the stigma is initially shorter than the anthers and elongates through the anthers over development until it eventually protrudes beyond them (Kalisz et al., 1999; Armbruster et al., 2002). Stigmas positioned below the anthers are unlikely to have had self pollen deposited, while those within or beyond the “pollen zone” (the area between the shortest and longest anthers to have dehisced) have likely been subjected to the possibility of autonomous self pollination. Therefore, we divided herkogamy into two categories: possibly selfed (stigmas within or beyond the pollen zone), and not selfed (those that are still below the pollen zone). Herkogamy was not determined for stage 0 flowers, since no anthers are dehisced at this stage.

**Predicting timing of autonomous selfing from floral development**—We used data on anther dehiscence, stigma receptivity, and herkogamy to predict the timing of seed production via autonomous autogamy. For each population, we calculated the probability of autonomous selfing for four time periods: 24 h before flowers opened, at the time flowers opened, and 24 and 48 h after flowers opened. For each population and stage of anther dehiscence (1–4; stage 0 flowers could not have been selfed), we multiplied the probability that stigmas were receptive at that stage by the probability that stigmas were within or beyond the pollen zone at that stage (data in Fig. 2A and 2B). We then used the observed distribution of floral stages at each of the time periods of interest (–24 h, 0, 24, 48 h; data summarized in Fig. 1) to calculate, for each of the 25 flowers followed through time, the probability that each could have been self-pollinated based on the combination of floral stage, stigma receptivity, and herkogamy. These values were averaged to estimate the predicted probability of selfing for each population and floral stage.

**Timing of selfing**—We determined the potential for autonomous selfing for each population under pollinator-free conditions and manipulated flowers to differentiate between prior, competing, and delayed selfing using a modification of the methods espoused by Schoen and Lloyd (1992) (Table 2). Our manipulations were performed under pollinator-free conditions in the growth chamber and so represent the potential for autonomous selfing at different times during floral life rather than the realized selfing rate (which should be measured under field conditions using genetic markers). The timing of application of the treatments was based on results of the “Anther dehiscence and floral lifetime” experiment (Fig. 1). The manipulations were timed to prevent selfing once the flower opened (emasculature of buds just prior to corolla expansion, E treatment), or at the end of floral life (inactivating the stigma of flowers about one day before the corolla was shed by cutting off the tip of the style, the S treatment, as in Lankinen and Kiboi, 2007). Stigmas were inactivated after 2, 3, and 4 d for TL-S, JP-M, and EF-L respectively. Our manipulations therefore allowed only prior selfing (E) or prior plus competing selfing (S). Seed number

TABLE 2. The four experimental treatments to distinguish between the different modes of autonomous selfing. Treatments were modified from those suggested by Schoen and Lloyd (1992), in that they were performed in a pollinator-free environment, and so seed production represents the potential for autonomous selfing rather than realized selfing rate.

Experimental treatments	Manipulation	Modes of autonomous selfing
Bud emasculature (E)	Remove anthers before corolla opens	Prior
Stigma inactivation (S)	Remove end of pistil 1 d before corolla is shed	Prior, competing
Unmanipulated (U)	None	Prior, competing, delayed
Hand pollinated (P)	Add self pollen, otherwise none	Maximum seed set possible

from these manipulated flowers was compared to seeds produced via autonomous autogamy in unmanipulated flowers (U), which allowed prior, competing, and delayed selfing, and to seeds produced in hand-self-pollinated flowers (P), which represent the maximum seed set possible from self (rather than outcross) pollen under our growing conditions (Table 2). Twenty-five experimental plants were used for JP-M and EF-L, and 23 for TL-S due to mortality. Each plant received each of the four treatments replicated twice with two exceptions (both in EF-L) where a plant had no replication for a single treatment (a different treatment unreplicated for each of two plants). Treatment order was regularized, but each plant was randomly assigned the first treatment applied.

The number of seeds produced in replicate treatments within individuals was averaged. We then calculated per-flower autonomous selfing ability and the contribution of each mode of selfing to the total for each plant as follows. In each case, we relativized the data by dividing treatment-specific seed numbers by the number of seeds in the hand-self-pollinated treatment (P) because populations were known to differ significantly from each other for ovule number (Parachnowitsch and Elle, 2004). Total autonomous selfing was U/P, representing the maximum number of seeds produced without a pollinator. Prior selfing was calculated as E/P, which includes only those seeds produced prior to corolla expansion (in buds), which is when the flowers were emasculated. Competing selfing was (S-E)/P, which subtracts those seeds produced prior to corolla expansion from those from the stigma inactivation treatment, i.e., seeds produced up to corolla abscission. Delayed selfing was calculated as the difference between unmanipulated flowers (total potential autonomous selfing) and those whose stigmas were inactivated just prior to corolla abscission, (U-S)/P.

We also performed manipulation controls to ensure that our floral surgeries (bud emasculatures and stigma inactivations) did not reduce seed set. Manipulations were performed as already described, but flowers were also hand self-pollinated, and seed production was compared to unmanipulated self-pollinated flowers. In a final treatment, buds were emasculated before any anthers dehisced to determine whether seeds could be produced via apomixis in this species. This treatment was similar to the E treatment, but was usually performed earlier in floral development (especially in TL-S, see Results), and any buds with dehiscent anthers were not used. These four control treatments were performed on 10 additional plants per population (11 for TL-S), with each treatment replicated twice per plant, as described.

**Statistical analysis**—All analyses were performed in the program SAS v. 9.2 for Windows (SAS Institute, Cary, North Carolina, USA.), and we present means  $\pm$  SE throughout. To analyze general timing differences among populations, we performed an ANOVA testing for differences among populations in the total number of hours flowers lasted, from the time the first anther dehisced (or the corolla opened, whichever was first) until the corolla was shed, followed by the Ryan-Einot-Gabriel-Welsch posthoc *Q* statistic (“Ryan’s *Q*”; Day and Quinn, 1989). We also assessed differences among populations in developmental stage at the time of flower opening by constructing population by flower stage contingency tables at time 0.

To analyze stigma receptivity, we constructed two types of contingency tables. We examined how the proportion of receptive stigmas varied with floral stage within populations by constructing stage by receptivity contingency tables separately for each population. We then examined how populations differed in the proportion of stigmas receptive at each stage by constructing



population by receptivity contingency tables for each floral stage. To determine whether herkogamy varied among stages within populations, or among populations within stages, contingency tables were constructed similarly.

We performed mixed-model analysis of variance (ANOVA) to compare seed production among treatments, populations, and their interaction, with treatment and population considered fixed effects, and plant included in the model as a random effect. Seed number was log transformed to eliminate heteroscedasticity for these analyses, and control and experimental plants were analyzed separately. Because populations were known to differ in ovule number (Parachnowitsch and Elle, 2004), seed number was expected to differ among them as well. So, to aid in interpretation of the significant interaction effects (see Results) we performed additional ANOVAs followed by Ryan's  $Q$  for each population separately to illustrate patterns. We performed a mixed-model ANOVA to compare the amount of autonomous selfing under pollinator-free conditions due to different modes (prior, competing, delayed, and total) in different populations, and their interaction, again including plant as a random factor. To aid in interpretation, we again performed ANOVAs by population and used  $t$  tests to determine whether relative seed production from the different modes differed significantly from zero.

## RESULTS

**Anther dehiscence and floral lifetime**—Populations differed significantly in total floral lifetime ( $F_{2,72} = 53.25$ ,  $P < 0.0001$ ). Flowers from EF-L (large-flowered) lasted significantly longer ( $153.9 \pm 4.0$  h) than flowers from JP-M (111.7  $\pm$  2.8) or TL-S (102.7  $\pm$  4.3), but JP-M and TL-S did not differ from each other. Populations also differed in the proportion of flowers that were at each stage when the flower opened ( $\chi^2 = 46.53$ ,  $df = 8$ ,  $P < 0.0001$ ). Flowers from TL-S (small-flowered) had their anthers start dehiscing sooner than JP-M and EF-L (Fig. 1). Almost half of TL-S flowers (12 of 25) were in stage 4 when they opened, but the majority of JP-M and EF-L flowers (20 and 24 respectively) were in stages 1 or 2 when they opened.

**Stigma receptivity and herkogamy**—Within all populations, a higher proportion of stigmas from later floral stages were receptive than stigmas from early stages (EF-L,  $\chi^2 = 72.22$ ,  $P < 0.0001$ ; JP-M,  $\chi^2 = 46.65$ ,  $P < 0.0001$ ; TL-S,  $\chi^2 = 27.91$ ,  $P < 0.0001$ ; all  $df = 4$ ). Among populations, the proportion of recep-

tive stigmas differed significantly for stages 0–3 (stage 0,  $\chi^2 = 18.73$ ,  $P < 0.0001$ ; stage 1,  $\chi^2 = 7.331$ ,  $P = 0.03$ ; stage 2,  $\chi^2 = 20.02$ ,  $P < 0.0001$ ; stage 3,  $\chi^2 = 24.03$ ,  $P < 0.0001$ ; all  $df = 2$ ). Populations did not differ at stage 4, because most stigmas were receptive by this stage ( $\chi^2 = 2.14$ ,  $P = 0.34$ ). EF-L (large-flowered) did not have any receptive stigmas until stage 3, but TL-S (small-flowered) had a high proportion of receptive stigmas even at stages 0 and 1. JP-M (mid-flowered) was intermediate, with a few receptive stigmas at stages 0 and 1, and the majority of stigmas becoming receptive at stages 2 and 3 (Fig. 2A).

Relative herkogamy varied with floral stage in some, but not all populations. JP-M and EF-L had a low proportion of flowers with stigmas within or above the pollen zone at stage 1; this proportion increased with floral stage (EF-L,  $\chi^2 = 35.93$ ,  $df = 3$ ,  $P < 0.0001$ ; JP-M,  $\chi^2 = 27.11$ ,  $df = 3$ ,  $P < 0.0001$ ; Fig. 2B). In contrast, a high proportion of TL-S flowers had stigmas within the pollen zone at stage 1, and levels remained similar throughout floral development ( $\chi^2 = 6.139$ ,  $df = 3$ ,  $P = 0.10$ ). Populations only differed significantly in the proportion of possibly selfed flowers at stage 1. By stage 2, there were no significant differences (stage 1,  $\chi^2 = 20.04$ ,  $P < 0.0001$ ; stage 2,  $\chi^2 = 4.37$ ,

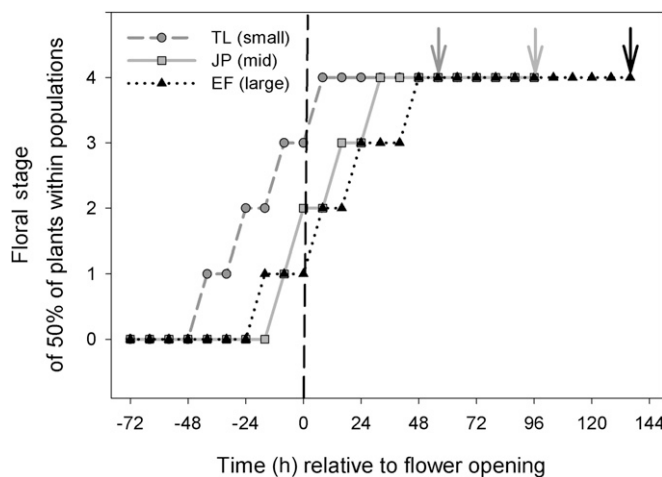


Fig. 1. Anther dehiscence for three populations of *Collinsia parviflora* that differ in flower size and time to first flower. Times are in hours where 0 indicates corolla opening, and the graph indicates when half of the sample ( $N = 25$  per population) has progressed from one stage to subsequent stages, where stage is the number of dehiscing anthers. The arrows indicate when half the plants have shed their corollas for each population.

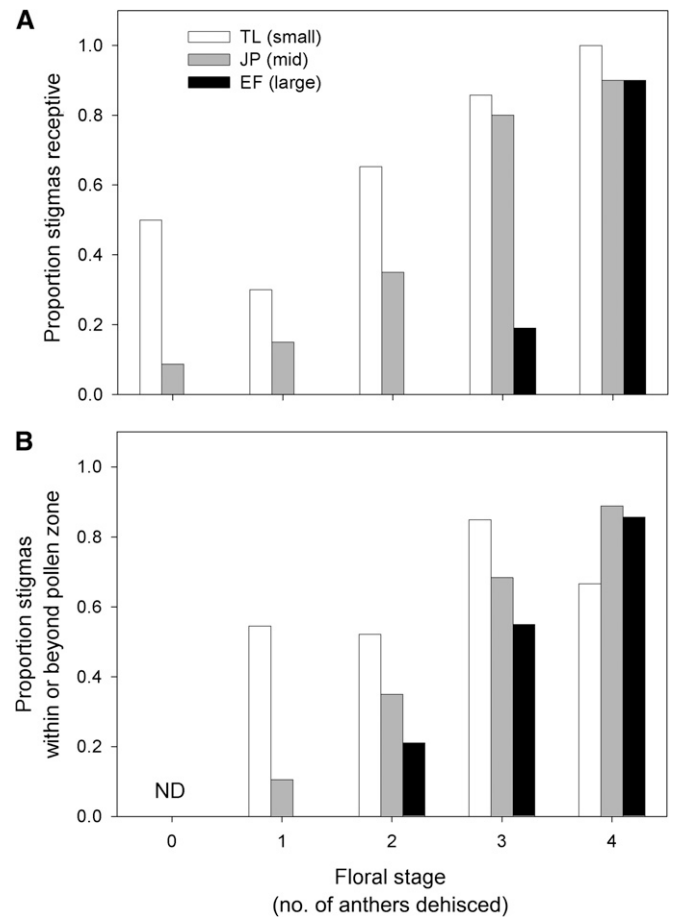


Fig. 2. Changes in (A) stigmatic receptivity and (B) position of the stigma relative to the anthers for three populations of *Collinsia parviflora* that differ in flower size. The pollen zone is the area between dehiscing anthers, and floral stage refers to the number dehiscing at the time of measurement. ND is no data; pollen zone cannot be assessed if no anthers are dehiscing.

$P = 0.11$ ; stage 3,  $\chi^2 = 4.260$ ,  $P = 0.12$ ; stage 4,  $\chi^2 = 3.6508$ ,  $P = 0.16$ ; all  $df = 2$ ).

**Predicted time of autonomous selfing**—Given information on floral development, we predicted that a majority (>60%) of TL-S (small) flowers would be autonomously selfed by the time flowers opened, JP-M (mid) flowers about a day later, and EF-L (large) flowers by 48 h after flowers opened (Fig. 3).

**Realized timing of autonomous selfing**—Seed production in experimental plants differed among populations, treatments, and their interaction (Table 3). Seed production was inversely related to the flower size of populations, and populations differed in the number of seeds produced over flower lifetime (Table 4). Within populations, the self-pollinated treatment produced the most seeds, followed by the unmanipulated and the stigma inactivated treatments (Table 4; TL-S:  $F_{3,88} = 5.08$ ,  $P = 0.003$ ; JP-M:  $F_{3,96} = 66.67$ ,  $P < 0.0001$ ; EF-L:  $F_{3,96} = 85.39$ ,  $P < 0.0001$ ). Emasculated flowers produced the least number of seeds, usually zero for the mid- (JP-M) and large-flowered (EF-L) populations, but more than half the maximum possible seed production for the small-flowered (TL-S) population because anthers dehisced before corollas expanded and the emasculation treatment was applied. Our control treatments indicated no effect of floral surgery for any population. Self-pollinated flowers in the emasculation control and stigma inactivation control treatments did not have significantly reduced set compared to unmanipulated flowers (Table 4). The lack of seed production by flowers emasculated before any anthers started dehiscing indicates there is no apomixis in this species.

Autonomous selfing ability varied with mode (prior, competing, delayed, and total autonomous), population, and their inter-

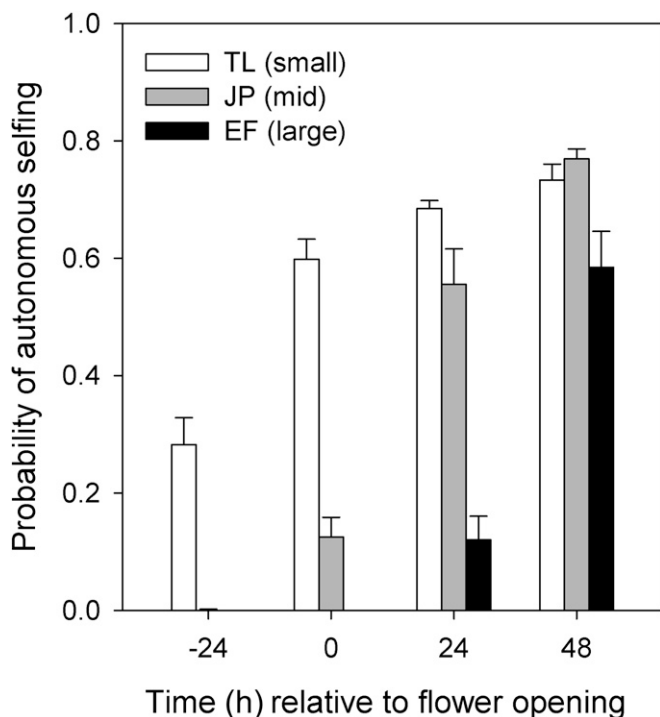


Fig. 3. Predicted probability of autonomous selfing at four time periods for plants from three populations of *Collinsia parviflora*. Probabilities are based on data in Figs. 1 and 2.

TABLE 3. Mixed-model analysis of variance (ANOVA) results for the timing of autonomous selfing experiment, performed under pollinator-free conditions on three populations of *Collinsia parviflora* that differ in flower size and time to sexual maturity

Source of variation	Num. df	Den. df	F	P
A) Seed number by experimental treatment				
Treatment	3	210	112.97	0.0001
Population	2	70	62.44	0.0001
Treatment × Population	6	210	11.68	0.0001
B) Contribution of modes of selfing				
Selfing Mode	3	210	27.59	0.0001
Population	2	70	6.49	0.0026
Mode × Population	6	210	9.16	0.0001

Notes: We tested for differences in (A) seed production for different experimental treatments (bud emasculation, stigma inactivation, unmanipulated, and hand selfed), populations (small-, mid-, and large-flowered), and their interaction, and (B) resulting autonomous selfing under pollinator-free conditions for different modes (prior, competing, delayed, and total within-flower autonomous), populations, and their interaction. Plant was included as a random factor in each model.

action (Table 3, Fig. 4). The small- and mid-flowered populations had equivalent amounts of within-flower autonomous selfing, while the large-flowered population had reduced potential for autonomous selfing. The amount of prior selfing was higher and that of delayed selfing lower in the small-flowered (TL-S) population compared to the others.

Within-population ANOVAs demonstrated that in all populations, the contributions of the different modes of selfing varied (TL-S:  $F_{3,88} = 12.20$ ,  $P = 0.002$ ; JP-M:  $F_{3,96} = 22.25$ ,  $P < 0.0001$ ; EF-L:  $F_{3,96} = 14.16$ ,  $P < 0.0001$ ; Fig. 4). TL-S (small-flowered) had high amounts of total autonomous selfing and prior selfing ( $0.84 \pm 0.08$  and  $0.65 \pm 0.10$ , respectively), which did not differ significantly from one another. The amounts of competing and delayed selfing ( $0.13 \pm 0.14$  and  $0.06 \pm 0.11$ , respectively) differed significantly from prior and autonomous

TABLE 4. Mean  $\pm$  SE seed production for treatments used to investigate the timing of autonomous selfing for three populations of *Collinsia parviflora* grown under pollinator-free conditions.

Treatments	Population		
	TL-S (Small)	JP-M (Mid)	EF (Large)
Experimental			
Bud emasculated (E)	2.7 $\pm$ 0.29 <sup>A</sup>	0.3 $\pm$ 0.14 <sup>A</sup>	0.1 $\pm$ 0.06 <sup>A</sup>
Stigma inactivated (S)	3.5 $\pm$ 0.37 <sup>A</sup>	1.2 $\pm$ 0.31 <sup>B</sup>	0.0 $\pm$ 0.02 <sup>A</sup>
Unmanipulated flower (U)	3.9 $\pm$ 0.40 <sup>AB</sup>	3.3 $\pm$ 0.24 <sup>C</sup>	1.2 $\pm$ 0.22 <sup>B</sup>
Hand self-pollinated (P)	4.6 $\pm$ 0.27 <sup>B</sup>	4.2 $\pm$ 0.20 <sup>C</sup>	2.7 $\pm$ 0.17 <sup>C</sup>
Control			
Emasculated, no anthers dehisced	0.0 $\pm$ 0.00 <sup>A</sup>	0.0 $\pm$ 0.00 <sup>A</sup>	0.0 $\pm$ 0.00 <sup>A</sup>
E + Pollination	4.9 $\pm$ 0.26 <sup>B</sup>	3.6 $\pm$ 0.36 <sup>B</sup>	2.8 $\pm$ 0.20 <sup>B</sup>
S + Pollination	5.0 $\pm$ 0.27 <sup>B</sup>	3.8 $\pm$ 0.49 <sup>B</sup>	2.5 $\pm$ 0.33 <sup>B</sup>
Hand self-pollinated (P)	5.1 $\pm$ 0.24 <sup>B</sup>	4.0 $\pm$ 0.39 <sup>B</sup>	3.1 $\pm$ 0.17 <sup>B</sup>

Notes: Within each population, means with the same letter are not significantly different from each other within category (experimental vs. control); ANOVA on log-transformed seed number followed by Ryan's  $Q$ . Two replicate flowers per treatment of each experimental ( $N = 23$  for TL-S, 25 for JP-M and EF-L) and control ( $N = 11$  for TL-S, 10 for JP-M and EF-L) plant. TL-S = Thetis Lake Regional Park (small-flowered); JP-M = Jack Point Park (mid-flowered); EF = Elk Falls Provincial Park (large-flowered)

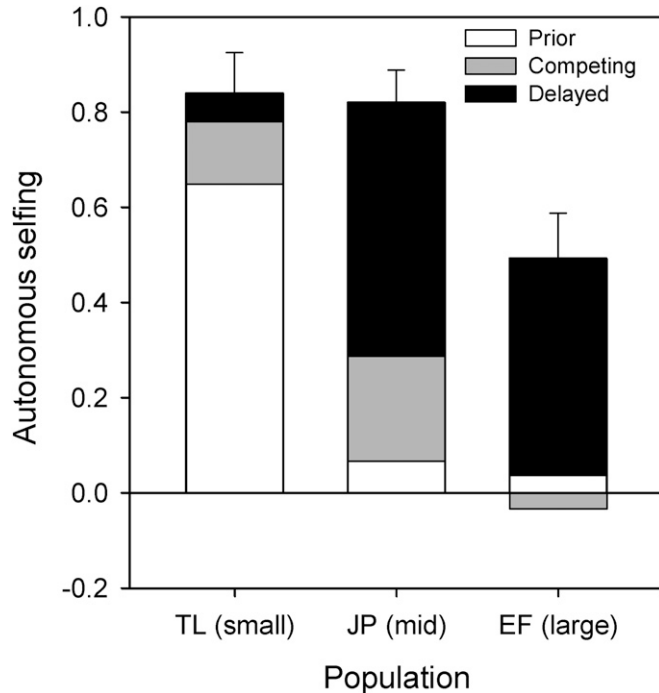


Fig. 4. Mean  $\pm$  SE autonomous selfing for three populations of *Collinsia parviflora* that differ in flower size and time to first flower. The contributions of prior, competing, and delayed selfing under pollinator-free conditions are shown.

selfing, but not from each other, and were not significantly different from zero (competing:  $t_{22} = 0.95$ ,  $P = 0.35$ , delayed:  $t_{22} = 0.52$ ,  $P = 0.61$ ). Thus, autonomous selfing in this population is prior to opportunities for outcrossing, with effectively no contribution of competing or delayed selfing to the overall potential for autonomous selfing.

JP-M (mid-flowered) had low amounts of prior and competing selfing ( $0.07 \pm 0.03$  and  $0.22 \pm 0.07$ , respectively). These amounts were not significantly different from each other but were significantly less than delayed selfing ( $0.53 \pm 0.10$ ). Prior selfing was not significantly different from zero, though this was marginal ( $t_{24} = 2.0$ ,  $P = 0.06$ ), while competing selfing and delayed selfing were both greater than zero (competing:  $t_{24} = 3.03$ ,  $P = 0.006$ , delayed:  $t_{24} = 5.55$ ,  $P = 0.0001$ ). Thus, within this population most autonomous selfing ( $0.82 \pm 0.07$ ) resulted from delayed selfing with some contribution from competing selfing and virtually none from prior selfing.

For the EF-L (large-flowered) population, prior and competing selfing ( $0.04 \pm 0.03$  and  $-0.03 \pm 0.03$ , respectively) were not significantly different from zero (prior:  $t_{24} = 1.33$ ,  $P = 0.19$ , competing:  $t_{24} = -1.10$ ,  $P = 0.28$ ). All the autonomous selfing ( $0.46 \pm 0.09$ ) in this population resulted from delayed selfing ( $0.45 \pm 0.10$ ), which differs from zero ( $t_{24} = 4.76$ ,  $P < 0.0001$ ).

## DISCUSSION

Much of our understanding of how morphology covaries with autonomous selfing ability comes from comparisons of related species, as first imagined by Darwin (1876). Within-species variation in autonomous selfing ability or the selfing rate has been documented frequently (e.g., *Mimulus guttatus*,

Ritland, 1990; three *Amsinckia* species, Johnston and Schoen, 1996; *Collinsia heterophylla*, Mayer et al., 1996; *Crepis sancta*, Cheptou et al., 2002; *Collinsia verna*, Kalisz et al., 2004). However, this variation has been linked to among-population differences in morphology (usually anther–stigma separation) less frequently (e.g., *Eichhornia paniculata*, Barrett and Husband, 1990; *Leavenworthia crassa*, Lyons and Antonovics, 1991; *Clarkia tembloriensis*, Holtsford and Ellstrand, 1992; *Hibiscus laevis*, Klips and Snow, 1997; *Aquilegia canadensis*, Herlihy and Eckert, 2004). The extent to which among-population variation in morphology translates into differences in the timing of autonomous selfing is relatively unknown. In *Arenaria uniflora*, large-flowered populations are outcrossing, but small-flowered populations, which co-occur with *Arenaria glabra*, have prior selfing for hybridization avoidance (Fishman and Wyatt, 1999). Populations of *Leptosiphon jepsonii* vary in the timing of breakdown of genetic self incompatibility, and populations with early breakdown have smaller flowers and higher selfing rates (Goodwillie and Ness, 2005). In the present study, we found that *C. parviflora* populations that differed in flower size and time to reproductive maturity also differed in development time of individual flowers and in the timing and potential for autonomous self-fertilization. As time to maturity decreased, floral development was faster, and autonomous selfing was greater and occurred earlier.

Differences in time to reproductive maturity are often related to environmental variation. High-elevation, higher-latitude, and desert species or populations frequently have rapid development due to the ephemeral nature of the environments where they occur. In cases where development has been studied in detail, earlier flowering is often associated with smaller flowers that are often of a more juvenalized form than populations or species from which they are evolutionarily derived (Guerrant, 1989; Diggle, 1992; Hill et al., 1992; Fenster et al., 1995; Runions and Geber, 2000). Although our research did not include a detailed description of whole-plant development, we show that flower size, known to be inversely related to whole-plant time to reproductive maturity and moisture availability in *Collinsia parviflora* (Elle, 2004), is also inversely related to within-flower development rates and total flower lifetime.

Reductions in dichogamy and herkogamy are commonly associated with the evolution of selfing (Stebbins, 1957; Lloyd and Schoen, 1992). We found variation in the timing of stigmatic receptivity, herkogamy, and anther dehiscence among our populations, congruent with sexual maturity prior to flowers opening in the small-flowered population and with sexual maturity at or after flower opening in the mid- and large-flowered populations. Our estimates of the timing of autogamy from these data suggested that most flowers in our small-flowered population would be selfed by the time flowers opened, but opportunities for self-pollination would occur later in our mid- and large-flowered populations. Because all populations have one or more anthers dehiscing by the time flowers open and at least some flowers have stigmas in the pollen zone once anthers dehisce, stigmatic receptivity may be the most important contributor to variation in the timing of autonomous selfing ability in our populations. Research on *Collinsia verna* and *C. heterophylla* suggests that pollen remains viable for an extended period and that pollen deposited on stigmas can remain viable until those stigmas become receptive (Kalisz et al., 1999; Lankinen et al., 2007). If pollen viability is similar in *C. parviflora*, autonomous selfing should be possible in all populations once stigmas are receptive, ranging from 24–48 h before corolla



expansion in our small-flowered population (TL-S) to more than 24 h after corolla expansion in our large-flowered population (EF-L).

The premise of the time limitation hypothesis (Aarssen, 2000) is that selection for rapid development results in greater selfing rates, especially in annual plants with a single opportunity for reproduction. We have previously demonstrated genetic correlations between development time and flower size in *C. parviflora* (Elle, 2004), suggesting that selection on time to sexual maturity may result in greater autonomous selfing ability via a correlated change in flower morphology. Indeed, artificial selection for rapid development results in a correlated reduction in flower size and increase in autonomous selfing ability in some populations (E. Elle, unpublished data). Here, we demonstrate that not only does the most rapidly developing population we studied have the highest potential for autonomous selfing under pollinator-free conditions, most of that self-pollination occurs early, prior to flowers opening. Our mid-flowered population, with an intermediate development time, selfs while flowers are open, contingent with the timing of opportunities for outcrossing that would occur under natural pollination conditions. And although we predicted that our large-flowered population would also demonstrate competing selfing, based on within-flower development (Fig. 3), essentially all autonomous selfing in this population was delayed (Fig. 4). There was effectively no realized competing selfing in flowers up to 4 d old—even though our morphology- and receptivity-based estimates suggested that close to 60% of flowers have the potential to self-pollinate within 2 d. This result could be explained if there is poor contact between stigmas and anthers within open flowers, even when stigmas are within or beyond the pollen zone. The shedding of corollas (with attached, epipetalous anthers) after wilting may allow for more contact than occurs within open flowers (as in Dole, 1992). The difference between our predicted and realized rates of autonomous selfing suggest that comparison of floral development rates alone (as in the cross-species comparison of Armbruster et al., 2002) may not always be adequate for predicting when autonomous selfing potentially occurs. Our results provide support for the idea that rapidly developing populations from more ephemeral habitats can self-pollinate earlier than slower-developing conspecifics from more mesic environments.

Prior selfing is not usually considered to provide reproductive assurance in the classic “bet-hedging” sense because of costs associated with seed and pollen discounting (Lloyd, 1992). Under extreme pollen limitation, however, these costs are reduced and prior selfing can more easily evolve (Davis and Delph, 2005; Morgan and Wilson, 2005). Our small-flowered population relies almost exclusively on autonomous selfing for reproduction; experimentally emasculated flowers produce effectively no seeds under natural pollination conditions (Elle and Carney, 2003; Kennedy and Elle, 2008b). Given the low level of inbreeding depression expressed in our TL-S population (Kennedy and Elle, 2008a) and extremely low pollinator visit rates (Kennedy and Elle, 2008b), ovule discounting does not act as a barrier for the evolution of prior selfing (Lloyd, 1992). In addition, the growing season on the rock outcrops where this population occurs can be quite short, potentially selecting for fast seed production. Taken together, these conditions should favor prior selfing more than the conditions at our large-flowered population (EF-L), where both pollinator visit rates and rainfall (and hence, the length of the growing season) are higher. We argue that research on the evolution of autogamy

should more frequently consider ecological conditions that reduce or remove barriers for the evolution of prior selfing.

Delayed selfing for reproductive assurance may be important under some ecological circumstances for JP-M and EF-L. Neither population exhibits the potential for selfing autonomously prior to flowers opening, although JP-M (mid) can self contingently with opportunities for outcrossing. In years when the pollination environment is adequate, large-flowered populations may be completely outcrossed, as we have demonstrated in multiple sites and years (Elle and Carney, 2003; Kennedy and Elle, 2008b). These populations, from more mesic sites, may use autonomous selfing in the bet-hedging sense that reproductive assurance selfing was first proposed to fill (Stebbins, 1957), in contrast to TL-S (small), which is effectively obligately selfing even when pollinators are present. The actual selfing rates for EF-L and JP-S may vary from our measured values, however. We estimated autonomous selfing ability of individual flowers under pollinator-free conditions. Under natural pollination conditions, outcross pollen could be better at fertilizing ovules than self pollen (“prepotency” of Darwin [1876]) and so could reduce the contribution of competing or delayed selfing to seed production. Pollinators could also increase the selfing rate if they facilitate autogamy or cause geitonogamy. A manipulative experiment under natural pollination conditions, with selfing rate measured using genetic markers, would be necessary to evaluate whether the variation in timing of autonomous selfing ability we document here translates into variation in selfing rate for natural populations.

A recent review by Eckert et al. (2010) discusses the impacts of human disturbance on the evolution of plant mating systems and demonstrates that the selfing rate is significantly increased in disturbed relative to undisturbed habitats. This increase is expected when anthropogenic activity leads to loss or conversion of natural areas, resulting in reduced plant or pollinator abundance or diversity and increased pollen limitation (Knight et al., 2005). Under conditions of extreme pollen limitation, recent theory predicts few barriers to the evolution of prior selfing, especially in annuals (Morgan and Wilson, 2005). Our research suggests that abiotic factors (in our case, growing season length as mediated through rainfall) may also select for prior selfing. Anthropogenic changes in climate have already lead to more rapid phenologies in plants (e.g., Fitter and Fitter, 2002; Parmesan and Yohe, 2003; Franks et al., 2007). We propose that changes in climate and phenology may, like other aspects of anthropogenic change, also result in mating system divergence.

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