BENEFITS OF AUTONOMOUS SELFING UNDER UNPREDICTABLE POLLINATOR ENVIRONMENTS

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Abstract. Pollinator unpredictability favors evolutionary shifts from outcrossing to autonomous selfing, which provides reproductive assurance. Our goal was to quantify the reproductive assurance benefit of autonomous selfing and the stochastic nature of pollinator-mediated pollen receipt using three wild populations of the annual species *Collinsia verna* (Scrophulariaceae) over three years. The timing of autonomous self-pollination in *C. verna* ranges from competing to delayed, with more than half of the autonomous pollen arriving late in floral life. Floral density, identity of insect floral visitors, and visitation rate varied within and among years, with significant variation in pollinator community between years within populations. Pollinator failure reduced fruit set within and among populations across years. However, reproductive assurance selfing increased fruit set on average by 8% overall and up to 30% in one population. Despite this fitness benefit, fruit set ($=\Sigma$fruits/Σflowers) estimated in pollinator-exclusion cages was significantly less than the fruit set in paired open-pollinated plots. In a series of experiments, we found no difference in fruit set from self- vs. outcross pollen, ruling out early inbreeding depression. However, flowers, hand-outcrossed at the same time that autonomous selfing naturally occurs, produced significantly more fruits relative to flowers that received pollen only through autonomous selfing. These results suggest that pollen type (self/outcross) is less important to fruiting success than is the timing or efficiency of autonomous pollination relative to vector-mediated pollination. Our results support the reproductive assurance hypothesis and suggest that autonomous selfing in *C. verna* is adaptive under variable pollinator conditions. Fruit set results from both pollinator-delivered and autonomous self-pollen, suggesting a ''best of both worlds'' scenario, with mixed mating a likely outcome.

Key words: autonomous selfing; Collinsia verna; *competing selfing; delayed selfing; mating-system evolution; mixed mating; pollen limitation; pollinator failure; reproductive assurance; resource limitation; selection on floral traits; self-pollination.*

INTRODUCTION

The evolution of selfing has traditionally been framed in the context of two opposing genetic forces: the transmission advantage of selfing (Fisher 1941) vs. the disadvantage of inbreeding depression, which lead to the expectation of selfing or outcrossing as equilibrium states (e.g., Lande and Schemske 1985). Both empirical work and theoretical work have underscored the importance of the genetics advantages and/or disadvantages in evolution (see reviews of theory and experiments by Charlesworth and Charlesworth [1987], Jarne and Charlesworth [1993], Uyenoyama et al. [1993], and Husband and Schemske [1996]). In addition, ecological factors, both abiotic and biotic, can influence mating-system evolution and the degree of self-pollination expressed by individual plants (Darwin 1877, 1888, Jain 1976, Lloyd 1979, 1988, Uyenoyama 1986, Holsinger 1991, 1992, Dole 1992, Lloyd and Schoen 1992, Uyenoyama et al. 1993, Barrett and

Harder 1996, Johnston 1998). Recent empirical work with natural populations (e.g., Dudash and Fenster 1997, Fishman and Wyatt 1999, Eckert 2000, Fausto et al. 2001, Goodwillie 2001, Herlily and Eckert 2002) underscores the importance of the ecological context in shaping mating-system evolution.

In animal-pollinated species, the likelihood of outcross-pollen receipt or reliability of pollinators can play a central ecological role and determine if plant populations are primarily outcross or self-pollinated (Levin 1972, Jain 1976, Lloyd 1979, 1980, 1992, Holsinger 1992, 1996, Schoen et al. 1996, Johnston 1998, Takebayashi and Morrell 2001, Vogler and Kalisz 2001). Pollinator absence or low pollinator abundance during some periods within or among flowering seasons favor shifts from outcrossing to autonomous self-fertilization because selfed seeds provide reproductive assurance (Darwin 1877, Müller 1883, Baker 1955, 1965, Lloyd 1979, 1992, Takebayashi and Morrell 2001). The fitness benefit of selfing can differ among life histories: variance in fitness and inbreeding depression among years can present a cost to selfing and maintain annual populations as mixed mating (Cheptou and Schoen 2002) while factors that decrement the fitness advantages (i.e., pollen and seed discounting and inbreeding de-

pression) of autonomous selfing for perennials (Morgan et al. 1997, Herlihy and Eckert 2002) are expected to be greatly reduced in annual plant species due to the overarching importance of reproductive assurance for this life history (Schoen and Brown 1991, Lloyd 1992, Lloyd and Schoen 1992).

While the reproductive assurance hypothesis predicts that within-flower autonomous selfing can evolve if *pollinators are unreliable in delivering outcross pollen,* the degree of unreliability at the individual flower level can favor different modes of selfing and these in turn have different consequences for mating-system evolution (Lloyd 1992, Schoen et al. 1996). Prior, competing, and delayed selfing are defined as autonomous, within-flower self-pollination that occurs before, during, or after the opportunity for outcross-pollen receipt for that flower has occurred, respectively (Lloyd 1992). At one extreme, selfing early in a flower's life (prior) is favored when a population requires pollinators for fruit set but pollinators are chronically absent (Lloyd 1992), a population's size is so low as to be undetectable by pollinators (Lloyd 1992, Fausto et al. 2001, Goodwillie 2001), or when a population experiences high levels of interspecific pollen flow (Fishman and Wyatt 1999). In addition to the reproductive assurance benefits, prior selfing could be favored since it reduces the costs associated with the longer floral maintenance time required for outcrossing and sets the stage for the evolution of reduced investment in cues for pollinators and the amount of pollen per flower. At the other extreme, autonomous selfing late in a flower's life (delayed) is favored when pollinator service and thus outcross-pollen receipt is unpredictable, such as when environmental variation compromises the ability of pollinators to visit flowers (e.g., Schoen and Brown 1991, Yahara 1992, Schoen et al. 1996), or when floral densities are exceedingly high, creating a relative scarcity of pollinators (e.g., Husband and Barrett 1992). In contrast with early selfing, later-selfing species will retain floral traits and costs associated with outcrossing (i.e., cues to attract pollinators, pollinator rewards, and prolonged floral maintenance relative to prior selfing species). Since the pollinator environment experienced by any single plant population varies temporally and spatially and can range from pollinators absent to pollinators unpredictable to pollinators generally reliable, the timing of within-flower autonomous selfing favored in that population can occupy a range along the selfing continuum (prior to competing to delayed) that balances physiological, ecological, and genetic constraints (Waller 1986, Schoen et al. 1996).

Population-level outcrossing rates (reviewed in Barrett 2002) provide estimates of the realized balance of genetic factors (e.g., inbreeding depression, transmission advantage) and ecological factors (e.g., pollinator environment, resource limitation) in natural populations. More than half of the animal-pollinated species in a recent analysis of outcrossing rates (Vogler and

Kalisz 2001) produce a mixture of selfed and outcrossed progeny within a season (i.e., have mixed mating). This result suggests that nongenetic factors, like stochastic pollinator visitation or pollinator foraging behavior, are likely to be influencing these population outcrossing rates to be neither pure selfing nor pure outcrossing. These environments can favor floral traits that confer outcrossing when pollinators are present but autonomous selfing when they are not (Darwin 1862, Müller 1883, Lloyd 1992), resulting in a mixed strategy with "the best of both worlds" (Bowman 1987, Becerra and Lloyd 1992, Lloyd and Schoen 1992). Unreliability of pollen receipt, both within and among floral seasons, is common in wild populations of animal-pollinated species (reviewed in Ehrlen 1992, Burd 1994, Erhlen and Eriksson 1995, Larson and Barrett 2000) and may contribute to mixed mating as a stable state (Vogler and Kalisz 2001). If pollinator failure contributes to mixed mating through reproductive assurance, then autonomous selfing can be adaptive. However, if mixed mating is due to geitonogamy, selfing is not adaptive, because here mixed mating is a by-product of floral attraction and pollinator behaviors (Lloyd 1992, Lloyd and Schoen 1992).

In populations with autonomous selfing, a flower may fail to set fruit for reasons other than pollinators' failure. These include insufficient resources to mature fruits and seeds; insufficient pollen loads, inability of some individual plants to autonomously self; and/or other causes including sterility and inbreeding depression (Husband and Schemske 1996). Resource limitation can co-occur with outcross-pollen limitation (e.g., Campbell and Halama 1993, Juenger and Bergelson 1997) and can magnify variation in fruit set due to selective abortion of selfed seeds (Stephenson 1981, Marshall and Diggle 2001), increase the level of inbreeding depression expressed by selfed progeny (Levri and Real 1998), or preclude fruit production even when a flower is not outcross-pollen limited (reviewed in Diggle 1995, 1997), which may negate selection favoring autonomous selfing. Thus the fruit set gained by reproductive assurance selfing can be altered by resource limitation and other factors. In addition, there may be physiological costs (i.e., water loss, respiration) associated with maintaining a corolla (Ashman and Schoen 1996, Galen 1999, 2000, Galen et al. 1999) past the time of probable outcross-pollen receipt, which is required to enable selfing late in floral life (i.e., delayed selfing).

Our main goals were to test the role of variable pollinator environments in favoring autonomous self-fertilization and reproductive assurance in natural populations. We present the results of field experiments designed to quantify the phenotypic distribution of autonomous selfing and the ecological contexts favoring autonomous selfing in three wild populations. Specifically we (1) used a common garden study to quantify the timing of autonomous self-pollen vs. outcross-pol-

len receipt in one population; (2) determined the frequency distribution of autonomous fruit set in the absence of pollinators in three field populations to determine the within- and among-population variation in realized autonomous selfing rates; (3) quantified the relative effects of pollinator failure and resource limitation and other factors on fruit set over three years of field experiments using a novel field bioassay. We determined actual pollinator failure rates on a per-flower basis across the flowering season and years, while accounting for other factors limiting fruit set; (4) quantified pollinator environments in the field to determine the extent to which it varied within and among years and thus might favor autonomous selfing individuals. We identified floral visitors and quantified floral visitation rates through direct observations of insect visitors to individual flowers and (5) conducted field experiments addressing two factors that can potentially diminish the fitness benefits of autonomous selfing: the efficiency and timing of delayed pollen receipt effect on fruit set and early inbreeding depression through the relative production of selfed and outcrossed fruits.

Materials and Methods

Study species and sites

Collinsia verna, blue-eyed Mary (Scrophulariaceae), is an ideal organism for studying the evolution of autonomous selfing and floral forms. Members of this self-compatible genus range from small-flowered, putatively highly selfing taxa to large-flowered, more outcrossing taxa (Weil and Allard 1964, Charlesworth and Mayer 1995, Mayer et al. 1996, Armbruster et al. 2002). *C. verna* plants mature acropetally (flowers on lower whorls mature first), produce from 3 to 7 flowers/whorl and flower over 4–6 wk in the spring. Within individual flowers there is temporal and spatial separation of anther and stigma development that precludes prior selfing. Herkogamy declines as the protandrous flowers age, which contributes to autonomous selfing ability (Kalisz et al. 1999). Flowers are open for a mean of 5–7 d in the field. The anthers and style are enclosed in a folded keel petal. The four anther filaments elongate one at a time (~1 d apart), the anthers sacs dehisce at the front of the keel, and later in floral life the style elongates and the stigma picks up self-pollen in the front of the keel. *C. verna* has floral attributes typical of an outcrossing species, including relatively showy flowers that produce both nectar and scent, but lifetime inbreeding depression values for this species (δ = 0.3; Kalisz 1989) are lower than the theoretical expectation for a purely outcrossing species (Lande and Schemske 1985). The flowers are visited by a variety of pollinators, many of them short-lived, specialist bees (Rust and Clement 1977, Appendix A). We found that insect pollinators did not avoid flowers whose anther sacs had been removed prior to anthesis and visited flowers at all developmental stages with equal frequency (S. Kalisz and D. W. Vogler, *unpublished data*); therefore, floral manipulations necessary to test for pollinator failure are possible. High pollen viability (>90%) and nectar production are seen throughout a flower's lifetime (Kalisz et al. 1999).

The relative timing of self-pollen vs. nonself-pollen receipt has not been described for this species. However, pollen receipt affects floral longevity. In greenhouse studies that paired flowers by stage, hand-pollinated flowers dropped their corollas 1–2 d earlier than flowers that were not hand-pollinated and only received pollen from autonomous selfing (S. Kalisz and D. W. Vogler, *unpublished data*). In addition, individuals within populations differ in their ability to produce selfed fruits in the absence of pollinators (Kalisz et al. 1999). A recently completed growth chamber study with the populations used in this study indicates significant variation among genotypes in autonomous selfing ability in the absence of pollinators (S. Kalisz and D. W. Vogler, *unpublished data*).

This winter annual species blooms coincident with the spring ephemeral flora. For such spring ephemeral species, resource availability (i.e., light and soil water) typically decreases through the growing season as the canopy trees leaf out, suggesting that fruit maturing late in the flowering season will be more resource limited than that maturing earlier. Fruits typically contain four seeds (Kalisz 1989), but fruits with fewer seeds (1–3) are also produced. Therefore we used fruit set (i.e., evidence of any pollination) rather than seed set as our per-flower estimator of pollinator visit in all experiments and individual flowers as the unit of experimental replication.

Three large populations of *C. verna* (≫1 × 10^6 plants) in southwestern Pennsylvania, USA, were chosen for the study: Braddock Trail (BT; GPS 40.18.18 N, 79.46.32 W, 179 m altitude) in Westmoreland County, Ten Mile Creek (TMC; GPS 40.01.86 N, 80.14.08 W, 360 m altitude) in Washington County located on Ten Mile Creek in the Ohio River watershed, and Enlow Fork (EF; GPS 41.35.32 N, 80.21.35 W, 231 m altitude) in Washington County located on the Enlow Fork of Wheeling Creek in the Monongahela River watershed. These populations differ in their physical aspects and date of first flowering by as much as 20 d (Appendix A) and thus were expected to experience different pollinator environments and abiotic conditions.

Pollen deposition by pollinators vs. autogamous selfing

To quantify when autonomous self- vs. outcross pollen is deposited on stigmas, we established a common garden population of *C. verna* in November 1999 in Mercer County, Pennsylvania. Each of six 1-m² raised-bed plots contained ~200 seedlings from the EF population. The following spring (2000), each plot was thinned to 72 plants, and half of the plots were enclosed by frames constructed of PVC pipe and fiberglass win-

dow screen to exclude pollinators and tall enough to ensure that the screens did not contact the plants; the other half of the plots were accessible to pollinators. To ensure maximal pollination of the exposed plants, two honey bee hives were set up within 50 m of the plots. Other insects, including carpenter bees and bumble bees, were common floral visitors (D. W. Vogel, *personal observation*). Flowers in the pollinator-exclusion plots received only autonomous self-pollen, while those open to pollinators received pollen delivered by floral visitors (both outcross and self-pollen), selfing facilitated by floral visitors and autonomous self-pollen.

To quantify differences in the amount of pollen received by flowers in the exclosure vs. open-pollination plots and to determine the relative floral stage at which a sufficient pollen load arrived in both, we used the following experimental design. Flowers from the open-pollinated and pollinator-exclusion plots were collected on four dates over the flowering season for a total of 30 flowers per stage per treatment (30 flowers × 6 stages × 2 treatments.) To ensure that 30 flowers of each of the six floral-developmental stages were sampled, the keel of each collected flower was carefully opened and the stage (as described in Kalisz et al. [1999]) determined under a dissecting microscope (stage 0 = no anthers dehisced, stage 1 = 1 anther dehisced, stage 2 = 2 anthers dehisced, stage 3 = 3 anthers dehisced, stage 4 = 4 anthers dehisced, stage 5 = corolla drop) The keel was held open and the anthers and stigma held apart by pressing each of the lower petals to the adhesive section of adhesive-backed note paper. The stigma was clipped and placed on a slide with 1% acetocarmine solution. Pollen grains adhering to the stigmatic tip were counted under a light microscope (100×). Using the mean expected pollen load per ovule necessary for fruit set derived by Ashman and Schoen (1996), we estimate that a pollen load of 12 grains is sufficient to initiate fruiting in this species, which has four ovules per flower. We used one-way ANOVA to assess both the differences by stage and treatment in the quantity of pollen received and to determine the relative floral stage at which a sufficient pollen load (i.e., 12 pollen grains) arrived between treatments.

Variation among individuals in autonomous selfing in the field

On average, if autonomous selfing compensates for a lack of outcross pollen, we would expect no difference in the mean fruit set of plants in pollinator exclosures (autonomous selfing only) relative to open-pollinated plants (where vector-mediated pollen receipt, facilitated and autonomous selfing contribute to fruit production). To determine the degree of variation among individuals in set fruit by autonomous pollination, we established paired pollinator-exclusion cages and pollinator-access plots in the field. Paired plots

provide estimates of fruit production for multiple individuals per plot pair that experienced a common microenvironment. Over a three-year period (1998–2000), ~100 PVC pipe frames (0.3 m^2) were placed in each of the three populations. In early spring, prior to flowering, half of the paired frames were completely covered in fiberglass window screening to exclude pollinators while the other half remained open to pollinators with screen tops only. All screens were left in place only until the plants had finished flowering (approximately four weeks later) and were then removed. At the time of fruit maturation (approximately four weeks after last flowering), all *C. verna* plants in all plots were individually collected, bagged, labeled, and transported to the laboratory (n = 200–450 plants per treatment per population). The total number of fruits and the total number of flowers produced by each plant were counted. Fruiting success rate per plant was calculated as the total number of fruits divided by the total number of flowers. Only the data from whorls 2–6 were used to ensure developmental consistency of the fruits sampled. Differences in mean fruit set per flower for plants in exclosures vs. controls were compared within populations using one-way ANOVA.

Pollinator visitation rates and pollinator identification

To estimate the variation in per-flower visitation rates in the field and changes in the identity of floral visitors across years, we conducted pollinator observation experiments. In 1997 and 1999, 6–12 1-m^2 plots were set up at each field site, and the number of *C. verna* plants and number of open flowers per plot per sampling date were recorded. In each population across the flowering season, on 3–5 days (depending on weather conditions) between 1000 hours and 1430 hours, the total number of floral visitors per plot and the number of flowers visited were counted for 20 min to 1 h, 2–4 times per day. Floral visitors were assigned to one of three groups based on size, color, and behavior: native bees, honey bees, or nonbees. These field data were used to calculate the number of floral visits per square meter and per flower. On other days, and near the same plots, floral visitors were collected from *C. verna* flowers and preserved. The collection was sent to the Museum of Natural History at the University of Kansas and identified by Drs. R. Brooks and C. Mitchner (Appendix B). Voucher specimens were deposited in the University of Kansas' Museum of Natural History collection of the Division of Entomology.

Floral experiments to determine causes of fruit failure

Because failure to set fruit can have multiple causes, we conducted field experiments to quantify and isolate the separate impacts of pollinator failure and all factors other than pollinator failure including resource limitation, inability to autonomously self, and/or dispro-

TABLE 1. (A) Floral treatments used to distinguish causes of fruiting failure in *Collinsia verna* in southwestern Pennsylvania, USA. (B) Assumptions and expectations of factors affecting fruit set under open pollination. (C) Assumptions and expectations of factors affecting fruit set under pollinator-excluded conditions.

A) Combinations of treatments and experimental condition used in field experiments (O_1–O_3 and E_1–E_4)

	Experiment						
Treatment	O_1	O_2	O_3	E_1	E_2	E_3	E_4
Floral treatment	intact	emasculated	intact	emasculated	emasculated	intact	intact
Pollinator access	open	open	open	excluded	excluded	excluded	excluded
Hand pollination?	yes	no	no	yes	yes	no	yes
Type	outcross	none	none	outcross	self	none	outcross
Autonomous selfing possible?	yes	no	yes	no	no	yes	yes

B) Fruit set failure experiments (field open pollination conditions): resource limitation + other factors (RL+), pollinator failure (PF), and no autogamous selfing ability (no AS)

Experiment	Fruit failure a function of:	Expectations
O_1	(RL+)	resources become limiting later in growing season
O_2	(RL+) and PF	PF varies across season/years
O_3	(RL+) and PF and no AS	AS contributes to fruiting success when pollinators fail and AS ability differs among plants

C) Costs/limits of autonomous selfing (field pollinator-exclusion conditions)

Flower treatment	Test	Expectations
E_1 vs. E_2	Experiment 1: Early inbreeding depression?	self-pollination (E_1) will have lower fruiting success relative to outcross (E_2)
E_3 vs. E_4	Experiment 2: Cost of floral maintenance?	selection against delayed selfing via cost of floral maintenance (E_3) relative to outcross (E_4)

portionate abortion of selfed fruits. Seven different experimental combinations of floral treatment, pollination environment, and hand-pollination treatment, described below, were used in our field experiments during the 1998–2000 field seasons. Flowers used in all experiments were chosen at the bud stage when all four anther sacs were developed but not yet opened. We marked all flowers used in our experiments on the underside of the calyx with a dot of nontoxic acrylic paint. Separate colors were used to indicate specific floral and pollination treatments (described below) and time in the flowering season (early, middle, late) when the experiment was conducted.

For each flower in our experiments we used one of two floral treatments: (1) emasculated, in which the keel petal of flowers was opened and the four anthers were removed using forceps; or (2) intact, in which the keel petal of flowers was opened but the anthers were not removed. Each floral treatment was under one of two pollinator-access environments: (1) open to pollinators, in which natural floral visitors had full access to flowers; or (2) excluded pollinators, in which floral visitors were excluded with cages. Finally, each treated flower received one of three pollination treatments: (1) hand-outcrossed, in which 5 d after assignation to a floral treatment, flowers were hand-pollinated with outcross pollen collected from 10–20 donor plants located at least 0.5 m away from recipient plant; (2) hand-selfed, in which 5 d after assignation to floral treatment, flowers were hand-pollinated with self-pollen collected

from other flowers on same plant; or (3) none, in which no hand-pollination occurred, but depending on floral treatment and pollinator environment, these flowers could receive pollen from either natural floral visitors or autonomous selfing or both. The combinations of floral treatments, pollinator treatments, and pollination treatments used in are summarized in Table 1A and Fig. 1.

In our open-pollination experiments (Table 1B), plants growing along four or five permanently marked 25-m transects in each population were chosen at ~0.5-m intervals (40–50 plants per transect). A small, green bamboo stake was placed 10 cm from each plant so it could be relocated ($N = 200$ plants/population). The same 200 plants/population were used throughout the entire experiment: with flowers contributing to early (whorls 2–3), mid-season (whorls 3–4), and late (whorls 5–6) estimates. If a plant failed to flower across the entire flowering season, then an additional plant was selected so that a sample size of 200 flowers per time in the flowering season per population was maintained in each experiment. For most plants used in the study, at least two of three open-pollination experiments (O_1, O_2, and O_3 in Table 1A, Fig. 1) could be applied to flowers of the same whorl during early, middle, and late portions of the flowering season.

In 1999, we attempted to include all three treatments on the same plant but found that we frequently could not. Therefore an additional, parallel transect was included in 1999 and 2000 to provide more experimental
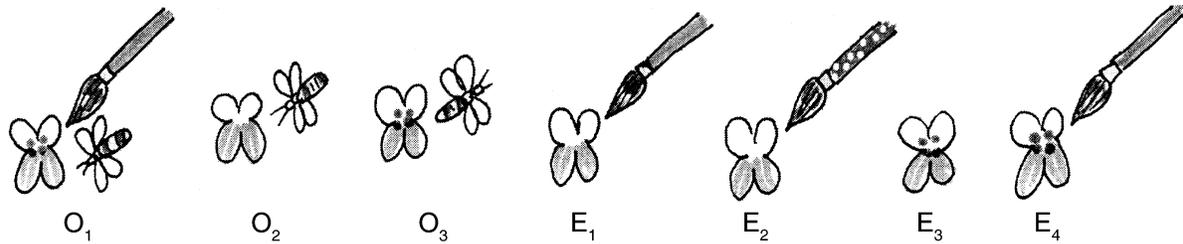
FIG. 1. Floral treatments used to distinguish causes of fruiting failure in *Collinsia verna* in southwestern Pennsylvania, USA. See Table 1 for details.

plants. To test if there were differences in fruit set between our parallel transects, we conducted another study in 1999. In one transect, using 1293 plants, an intact, hand-outcrossed flower (O_1) was paired with an experimental flower that had its anthers removed (O_2). In the parallel transect, using 1379 plants, a hand-pollinated intact flower (O_1) was paired with an intact flower (O_3). We found no significant differences in fruit success:failure of hand-pollinated flowers (O_1) from parallel transects for any of the three populations (ANOVA, $P = 0.11$, 0.98, and 0.89, for BT, EF, and TMC, respectively). Consequently, we use the fruit set of the O_1 flowers in parallel transects in each population for each time interval in 1999 and 2000 seasons to quantify the mean limitation of fruit set caused by factors other than pollinator failure. Over all years and sites, we successfully marked and recorded fruit set from 2710 hand-outcrossed intact flowers (O_1), 3567 naturally pollinated emasculated flowers (O_2), and 4113 intact flowers under open-pollination conditions (O_3). For each sampling period and year, data were pooled by transect to calculate a mean and standard deviation of fruit set.

Separating the causes of fruit failure

Our floral treatments were planned to simultaneously identify single or multiple factors altering fruit success or failure (rationale detailed below and summarized in Table 1B). In brief, failure of O_1 flowers to set fruit is used to estimate resource limitation plus other nonpollinator-related factors, failure of O_2 flowers to set fruit is used to estimate pollinator failure, and the success of O_3 relative to O_2 flowers is used to estimate the fruit set benefit of autonomous selfing.

Resource limitation plus other factors (RL+), excluding pollen

We experimentally removed pollen as a factor limiting fruit set for O_1 flowers. We did this by both hand-outcrossing and allowing natural pollinator access. Since O_1 flowers are not emasculated, self-pollination could also have occurred by facilitated and/or autonomous selfing. The remaining factors that could limit fruit set of O_1 flowers include resource limitation, as well as the genetic propensity for low fruit set, sterility, or fruit abortion unrelated to resource limitation. Based on the importance of resource limitation in fruit set for

annual plants (Stephenson 1981), we expect fruit set failure of our O_1 flowers to be primarily due to resource limitation, but we recognize that additional factors of smaller expected effect may decrease fruit set. We define RL+ and calculate mean fruit failure attributable to these factors as

$$\text{RL+} = [1 - (\text{no. } O_1 \text{ flowers setting fruit}) \div (\text{total no. } O_1 \text{ flowers})]. \quad (1)$$

Since not all plants or whorls survived to the end of the experiment, our total sample size of intact, hand-outcrossed O_1 flowers for 1999 and 2000 was 2710. We used χ^2 tests of heterogeneity to test for differences in RL+ within years. We expect that resource limitation could increase fruit failure across the flowering season while the other factors contributing to RL+ listed above are not expected to change in a predictable way.

Pollinator failure (PF)

If an O_2 (emasculated) flower sets fruit, then it must have received pollen from a pollinator. However, if an O_2 flower *fails* to set fruit, both pollinator failure and/or resource limitation plus other factors (RL+ above) could be the cause. Using Eq. 1, we can adjust for the mean RL+ experienced by O_2 flowers to determine if fruit failure is caused by pollinator failure. We define fruit set failure caused by pollinator failure (PF) and calculate mean fruit failure attributable to pollinator failure as

$$\text{PF} = [1 - (\text{no. } O_2 \text{ flowers setting fruit}) \div (\text{total no. } O_2 \text{ flowers})] - \text{RL+}. \quad (2)$$

Significant PF was evaluated by χ^2 tests comparing the fruiting success:failure of 2710 hand-pollinated intact flowers (O_1) with that of 2765 anther-removed flowers (O_2) for each time period (early, middle, late) in each population for 1999 and 2000. We used χ^2 test of heterogeneity to determine differences in PF within years. We expect that pollinator failure will vary across the flowering season and among years, which will favor autonomous selfing.

Because our 1998 field experiments did not include O_1 flowers, we estimated 1998 resource limitation from the fruiting failure of O_3 (intact) rather than O_1 flowers.

We also used this comparison in our test of heterogeneity of pollinator failure across the flowering season. The 1998 O_3 data will overestimate the impact of resource limitation and underestimate the impact of pollinator failure whenever both resource limitation and pollinator failure are occurring. Consequently, our 1998 estimate of pollinator failure is a conservative one. We tested for heterogeneity in pollinator failure within seasons by comparing the pooled χ^2 value against the summed χ^2 for the three time periods. This comparison was made for each of the three years at each of the three populations. The χ^2 tests for pollinator failure (O_1 vs. O_2 1999–2000 or O_3 vs. O_2 1998) were adjusted for multiple comparisons using a sequential Bonferroni correction (Sokal and Rohlf 1995).

Contribution of autonomous selfing (AS) to fruit set in the field

We assume that fruit production in an intact flower occurs when either successful insect pollination or autonomous selfing has occurred and there are sufficient plant resources to mature the fruit. We quantified the relative contribution of autonomous selfing to fruit set by comparing fruit success:failure of intact flowers (O_3) with that of emasculated flowers (O_2) under open-pollinated conditions across the flowering season. Because flowers in this comparison are under equal resource constraints as well as equal pollinator environments, fruiting success should differ between the two groups when autonomous selfing is the only source of pollen. This occurs when pollinators fail to visit flowers and autonomous self-pollination is successful (i.e., no fruit set in emasculated flowers and autonomously selfed fruit set in the intact flowers). Significant contribution to seed set by autonomous selfing was evaluated by χ^2 tests comparing the fruiting success:failure of 4113 intact open-pollinated flowers (O_3) with that of 3567 anther removal open-pollinated flowers (O_2) analyzed by time period (early, middle, late) in each population by year. The χ^2 tests for autonomous selfing (O_2 vs. O_3) were adjusted for multiple comparisons using a sequential Bonferroni correction (Sokal and Rohlf 1995).

Tests of self vs. outcross and outcross vs. delayed self in fruiting success

In each of the three populations in 2000, we set up pollinator-exclusion cages. Naturally occurring groups of *C. verna* plants were caged just prior to flowering. A mean of 50 plants were enclosed in each $1 \times 0.5 \times 0.5$ m PVC frame cage covered with fiberglass window screen ($n = 10$ cages/population $= 500$ plants/population). Half of these cages were used in the self vs. outcross experiment (Experiment 1) and half were used in the outcross vs. delayed selfing experiment (Experiment 2). At intervals over the flowering season, the screen on one side of cage was opened, flowers were manipulated as detailed below (E_1–E_4, Table 1A, Fig. 1), and the cage resealed.

Experiment 1.—The benefit of selfing may be decremented if self-pollen does not fertilize ovules in proportion to its deposition or if fruit resulting from selfing are aborted disproportionately. In 2000, we emasculated and marked two flowers per plant on 10 plants per cage using the protocols described previously. Five days later, we hand-pollinated each flower using either (1) outcross pollen from a flower of a plant at least 0.5 m away for E_1 flowers or (2) self-pollen from another flower on the same plant for E_2 flowers. Pollen was gathered from the appropriate donor flower onto a fresh toothpick and applied directly to the stigmatic tip of the marked recipient flowers. These experiments were done twice during the flowering season in each population (sample size $= 10$ flowers per treatment $\times 5$ cages $\times 2$ flowering dates $= 100$ flowers per treatment per population). Fruiting success was scored for each flower in the experiment. Significant differences in fruit set of hand-selfed (E_2) vs. hand-outcrossed flowers (E_1) was evaluated by χ^2 tests comparing the fruiting success : failure of self vs. outcross flowers by date in each population. Across all populations 350 outcross-pollinated (E_1) flowers and 340 self-pollinated (E_2) flowers were compared. If inbreeding depression, lower fertilization rates of self-pollen, and/or high abortion of self-fruit occurs, then we would expect to observe lower fruit set in self- vs. outcross flowers.

Experiment 2.—Delayed selfing could extract a physiological cost through the extended maintenance of flowers beyond the normal period for outcrossing. To determine if flowers receiving delayed self-pollen are less likely to produce fruits relative to flowers receiving outcross pollen, in 2000 we compared the fruiting success of intact unmanipulated flowers (E_3) with that of intact hand-outcrossed flowers (E_4). At each sampling date we marked approximately 100 intact flowers in bud (stage 1) in pollinator-exclusion cages identical to those described above. Five days later we hand-pollinated half the marked intact flowers with an outcross pollen-laden paintbrush (in the same manner as those in the transects). Thus, the E_4 flowers were outcrossed at the same stage when autonomous pollination potentially begins (i.e., stage 4 with four anthers dehisced). Our design mimics what occurs in nature, where delayed selfing is exactly that: selfing that occurs after the opportunity for outcrossing. We left the other marked intact flowers unmanipulated, so fruit production by these E_3 flowers can only result from autonomous selfing. In the BT and TMC populations, the experiment was carried out for two replicates (early and mid-bloom periods), at EF the flowering period lasted long enough that we were able to carry out a third replicate (early, middle, and late). Fruiting success was scored for each flower in the experiment. Differences in fruit set between late outcross and delayed selfing were evaluated by χ^2 tests comparing the fruiting success:failure of late hand-outcrossed flowers E_4 with that of
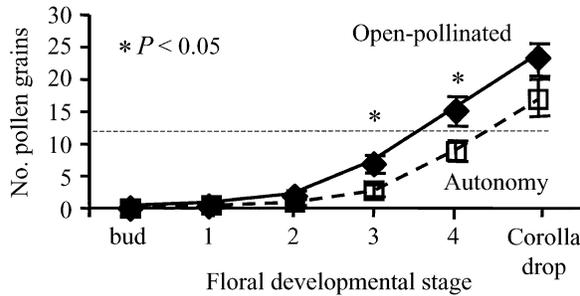
Fig. 2. Stage of pollen deposition on stigmas of *Collinsia verna* flowers from the Enlow Fork population in autonomous self-pollination plots (pollinator-exclusion, open squares) or open-pollinated plots (closed diamonds) (means ± 1 SE, where SE is visible beyond symbol). $N = 30$ flowers per floral developmental stage per treatment. Floral stages are bud (no anthers open), 1 = one anther dehisced, 2 = two anthers dehisced, etc. through corolla drop, with persistent style. An asterisk indicates significant differences in number of pollen grains per stigma for flowers at that stage between treatments. The dotted line at 12 pollen grains indicates the theoretical minimum number of pollen grains sufficient to fertilize all four ovules in a flower.

of E_3 flowers (delayed selfing only). Higher fruit set in hand-outcrossed flowers relative to delayed self-flowers suggests a cost to maintaining the flower to allow for delayed selfing and/or inefficient delayed selfing ability.

RESULTS

Pollen deposited by pollinators vs. autonomous selfing

Of the three types of autonomous pollination, Fig. 2 shows that prior selfing does not occur (no pollen was deposited in the bud stage) and that while competing selfing does occur, delayed selfing contributes more than half of the autonomously deposited pollen grains in the EF population. Little pollen is deposited on flowers prior to the third anther dehiscing (stage 3) in either the open or pollinator-excluded flowers (Fig. 2). However, flowers at both stages 3 and 4 in the open-pollinated treatment had significantly more pollen on their stigmas than did flowers of the same stages in the pollinator-exclusion treatments (Fig. 2). Open-pollinated flowers received the expected pollen load sufficient to initiate fruit set (dashed line) earlier in floral development than did flowers in the pollinator-exclusion treatments (stage 3 vs. stage 4, respectively; Fig. 2). There was no significant difference in the final number of pollen grains per stigma between treatments for flowers after their corollas dropped. These data show that pollen is deposited at earlier stages in open-pollinated flowers and that, in the absence of pollinators, flowers of *C. verna* deposit self-pollen loads equivalent in magnitude to that received in the presence of pollinators later, in part because of delayed selfing.

Within-population variation in autonomous selfing ability

There is significant variation within populations in their ability to produce fruits in the absence of pollinators (Fig. 3). In each population, mean fruit set for plants in the pollinator-excluded treatment was significantly less than mean fruit set for plants in the presence of pollinators (cages vs. control: 56% vs. 76% BT, 56% vs. 70% EF, and 50% vs. 62% TMC, one-way ANOVA $P < 0.0001$ for each population). These data indicate significant variation among individuals within populations in their ability to self autonomously, with many individuals in each population expressing a complete lack or low percentage of fruits matured in the cages (0–30%, Fig. 3). Despite the pairing of plots, which controls for microenvironments, the coefficient of variation in percentage of fruits per flower is consistently greater in the absence of pollinators than in their presence (CV of cages vs. control: 34.6 vs. 16.9 BT, 35.4 vs. 23.4 EF, 43.6 vs. 27.5 TMC), indicating a higher variance in fruit production among individuals in the absence of pollinators.
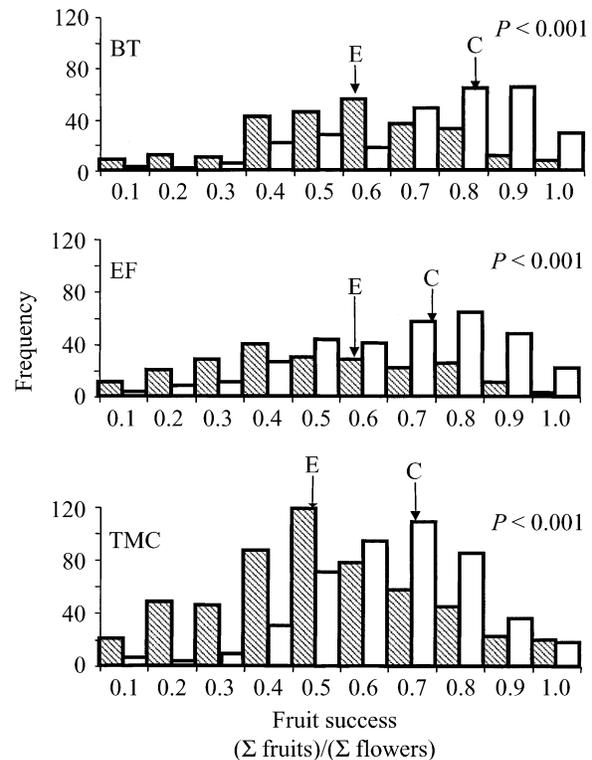

Fig. 3. Distribution of fruit production of *Collinsia verna* plants grown in paired exclosure (hatched bars) and control (open bars) plots in the field. Abbreviations are: BT, Braddock Trail; EF, Enlow Fork; TMC, Ten Mile Creek. Autonomous self-pollen was the only means of fertilization in the exclosure plots, while plants in control plots received pollen both from pollinators and autonomous selfing. Arrows indicate the mean fruit production rate for the enclosure (E) and control (C) plants in each population. Note the low frequency of the 0–30% fruit-success classes in C plants.

TABLE 2. Causes of fruiting success or failure in *Collinsia verna* in field open-pollination experiments.

Popula-tion	Year	Time in flowering season	O₁ hand-outcrossed intact flowers (RL+) success : failure		O₂ open-pollinated emasculated flowers (RL+ and PF) success : failure	
			Ratio	Percentage	Ratio	Percentage
BT	1998	early	...	...	27:45	37
		middle	...	...	39:56	41
		late	...	...	7:98	7
	1999	early	118:39	75	117:39	75
		middle	43:109	28	30:62	32
		late	8:151	5	4:143	3
	2000	early	154:28	85	120:45	72
		middle	169:9	95	121:37	76
		late	132:43	75	106:52	67
EF	1998	early	...	...	59:20	75
		middle	...	...	34:47	42
		late	...	...	35:97	26
	1999	early	107:46	70	104:33	76
		middle	26:133	16	30:120	20
		late	10:143	6	10:137	7
	2000	early	149:9	94	144:28	84
		middle	155:14	92	110:48	70
		late	75:80	48	49:55	47
TMC	1998	early	...	...	27:48	36
		middle	...	...	36:55	40
		late	...	...	9:63	12
	1999	early	43:122	26	44:122	26
		middle	9:140	6	4:164	24
		late	3:138	2	0:159	0
	2000	early	113:15	88	161:16	91
		middle	27:8	77	145:42	77
		late	13:129	9	21:143	13

Notes: Experimental flowers (see Table 1) were used to test for significant pollinator failure and reproductive assurance through autonomous selfing. Data for each period within population and year are pooled across ∼200 plants distributed across 4–5 transects. Values in boldface type indicate significant effects ($P < 0.05$) for individual χ^2 comparisons. An asterisk indicates a significant experiment-wide effect ($P < 0.05$ and $k = 27$ comparisons) following a Bonferroni correction for multiple comparisons. Abbreviations are: BT, Braddock Trail; EF, Enlow Fork; TMC, Ten Mile Creek.

Pollinator visitation rates and pollinator identification

The flowering season lasts approximately four weeks at each site. The density of flowers per square meter differed substantially among populations and among years (Appendix A). BT consistently had the highest density of flowers, and overall densities ranged from >1000 flowers/m² (at BT in 1997) to just over 200 flowers/m² (at TMC in 1999) at peak floral time. Insect visitation rates were highly variable over the season. Visitations per square meter for 1-h observation periods spanned 0–222 visits. However, floral density per square meter does not explain the differences in per capita visitation rate. In nearly every case, the seasonal mean per capita visitation rates overlap zero and there is no relationship between floral density and visit rate (Pearson correlation coefficient = −0.05, $R^2 = 0.003$, NS $P = 0.49$). Therefore other factors, such as pollinator abundance or identity, must vary across years or local weather conditions. Floral visitors were espe-

cially scarce during periods of cold, windy, or rainy weather, which are common weather conditions during the early spring flowering season (S. Kalisz and D. W. Vogler, *personal obervations*).

We grouped the floral visitors into three categories: native bees, honey bees, and other pollinators (Appendix A). However, the proportion of these types of floral visitors on *C. verna* flowers differed among years in all populations (Appendix A, $P < 0.001$ for all comparisons), indicating that the floral visitors are not constant for this species. Most notably the proportions of honey bees decreased between 1997 and 1999 at all sites. At EF and TMC the proportions of honey bees dropped from above 85% of all visitors in 1997 to below 20% in 1999.

Separating the causes of fruit failure

Resource limitation plus other factors (RL+).—The proportion O₁ flowers that failed to produce fruit (Eq. 1) is our metric of resource limitation plus other factors

TABLE 2. Extended.

χ^2 test for pollinator failure (PF) (O_1 vs. O_2)		O_3 open-pollinated intact flowers (RL+ and PF and AS) success : failure		χ^2 test for autonomous selfing (AS) (O_2 vs. O_3)	
χ^2	P	Ratio	Percentage	χ^2	P
...	...	52:27	66	12.11	**0.001***
...	...	57:33	63	9.1	**0.002***
...	...	24:77	24	35.2	**0.000***
0.001	0.974	129:39	77	0.141	0.707
0.51	0.475	59:103	36	0.374	0.541
1.08	0.298	13:156	8	3.81	0.051
21.7	**0.000***	158:35	82	4.28	**0.039**
23.8	**0.000***	156:33	83	1.89	0.169
2.83	0.093	122:65	65	0.130	0.718
...	...	66:7	90	6.4	**0.011**
...	...	55:24	69	11.6	**0.001***
...	...	62:61	50	23.9	**0.000***
1.30	0.254	108:51	68	2.31	0.129
0.692	0.406	24:119	17	0.50	0.478
0.009	0.926	12:180	6	0.04	0.838
9.26	**0.002***	172:15	92	5.79	**0.016**
25.9	**0.000***	143:38	79	3.92	**0.048**
0.040	0.841	81:81	50	0.211	0.646
...	...	50:22	69	16.47	**0.000***
...	...	32:49	39	0	0.994
...	...	16:43	27	4.48	**0.034**
0.003	0.953	55:105	34	2.38	0.123
2.6	0.101	9:144	6	2.53	0.112
3.4	0.065	4:140	3	4.47	**0.035**
0.58	0.445	170:170	92	0.242	0.623
0.003	0.959	254:27	90	14.7	**0.000***
1.02	0.331	29:157	16	0.553	0.457

(RL+) in the field (see Table 2, Hand-outcrossed column, 1999, 2000 data). The populations varied significantly across years in their fruiting success (Fig. 4A), and as expected, fruiting success declined consistently during the flowering season in all populations and years. Reductions in percentage of fruiting success from early to late season ranged from a low of 10% (BT, 2000, 85% to >75%) to 71% (TMC 2000, 88% to >9%). These data provide the background RL+ used in our estimates of pollinator failure.

Pollinator failure (PF).—We detected significant fruit failure due to pollinator failure (PF) in 2000 in BT and EF in early and mid-flowering season flowers, but none in 1999 in any population (Table 2). Pollinator failure varied significantly across the flowering season and among years within populations (Fig. 4B). The impact of PF ranged from no effect (e.g., EF 1999) to a greater than 30% reduction in fruit set during the flowering period (e.g., BT 1998). This effect can even be seen across a single flowering season in a single population (e.g., EF 2000 mid- to late). Tests of heterogeneity for fruit success:failure revealed significant heterogeneity ($\chi^2 > 5.9$, $P < 0.05$) in all three populations in 1998 and BT and EF in 2000. In the latter case the pattern was the same, with significant differences in fruit set in the early pollination period, but no significant difference in the last pollination period (Table 2). The year of highest mean PF in all three populations was 1998 (Fig. 4). In contrast, 1999 was a year of low PF and high RL+ across all populations (Fig. 4). High levels of RL+ in TMC in 1999 and 2000 likely made PF essentially zero in those years.

Contribution of autonomous selfing (AS) to fruit set in the field.—Comparison of intact O_3 vs. emasculated O_2 flowers' fruit set indicates that autonomous selfing significantly increases fruit set (Table 2) in more than a quarter of the sample dates. This positive effect of autonomous selfing on fruiting success occurred across all levels of resource availability during the flowering season and was often substantial. For example, autonomous selfing increased fruit set 30% in BT in the 1998 early season. Pooling the data across all seasons, years, and populations, our data indicate that autonomous selfing increases the overall mean fruit set by 8%. The mean effect for each population ranged from 10.4% at BT to 8.2% at EF to 5.4% at TMC.
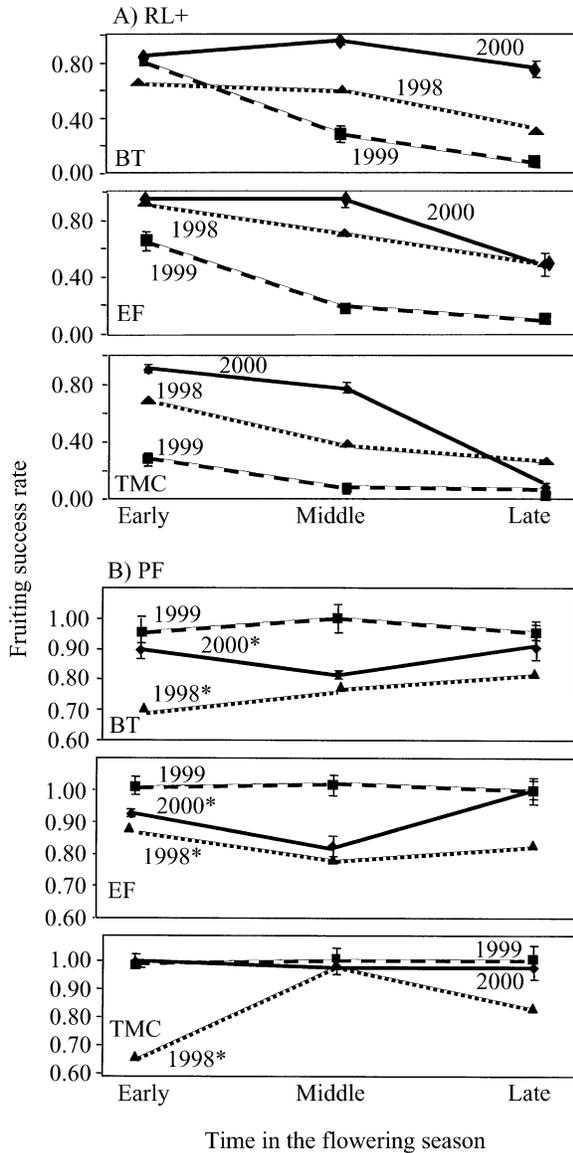
FIG. 4. Change in population mean fruiting success as a function of (A) resource limitation plus other factors (RL+) and (B) pollinator limitation (PF) in the field in three populations of *Collinsia verna* (means ± 1 SE, where SE is visible beyond symbol). Triangles, squares, and diamonds represent 1998, 1999, and 2000, respectively. An asterisk indicates significant within-year heterogeneity (χ^2 tests for heterogeneity, $P < 0.05$). Abbreviations are: BT, Braddock Trail; EF, Enlow Fork; TMC, Ten Mile Creek.

Tests of self vs. outcross and outcross vs. delayed self on fruit set

Within our field pollinator-exclusion cages, we detected no differences in the fruiting success of hand-outcrossed E_1 vs. hand-self-pollinated E_2 flowers at any time during the flowering season in any population (Fig. 5A). Similarly, intact flowers that were hand-outcrossed five days after opening (E_4) vs. intact flowers that were not hand-pollinated (E_3) within the cages did

not differ in fruit set early in the flowering season (Fig. 5B). However, E_4 flowers had significantly higher fruit set later in the flowering period at two of our three study populations ($P < 0.01$; Fig. 5B). The E_3 and E_4 flowers differ by 10–20% in fruit set. Lower fruit set of the E3 flowers was seen mid-season at BT and EF as well as late season at EF. Thus late in the season, when plants appear to be most resource limited, flowers that could only receive pollen via autonomous pollination have lower fruit set in some cases.

DISCUSSION

Collinsia verna flowers attract pollinators but they can also autonomously self, which provides reproductive assurance, if pollinators fail to visit, and the potential for mixed mating (Cruden and Lyon 1989, Schoen and Brown 1991, Lloyd 1992, Johnston 1998). The timing of receipt of self-pollen estimated in our common garden experiment (Fig. 2) ranged from coincident with pollinator-delivered pollen (competing) to potentially after the fact (delayed). Notably, more than half of the autonomous self-pollen is deposited at the floral stage preceding corolla dehiscence (Fig. 2), thus the EF population is weighted toward the delayed end of the autonomous selfing continuum. Similar variation in both the stage when self-pollen is autonomously deposited and the number of self-pollen grains deposited per stage were also seen in a greenhouse study using the EF population (Kalisz et al. 1999; Table 1), suggesting that there is variance within and/or
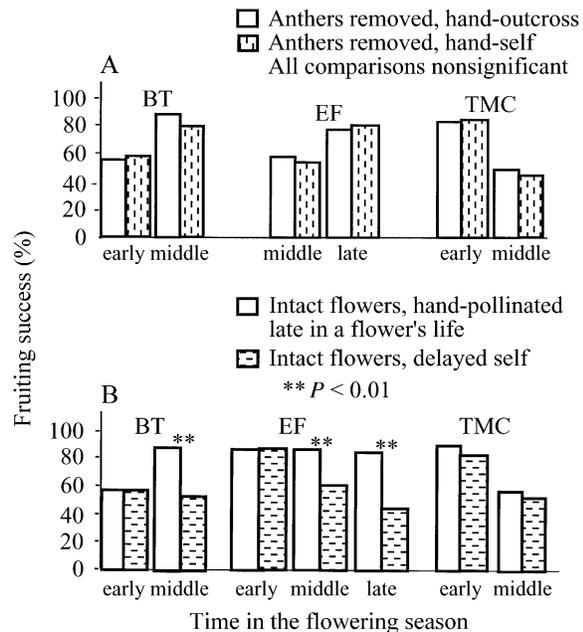

FIG. 5. Fruiting success in the field of (A) hand-outcrossed and hand-selfed flowers and (B) hand-outcross vs. autonomously selfed flowers of *Collinsia verna* across the flowering season. Two asterisks indicate significant differences between treatment pairs (χ^2 test, $P < 0.01$).

among individuals in the timing of within-flower selfing.

The reproductive assurance benefit of autonomous selfing for these *C. verna* populations is clear. Pooled over all populations and years, autonomous selfing boosted fruit production a mean of 8% and was found to increase fruit production by as much as 30% in one population in one year (TMC, 1998 early; Table 2). We can also use data from plants in the pollinator-exclusion cages (E plants, Fig. 3) to estimate reproductive assurance under experimental conditions that simulate the complete absence of pollinators. The E plants, which can only make fruit through autonomous self-pollination, attain nearly three-quarters of the fruit set observed by open-pollinated C plants (Fig. 3). Thus, these annual populations could set fruit in a range of pollinator environments to provide reproductive assurance.

The pollination environment experienced by flowers in our study populations varied significantly over the relatively short (4–5 wk) flowering season and between years. Several factors may contribute to stochasticity in the pollinator environment. First, both the absolute number of floral visitors and the proportional representation of the groups of floral visitors differed significantly across years (Appendix A). In both the EF and TMC populations, the proportion of native bees was higher relative to the proportion of honey bees (an introduced species) in the second year (proportion native bees 8.9% [1997] vs. 71% [1999] at EF; 2% [1997] vs. 58% [1999] TMC). Although we did not quantify the efficacy of the different floral visitors in delivering outcross pollen, visitor identity is known to alter pollination success in many species (e.g., Schemske and Horvitz 1984) and may affect pollen receipt in our study. Averaged over all types of floral visitors, per capita visitation rates ranged from a low of 0.02 (BT) to a high of 0.17 (EF) visits per flower per hour. Second, large numbers of simultaneously flowering individuals within a population can exaggerate times of low number of pollinators through competition among flowers for pollinators (e.g., Fritz and Nilsson 1994). The *C. verna* populations used in this study had variable floral densities within years (Appendix A), which could have increased pollinator failure for individual flowers on a local scale. Finally, our pollinator-failure experiments using emasculated flowers indicate significant variation in fruit set within years attributable to pollinator failure (Table 2, Fig. 4). Pollinator failure significantly decreased fruit set in all populations in 1998 and in BT and EF in 2000, with pollinator failure when present reducing fruit set from ~10–40%.

Pollinator failure did not alter fruit set in any population in 1999 or in TMC in 2000. In these instances the other aspects of the physical environment, including resource limitation (RL+), outweighed the effect of pollinator failure (Fig. 4). While fruit set failure due to pollinator failure exists in these wild populations,

the majority of emasculated flowers in our study set fruit equal to that of hand-pollinated flowers in 14 out of 18 periods tested (Table 2), suggesting that pollinators in these populations are generally reliable. Delayed selfing should be favored under these conditions (Schoen and Brown 1991, Lloyd and Schoen 1992, Schoen et al. 1996) with the mating system outcome of an intermediate outcrossing rate.

Despite the reproductive assurance benefit and the "best of both worlds" possibilities, not all plants in our populations set fruit in the absence of pollinators (Fig. 3). Individuals ranged from 0% to 100% autonomous fruit set when pollinators were excluded, and the mean fruit set by in the pollinator-exclusion cages was consistently 15–20% lower than fruit set of matched open-pollinated controls (Fig. 3) across all three populations. Since exclosure cages and open-pollination plots were paired in the field, resource status or other physical features of the habitat were the same across pairs, and we expected E (exclosure) and C (control) plants to be equally affected by these local environmental factors. A substantial fraction of the E plants across all three populations were in the lowest fruiting success classes (i.e., 0–30%) but these classes were rare among C plants (Fig. 3). The presence of individuals with low autonomous selfing ability in the E plots mirrors our results from a tandem growth chamber study of genotypes from these same three populations (i.e., 0–100% fruit set in the absence of pollinators; S. Kalisz and D. W. Vogler, *unpublished data*). If fruit set failure is due to innate inefficiency or inability of individuals to autonomously self, the individuals in the left tail of the E plants distributions may represent strictly outcrossing phenotypes.

Given the quantified reproductive assurance benefit, we did not expect to find so many individuals incapable of autonomous selfing. Even for plants that can autonomously self, the benefits could be diminished if self-pollen does not fertilize ovules in proportion to its deposition (e.g., Snow and Spira 1993), if self seeds are aborted during fertilization and fruit maturation (reviewed in Husband and Schemske [1996]) via early inbreeding depression, if there is a reduction of pollen or ovule viability with floral age (Nyman 1992) or change in per flower pollen production over the season, and/or if the cost of floral maintenance (Ashman and Schoen 1996, Galen 1999, 2000, Galen et al. 1999) required for complete delayed selfing is too high. Below we address each of these factors.

We found no evidence for inbreeding depression affecting fruit set (Experiment 1). These results match prior results for another population of *C. verna*, in which early inbreeding depression was negligible (Kalisz 1989). Self-pollen is equivalent to outcross pollen in the likelihood of fruiting success in all three populations. Second, the age of pollen in autonomously selfed flowers is unlikely to alter fruit success. These populations have high pollen viability, with >90% ger-

mination of all pollen tested, even pollen in corollas collected from the forest floor (Kalisz et al. 1999). We have no direct data to test the loss of ovule viability in late stage flowers. *C. verna* and other members of the Scrophulariaceae have persistent styles that remain attached to the ovary even after the corolla has dropped. We have obtained successful fruit production from hand pollinations of these naked styles (S. Kalisz, *personal observation*), suggesting that ovule age is not likely a cost.

In two of the three populations we found that intact flowers hand-outcrossed at the stage when outcross pollen is typically received had higher mean fruits set relative to intact flowers that were not hand-pollinated (Fig. 5B), suggesting an association with the timing of delayed selfing. Interestingly, this difference was not seen early in the flowering season, but mid- to late season, when populations were more resource limited (Fig. 4A). One explanation is that late-season flowers could produce less pollen than early-season flowers, thus reducing fruit set of autonomously selfed flowers through pollen limitation. We have no data to support this idea. Alternatively, fruit failure result could represent a cost of maintaining flowers (Ashman and Schoen 1996, Galen 1999, 2000, Galen et al. 1999) until autonomous selfing is completed. In a greenhouse study that paired flowers by stage, hand-pollinated flowers dropped their corollas 1–2 d earlier than flowers that were not hand-pollinated but could autonomously self (S. Kalisz and D. W. Vogler, *unpublished data*). Our experimental design may have exacerbated within-plant resource competition, since hand-pollinated and control flowers were typically on the same whorl. If hand-pollinated flowers began the process of fruit production earlier, they may have preempted resources needed to maintain autonomously selfed flowers, causing their failure later in the growing season when resources become limiting (Becerra and Lloyd 1992). In short, for *C. verna*, the type of pollen (self or outcross) is less important to fruiting success than is the timing or efficiency of autonomous pollination when compared to vector-mediated pollination.

Implications for mating system evolution

Geitonogamy, facilitated selfing and autonomous selfing (competing to delayed) can produce intermediate outcrossing rates via mixed mating (the production of both self and outcross progeny by individual plants). Both geitonogamous selfing and facilitated selfing are indirect consequences of traits favoring outcrossing, such as large multiflower displays and/or long-lived flowers (Emms et al. 1997, Barrett 2002). Geitonogamous and facilitated selfing typically reduce fitness through significant inbreeding depression and pollen and seed discounting, which decrease opportunities for outcrossing (Holsinger 1991, 1992). Thus, both geitonogamy and facilitated selfing, while producing intermediate outcrossing rates, provide no re-

productive assurance and are not adaptive. In contrast, all forms of autonomous selfing can be adaptive, and competing and delayed selfing can contribute to the evolution of mixed mating. Delayed selfing has the greatest potential fitness benefits (Lloyd 1979, 1992, Schoen and Brown 1991), allowing the best-of-both-worlds response to pollinator unpredictability through reproductive assurance. While competing selfing has variable costs that are context specific (Lloyd 1992), delayed selfing incurs no pollen or seed discounting costs (Holsinger 1991, 1992, 2000) and increases fitness even when there is substantial inbreeding depression (e.g., Schoen and Brown 1991).

True delayed selfing occurs after outcrossing opportunities have ended (Lloyd 1992). If this autonomous selfing occurs too early it becomes competing rather than delayed selfing, but if it occurs too late the efficiency of autonomous selfing may be reduced (i.e., the cost of waiting ''too long''). If autonomous selfing evolves in direct response to pollinator unpredictabilty, the timing of delayed selfing in natural populations is expected to vary in proportion to the reliability and timing of pollinator-mediated pollen receipt. Consequently, high variance in pollinator access or abundance either within or across seasons may explain variance in timing and perhaps the success rate of autonomous pollination. These factors can produce a continuum of autonomous selfing individuals in wild populations, which in any given season may appear to be wholly selfing (complete pollinator failure), largely outcrossing (unlimited pollinator access), or, more likely, mixed mating to some degree.

Although the proportion of mixed-mating species that utilize autonomous selfing is unknown, we do know that animal-pollinated species with intermediate outcrossing rates are not rare (Vogler and Kalisz 2001). In general, more studies that take an ecological approach to mating system evolution (Aide 1986, Uyenoyama 1986, Waller 1986) are needed to reveal both the distribution of autonomous selfing in nature and the ecological context favoring mixed mating in particular. Autonomous selfing and reproductive assurance may provide the evolutionary impetus for trips along the selfing–outcrossing highway (Stebbins 1957, Wyatt 1983, 1986, 1988) with some stops in between to watch the pollinators.

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APPENDIX A

A table presenting characteristics of the pollination environment for *Collinsia verna* at three populations in Pennsylvania, USA, 1997 and 1999 is available in ESA's Electronic Data Archive: *Ecological Archives* E084-077-A1.

APPENDIX B

A table presenting the observed visitors to *Collinsia verna* flowers in southwestern Pennsylvania, USA, is available in ESA's Electronic Data Archive: *Ecological Archives* E084-077-A2.