BEYOND SIMPLE REPRODUCTIVE ASSURANCE: CLEISTOGAMY ALLOWS ADAPTIVE PLASTIC RESPONSES TO POLLEN LIMITATION

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Historically, the persistence of mixed mating (reproduction via both self- and cross-fertilization) has presented a puzzle because classic theory predicts that mixed mating should be evolutionarily unstable. One mechanism that could contribute to the maintenance of mixed mating in cleistogamous species is the ability to invest in the appropriate type of reproduction (outcrossing vs. selfing) for a given pollination environment. We tested whether *Collomia grandiflora* plants responded to pollen limitation by plastically shifting relative investment in cleistogamous (obligately self-fertilizing) versus chasmogamous (potentially outcrossing) reproduction. We estimated reproductive effort (seed production) for chasmogamous and cleistogamous reproductive modes in hand-pollinated and unpollinated plants. We also investigated whether plastic responses to pollen limitation could be constrained by water availability or early nutrient fertilization. Pollen limitation significantly increased cleistogamous reproductive effort, and responses were consistent across gradients of water availability and soil fertility. Our results suggest that dimorphic cleistogamy can provide more than a fixed level of reproductive assurance, as plasticity in allocation to cleistogamy versus chasmogamy can simultaneously enable outcrossing when pollinators are abundant and increase selfing when pollinators are scarce. We argue that such plasticity could be a factor in the evolutionary maintenance of mixed mating in cleistogamous plants.

Keywords: "best of both worlds" hypothesis, mating systems, mixed mating, outcrossing, phenotypic plasticity, selfing.

Introduction

A persistent controversy in evolutionary biology concerns the mechanisms that stabilize mixed mating systems—those in which organisms reproduce by both self- and cross-fertilization (reviewed in Goodwillie et al. 2005). Classic theory presents strong opposing forces that act on outcrossing rate. Automatic selection advantage should promote selfing (Fisher 1941; Nagylaki 1976; Wells 1979), but the fitness of selfed progeny may be reduced by inbreeding depression in historically outcrossing species (Maynard Smith 1978). Additionally, outcrossing may be advantageous because recombination between genomes could allow for more rapid adaptation in subsequent generations (Stebbins 1957). Lande and Schemske (1985) demonstrated that inbreeding depression can change with the rate of selfing as deleterious alleles are purged, and they predicted that species will thus evolve either completely outcrossing or completely selfing mating systems. Yet mixed mating strategies are abundant; in plants, an estimated 42% of species studied to date exhibit mixed mating systems (Goodwillie et al. 2005), and 47% of hermaphroditic animals (excluding insects) have intermediate outcrossing rates (Jarne and Auld 2006). This mismatch between theory and reality has led to a focus

on other factors that may play a role in mating system evolution (reviewed in Goodwillie et al. 2005). For instance, reduced outcross siring success arising in species using pollen for self-fertilization ("pollen discounting"; Holsinger 1996) must be accounted for and may constrain the evolution of increased selfing. Reproductive assurance may also help maintain mixed mating systems: if outcrossing fails, self-fertilization ensures production of progeny (Jain 1976; Morgan and Wilson 2005). Despite the attention given to such factors, comprehensive explanations for the maintenance of mixed mating remain elusive. As many have noted (Goodwillie et al. 2005; Oakley et al. 2007), progress is currently limited by a lack of empirical tests of theory rather than by a lack of theory itself.

Dimorphic cleistogamy may prove useful in understanding the maintenance of mixed mating systems because the selective forces stabilizing dimorphic cleistogamy are expected to be especially strong (Oakley et al. 2007). In these systems, chasmogamous (CH) flowers (open and potentially outcrossing flowers) and cleistogamous (CL) flowers (closed and obligately selfing flowers) occur on the same plant, diverge during development, and differ in morphology (Culley and Klooster 2007). Although "cleistogamy" is sometimes used to refer to plants that produce only CL flowers, here we use "cleistogamy" interchangeably with "dimorphic cleistogamy" (Culley and Klooster 2007). While many self-compatible flowers may produce seed in the absence of pollinators, floral dimorphism allows CL flowers to be less costly to produce than CH flowers (via, e.g., reduced or absent floral parts; Oakley et al. 2007). Furthermore, inbreeding depression likely is low in cleistogamous species with high selfing rates because

deleterious alleles may be purged over successive generations of selfing (Oakley et al. 2007). The combined benefits of reproductive reliability and low cost in species with reduced genetic loads constitute a formidable advantage to reproduction via CL flowers. Indeed, the overall estimated fitness advantage of reproduction via CL flowers in comparison to CH flowers has been calculated for two species: 15% for *Impatiens capensis* and 231% for *Viola septembola* (Oakley et al. 2007). Thus, for reproduction via CH flowers to continue, the forces maintaining outcrossing must be strong enough to counter the advantage of reproduction via CL flowers. The ability to quantitatively estimate the costs and benefits of reproduction by CH and CL flowers makes dimorphic cleistogamy a useful system for empirical studies of factors that stabilize mixed mating systems (Oakley et al. 2007). However, relatively few models address the maintenance of mixed mating in dimorphic cleistogamy, and fewer have been tested (Oakley et al. 2007).

Schoen and Lloyd (1984) proposed a central model for the maintenance of dimorphic cleistogamy. Their "complex habitat" model indicates that mixed mating can be maintained if phenotypes are plastic and able to produce the appropriate flower type for the environment. Lack of pollinators provides a clear case of an environment in which CL flowers would be more advantageous than CH flowers; in such an environment, phenotypes that respond by an increasing investment in CL flowers would have an adaptive advantage due to reproductive assurance and the low cost of CL flowers. Conversely, in an environment with many pollinators, a phenotype that responds by producing more CH flowers may gain benefits associated with outcrossing (Morran et al. 2009).

The idea that cleistogamous plants might plastically produce the best-adapted flower morph for a given pollination environment has been invoked repeatedly in botanical literature (e.g., Uphof 1938; Heslop-Harrison 1966; Schoen and Lloyd 1984). Plastic production of CL flowers in response to CH fruit set is supported by correlative studies (Redbo-Torstensson and Berg 1995; Berg and Redbo-Torstensson 1998) but has not been shown experimentally.

While Schoen and Lloyd (1984) focused on CH versus CL flower number, plasticity in flower number is not the only way for a plant to reallocate resources from one reproductive strategy to another in dimorphically cleistogamous species. Plasticity in number or mass of seeds produced by each flower type may also be possible. While such plasticity would not result in cost savings via avoidance of production of expensive CH flowers in pollinator-limited environments, it would allow plants to increase fitness through increased CL reproduction. In observational studies, Berg and Redbo-Torstensson (1995) found that if *Oxalis acetosella* or *Viola hirta* plants had CH flowers that failed to produce seeds, then their CL inflorescences tended to produce not only more CL flowers but also more CL seeds per ramet. Examining data from more than 300 *Viola praemorsa* plants, Forrest and Thomson (2008) found a correlation suggesting a trade-off between CH fruit set and the number of surviving CL flowers (flowers actually or potentially setting fruit). However, experimental studies have failed to find such an effect. When pollinators were excluded from 38 *V. praemorsa* plants (CH flowers were enclosed in mesh bags), and half were hand-

pollinated, the unpollinated plants did not significantly alter CL fruit production relative to the plants that were hand-pollinated (Forrest and Thomson 2008). Likewise, Culley (2002) failed to find such an effect in a manipulative experiment. Outcrossing in *Viola pubescens* was manipulated via four treatments in the field (bagged plants, emasculated CH flowers, hand-pollinated CH flowers, and unmanipulated control plants), but there were no significant treatment differences in CL fruit or seed set.

The lack of plasticity in CH versus CL flower number and in CH versus CL seed number per flower in all studies that have manipulated pollinator environments provides a serious challenge to the hypothesis that plants can respond to the pollination environment via altered investment in CH versus CL modes of reproduction. However, few species have been tested, and all were perennials. Furthermore, previous tests could have been constrained by the particular environmental conditions that were present (water, nutrients, competition, etc.). In this study, we experimentally manipulated pollen limitation in an annual dimorphic cleistogamous species, *Collomia grandiflora*, to test the hypothesis of adaptive plastic investment in CH and CL modes of reproduction. Given that allocation to CH versus CL reproduction varies with abiotic environmental gradients such as moisture (Wilken 1982; Bell and Quinn 1987; Webster and Grey 2008), we tested the effects of pollen limitation across three water environments and two soil fertility environments to expand the generality of our results. Specifically, we asked, (1) Does pollen limitation affect investment in CH and CL reproduction? (2) If so, is reproductive investment altered via flower number or seed number? and (3) How does investment in CH and CL reproduction under pollen limitation vary across gradients of water and soil fertility?

Methods

Study Species

We tested the effects of pollen limitation on reproductive allocation in *Collomia grandiflora* (Polemoniaceae), an herbaceous annual native to North America. While its pollinator species have not been cataloged, other members of the family are pollinated by members of the Coleoptera, Diptera, Hymenoptera, and Lepidoptera (Wilken 2004). *Collomia grandiflora* forms compact terminal (and sometimes axillary) inflorescences of salmon- to cream-colored flowers (Wilken 2004). All CH (and some CL) flowers are found within the terminal inflorescence and in upper axillary inflorescences (Abrams and Ferris 1984; Wilken 2004). In the terminal inflorescence, flower morph is highly time dependent (and thus position dependent; Ellstrand et al. 1984; L. P. Albert and L. G. Campbell, personal observation). The first (topmost) flowers produced are usually CL. Next, CH flowers are exclusively produced, followed by a shift to CL flowers near the base of the terminal inflorescence. Only CL flowers are found in lower axillary inflorescences. This species was selected because it produces many CL flowers after producing CH flowers, potentially allowing an individual to alter investment in CL reproduction in response to the cue provided by pollination success rates of CH flowers. For pictures of the

two flower morphs, see Ellstrand et al. (1984). The CH flowers in *C. grandiflora* appear to be able to self-pollinate, as they have been shown to set small numbers of seeds (on average, less than one seed per CH flower) in the absence of insect visitors (Ellstrand et al. 1984). Fruits are explosively dehiscent (Wilken 2004).

Plant Care

Plants were grown in a greenhouse at Rice University (Houston, TX). Seeds were obtained from Seed Hunt (Pajaro Valley, CA) and were planted in 1 : 3 sand : Promix BX (Premier Horticulture, Quakertown, PA) in seedling trays (6 cells per 17.78×13.33-cm pack with 5.71 cm depth) on November 20, 2008. Seedlings were transplanted into 10.16-cm-diameter pots between December 12 and December 19. Plants were fertilized March 13 with 0-10-10 liquid fertilizer (10 mL/3.785 L) and April 13 with 24-8-16 liquid fertilizer (2.5 mL/3.786 L). To control spider mites and thrips, plants were sprayed with a mix of TetraSan 5 WDG Miticide (Valent USA, Walnut Creek, CA) and Avid 0.15EC Miticide/Insecticide (Syngenta Crop Protection, Greensboro, NC) on April 24.

Experimental Design

Plants were randomly assigned to one of three watering treatments (high, medium, or low), to a pollination treatment (hand-pollinated or unpollinated), and a soil fertility treatment (early fertilization vs. no early fertilization). Fifteen plants were assigned to each combination of water, soil fertility, and pollination treatment, for a total of 180 plants. To prevent soil disturbance to experimental plants, 30 additional sentry plants (10 per watering treatment) were reared for monitoring soil moisture but were excluded from the final analyses.

Watering treatments began December 22, 2008. The volumetric water content (VWC) of the sentry plants was measured each day (Field Scout TDR 100/200 moisture meter, Spectrum Technologies, Plainfield, IL) after watering the high-water-treatment plants. Plants in the high water treatment were watered daily. Plants in the medium and low water treatments were watered when soil VWC of the sentry plants fell to 65% and 35%, respectively, of that of the high water treatment. Watering treatments were terminated on May 16, when 50% of the plants had begun to senesce and the remaining plants were no longer initiating flowers. After this, plants were watered as needed until harvest.

Pollination treatments began on March 25. All open CH flowers in the hand-pollinated treatment were pollinated every 1–2 days for the duration of CH flowering, using pollen from a separate pool of 31 donor plants that were not included in the treatments or analyses. Because *C. grandiflora* CH flowers remain open for multiple days, each CH flower likely was pollinated at least once. Pollination was performed by removing an anther from a haphazardly selected donor plant with forceps and touching the anther to the stigma of a recipient plant until pollen was visibly present on the stigma. This method was well suited to simulate outcross-pollination without increasing self-pollination because the anthers are very small (generally less than 1.5 mm in length), allowing us to add pollen to the stigma in a controlled manner without distur-

bance to the surrounding anthers. Each anther was used to pollinate several open CH flowers on one or more recipient plants. While all of the plants were fertilized later in development ("Plant Care," above), half of the plants were randomly selected to receive an early fertility treatment on December 19 (24-8-26 liquid fertilizer, 2.5 mL/3.786 L).

Data Collection

Over the course of flowering, the numbers of CH and CL flowers in the terminal inflorescence and in lateral inflorescences with both CH flowers and CL flowers were counted. Occasionally, plants produced flowers that appeared intermediate between CH and CL morphologies. These were counted as CH flowers if the petals were open, potentially exposing the stigma and anthers, or as CL if not. The stem was also labeled with a jewelry tag at the point below which only CL flowers were produced (the "CL transition point").

Seeds per CH and CL flower for each plant were estimated by multiplying the proportion of fruits containing any seeds ("proportion fruits filled") by the average number of seeds per filled fruit ("seeds/fruit"). CL proportion fruits filled and CL seeds/fruit data were gathered from below the CL transition point (on average, 89% of the CL fruits occurred in that region). Between 4 and 111 CL fruits were sampled per plant (average number sampled was 42, average percent sampled was ~20%) to calculate the CL proportion fruits filled. Because fruit type (CH vs. CL) is not apparent in the mature terminal infructescence, the CH proportion fruits filled was estimated as $(F - L)/H$, where F is the number of filled fruits above the CL transition point (includes both CH and CL fruits), L is the number of CL flowers above the CL transition point, and H is the number of CH flowers above the CL transition point. We assumed that CL flowers above the CL transition point all set fruit for the following reasons: first, high CL fruit set in the terminal inflorescence is expected from previous observations (Ellstrand et al. 1984), and, second, the CL flowers and fruits above the transition point are formed roughly in synchrony with the CH flowers and fruits, so these CL fruits are unlikely to be affected by the pollination treatments. Using an alternative assumption that, for each plant, CL flowers above the transition point set fruit at the same rate as CL flowers from below the transition point (thus, CH proportion fruits filled = $F - [P \times L]/H$, where P is the CL proportion of fruits filled below the transition point) did not qualitatively change the patterns of reproductive allocation reported in the results. To estimate seeds/fruit in CH fruit, 1–10 CH filled fruits (mean = 7.4) were collected from the intermediate (exclusively CH) zone of the terminal inflorescence, and their seeds were counted. To estimate seeds/fruit in CL fruit, 5–10 CL filled fruits (mean = 9.7) per plant were collected from below the CL transition point, and their seeds were counted. In some cases, postdehiscence septa were used to determine how many seeds a fruit had contained (septa retain impressions of the seeds and can be used for accurate seed counts).

The CH and CL seeds from 33 plants distributed across treatments were weighed on a microbalance. Per-seed mass was then calculated for each type of seed (CH or CL) for each plant. Individual seeds from CH flowers had greater

mass than those from CL flowers (ANOVA, $F_{1,62} = 33.45$, $P < 0.0001$; mean $\pm$ SE, 4.76 ± 0.07 mg and 3.76 ± 0.16 mg, respectively); within a flower type, individual seed mass did not differ between pollination treatments ($F_{1,62} = 0.34$, $P = 0.561$). Reproductive effort allocated to CH or CL flowers was calculated as the product of each plant's flower number, each plant's seeds per flower, and mean individual seed mass for the appropriate flower type; thus, it represents investment in female function for that flower type. Finally, the number of branches and plant height were recorded at plant senescence.

Statistical Analysis

After we excluded plants with incomplete data, 131 *C. grandiflora* plants (71 in the hand-pollinated and 60 in the unpollinated treatment) were analyzed. ANOVA (Proc GLM; SAS Institute 2003) was used to test the effects of treatments (pollination, water, soil fertility, and all interactions) on total CH reproductive effort and total CL reproductive effort. In addition, *C. grandiflora* flowers were small, tightly clustered, and difficult to count, prompting us to include observer identity as another factor in the model. Because residuals were nonnormal in preliminary analyses, significance levels were assessed using a randomization procedure (Cassell 2002) with 10,000 replicates. Following a significant treatment effect on overall reproductive effort, we tested for treatment effects on components of reproductive effort (number of flowers, number of seeds per flower) using individual ANOVAs. By analogy to a significant overall MANOVA followed by protected ANOVAs (Scheiner 2001), we deemed correction for multiple comparisons overly conservative and report uncorrected *P* values.

The main focus of this article is the effects of pollen limitation on allocation to CL versus CH reproduction. Therefore, following a finding of no significant two- or three-way interactions between the pollination treatment and the water or soil fertility treatments for CL reproduction and only one significant interaction for CH reproduction (see below), we focus on the least squared means for the pollination treatment. Specific responses of CH and CL investment to water and soil fertility will be examined in detail elsewhere in an analysis focusing on mating systems and climate change (L. G. Campbell, L. P. Albert, and K. D. Whitney, unpublished manuscript).

Results

Pollen Limitation and Investment in CH and CL Reproduction

Regardless of pollination treatment, there was greater overall investment in CL reproduction than in CH reproduction, as measured by the aggregate mass of each seed type produced by a plant (fig. 1a). Pollination treatment significantly affected investment (fig. 1a; table 1): plants in the unpollinated treatment allocated 26% less to CH reproduction, as expected, but also plastically shifted more resources to CL reproduction, producing 26% greater aggregate CL seed mass than plants in the hand-pollinated treatment.
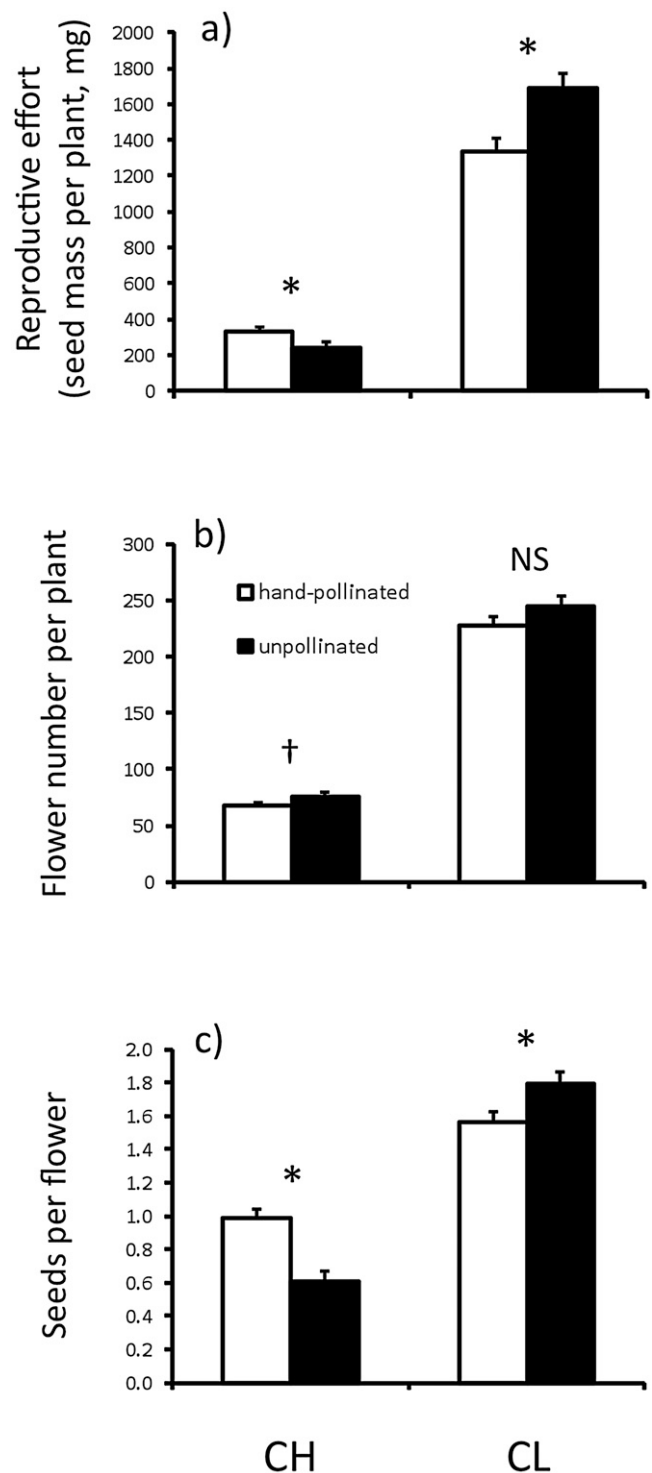

Fig. 1 Effects of pollination treatment on allocation to chasmogamous (CH) and cleistogamous (CL) reproduction in *Collomia grandiflora*. Bars represent least squares means from ANOVA models (table 1). Reproductive effort was calculated as the product of each plant's flower number, each plant's seeds per flower, and mean individual seed mass for the appropriate flower type. Comparisons between hand-pollinated versus unpollinated treatments (table 1): asterisk = significant at $P < 0.05$; dagger = marginally significant at $0.05 < P < 0.10$; NS = nonsignificant.

Table 1

Summary of *P* Values for Effects of Pollination, Watering, and Soil Fertility Treatments on Investment in Chasmogamous (CH) and Cleistogamous (CL) Reproduction in *Collomia grandiflora*, by Response Variable

Effect	CH reproductive effort	CL reproductive effort	CH flowers	CL flowers	Seeds per CH flower	Seeds per CL flower
Pollination	.0211[*]	.0060[*]	.0655[†]	.1616	<.0001[*]	.0160[*]
Water	.0236[*]	<.0001[*]	<.0001[*]	<.0001[*]	.0997[†]	.0106[*]
Pollination × water	.0226[*]	.1558	.7112	.4204	.1764	.8977
Soil fertility	.0173[*]	.0292[*]	.4412	<.0001[*]	.0109[*]	.2486
Pollination × soil fertility	.1223	.9763	.4656	.6686	.0875	.4807
Water × soil fertility	.0019[*]	.1846	.0011[*]	.0711[†]	.0341[*]	.7765
Pollination × water × soil fertility	.3167	.3095	.3477	.6993	.4954	.3614
Observer	.2047	.1270	.0234[*]	.3526	.8883	.3432

Note. Reproductive effort is the total seed mass produced by an individual plant. ANOVA results are based on 10,000 randomizations. $n = 131$ plants.

[*] $P \leq 0.05$.

[†] $0.05 < P \leq 0.10$.

Investment in Flower Number versus Seed Number under Pollen Limitation

There was no evidence that plants in the unpollinated treatment achieved higher CL reproduction via a plastic shift toward higher CL flower numbers. The number of CL flowers did not differ between pollination treatments, while the plants in the unpollinated treatment tended to produce slightly more CH flowers, a nonsignificant trend (fig. 1*b*; table 1). In contrast, plants in the unpollinated treatment had significantly fewer seeds per CH flower but significantly more seeds per CL flower relative to the hand-pollinated treatment (fig. 1*c*; table 1).

Allocation to CH and CL Reproduction under Pollen Limitation across Gradients of Water and Soil Fertility

Water availability and soil fertility significantly affected investment in CH and CL reproduction (table 1). However, the interaction between the watering and pollination treatments was never significant for CL reproduction, nor was there a significant interaction between the soil fertility treatment and the pollination treatment for CL reproduction (table 1). Thus, the effects of the pollination treatment on CL reproduction described above were consistent across gradients of water and soil fertility.

Effects of pollination treatment on CH reproduction were also consistent across the fertility treatment. However, a significant pollination × water interaction (table 1) indicated that the overall 26% drop in CH reproduction in the unpollinated treatment relative to the pollinated treatment included some variation in responses. Specifically, unpollinated plants in the low and medium water treatments had strong decreases in CH reproductive effort relative to pollinated plants (contrast $P = 0.009$ and $P = 0.012$, respectively), while CH reproductive effort in the high water treatment was relatively unresponsive to pollination treatment (contrast $P = 0.358$).

Discussion

Here, we present the first experimental demonstration that cleistogamous plants can plastically increase their investment in CL reproduction in response to pollen limitation. Such plasticity across pollinator environments (or across cues, such as day length, associated with pollinator environments) is necessary for some models describing the maintenance of mixed mating systems in CL plants (Schoen and Lloyd 1984). Furthermore, such plasticity provides an added degree of reproductive assurance in pollinator-limited environments. This plasticity was maintained across gradients of water availability and soil fertility, indicating a degree of robustness for the plasticity response.

Comparisons to Previous Observational and Experimental Studies

Previous studies seeking to discover whether cleistogamous plants exhibit plasticity in CH and CL allocation have yielded conflicting results. An observational field study of *Oxalis acetosella* and *Viola hirta* showed that ramets with unfertilized CH flowers produced more CL flowers and seeds than ramets with all CH flowers fertilized, and CH fertilization success did not influence total seed output per ramet (Redbo-Torstensson and Berg 1995; see also Berg and Redbo-Torstensson 1998). Likewise, an observational study of *Viola praemorsa* plants along an elevational gradient revealed that plants with three or more mature CH fruits had significantly fewer CL flowers surviving to set fruit than did those of the same size that produced fewer CH fruits (Forrest and Thomson 2008). In contrast to these observational studies, Forrest and Thomson (2008) found no difference in allocation to CL structures between unpollinated and hand-pollinated *V. praemorsa*. In another experimental study, Culley (2002) also found no effect of CH fruit set on CL flower or fruit production in *Viola pubescens*.

We offer two possible reasons that our study found plasticity in CL investment when previous experimental studies did not. First, CH fruit set may need to vary greatly between pollinated and unpollinated plants in order to trigger differences in CL investment across pollination treatments (Forrest and Thomson 2008). Insufficient variation in CH fruit set across pollination treatments was probably a factor in the studies by Forrest and Thomson (2008) and Culley (2002; discussed in Forrest and Thomson 2008). In our study of *Collomia*

grandiflora, unpollinated CH flowers averaged ~38% fewer seeds per fruit than pollinated CH flowers ($P < 0.0001$), demonstrating that the pollination treatment created a significant difference in seed set between pollinated and unpollinated CH flowers. A second possible explanation is that *C. grandiflora*, an annual, has only one season to reproduce. The two species used in previous experimental studies of CH versus CL allocation under pollen limitation, *V. pubescens* and *V. praemorsa*, are both perennials (Fabijan et al. 1987; Culley 2002). Annual plants are particularly likely to evolve strategies that maximize reproduction within one season because they cannot reallocate resources to future reproduction. Thus, the ability to plastically increase investment in CL reproduction in low-pollinator environments may be particularly apparent in cleistogamous annuals such as *C. grandiflora*.

Mechanisms of Plasticity

If a plant is capable of responding to pollinator availability by plastically reallocating resources between CH and CL reproduction, it could do so in several ways. One possibility is via alteration of flower number. In cleistogamous annuals, this option depends on temporal patterns of CH and CL flower production. For instance, if an annual plant produces all of its CL flowers before it produces any CH flowers, it would be impossible to adjust the number of CL flowers produced in response to CH pollination success. A review of CH and CL phenology examining 11 annual genera (with data from 13 species) revealed that all annual species begin with CL production followed by partial overlap of CH and CL production (Oakley et al. 2007). Some of these species, including *C. grandiflora*, follow this overlap with a second period of CL flowering (Oakley et al. 2007). Thus, other annuals with dimorphic cleistogamy probably also have the opportunity to plastically alter their flower production in response to the pollination environment. In perennials, plants may reallocate resources across seasons, so, in theory, cleistogamous perennials with any sequence of floral production within a season could respond to the pollination environment by altering numbers of CH and CL flowers in future years.

In *C. grandiflora*, although most of the CL flowers are produced later in the season than the CH flowers, plants exposed to a low-pollinator environment did not shift toward production of more CL flowers. Instead, *C. grandiflora* appears to employ a different mechanism of plasticity, maintaining relatively constant ratios of CH and CL flowers across pollinator environments but altering investment to maturing seeds within the two flower types. Pollen-limited *C. grandiflora* plants increased their allocation to seed production within CL flowers; seed numbers per CL flower were 1.56 and 1.79 in the hand-pollinated and unpollinated treatments, respectively, representing a 15% increase. We posit that plasticity in CH versus CL seed allocation (combined with a lack of plasticity in CH vs. CL flower number) could indicate that increased reproductive assurance is more important to the maintenance of cleistogamy in *C. grandiflora* than is the cost savings associated with producing fewer expensive CH flowers in low-pollinator environments.

Plasticity in CL Seed Production and the Maintenance of Dimorphic Cleistogamy

Schoen and Lloyd's (1984) model demonstrates that, for cleistogamous species, a phenotype producing the most appropriate flower type (CH vs. CL) for a given pollinator environment will be better adapted than a phenotype with no such phenotypic plasticity. Thus, plasticity in CH versus CL reproduction could yield an evolutionarily stable mixed mating system. As we found a shift in CH versus CL seed (but not flower) production in response to pollination environment for *C. grandiflora*, it would be informative to investigate whether retooling the model to include plasticity in CL seed number also yields a stable mixed mating system.

In this study, we looked for responses in reproductive investment to a direct manipulation of the pollination environment, but it is important to note that plants may use other environmental variables as proxies for pollination environment. Environmental variables have well-documented effects on CH and CL flower production. Allocation to CH and CL flowers has been shown to respond plastically to conditions such as light (e.g., Wilken 1982; Le Corff 1993; Cheplick 2005; Cortes-Palomec and Ballard 2006), season of flowering (Winn and Moriuchi 2009), and temperature (Connor 1998). Schoen and Lloyd (1984) have suggested that cleistogamous plants may respond to such cues in order to produce the appropriate flower type for the pollination environment. For example, for insect-pollinated species, temperature may serve as a reliable proxy for pollinator activity. We note that it will take careful experimental work to identify whether a given environmental variable is a proxy for pollination environment or whether it is a selective force driving the evolution of plasticity in CH and CL reproduction in its own right.

A major criticism of the Schoen and Lloyd (1984) model is that it requires a period when CH reproduction is advantageous, as a review of 14 species suggests that fitness of CL-derived offspring is, on average, 1.12 times higher than that of CH-derived offspring (Oakley et al. 2007). Combined with the greater costs of producing CH flowers, these patterns suggest that CH reproduction is not routinely favored. However, we suggest that definitive assessments are difficult to achieve because fitness differences between the CH- and CL-derived plants may not manifest at all life stages or may only become apparent in extreme conditions. For instance, in *Triodanis perfoliata*, Gara and Muenchow (1990) found no difference between percentage CH and CL seed germination, but they did discover that CH-derived offspring reached a significantly larger size than CL-derived offspring. Such effects will only be found in longer-term studies. Further, the greenhouse conditions under which many fitness estimates are made are unlikely to be representative of conditions in the wild; for example, natural conditions increase the expression of inbreeding depression in plants (Roff 1997). In studies where some measure of fitness has been estimated for plants in the field, CH-derived progeny sometimes do have an advantage. For example, CL-derived *Impatiens capensis* individuals had higher germination rates, but CH-derived individuals had higher fecundity (Steets et al. 2007). A study of *Calathea micans* found that when ants dispersed seeds, CH

recruitment was higher than CL recruitment in the understory (Le Corff 1996).

Experimental investigation of plasticity in CH versus CL flower and seed number in other cleistogamous species would help clarify the role of plasticity in mixed mating system evolution. With numerous such studies, it would be possible to investigate whether pollination-induced floral plasticity is associated with particular life-history traits or particular clades. For instance, one might predict that species with very "costly" CH flowers and "cheap" CL flowers have evolved greater plasticity than species with CH and CL flowers that require more equivalent investment. The biomass of *C. grandiflora* CL flowers is ~90% that of CH flowers, a higher percentage than that found in five other species that have been reviewed recently (Wilken 1982; Oakley et al. 2007). Future studies of species with greater differences in estimated investment in CH versus CL flowers would be informative.

CH : CL Plasticity in Relation to "Best of Both Worlds" Strategies

The plastic allocation strategy we found in *C. grandiflora* appears to parallel strategies found in other plants with mixed, but noncleistogamous, mating systems. For instance, self-pollinated flowers of *Phormium tenax* in close competition with outcross-pollinated flowers (i.e., on the same inflorescence) aborted at higher rates than self-pollinated flowers in less direct competition with outcross-pollinated flowers (i.e., on separate inflorescences; Becerra and Lloyd 1992). This mechanism is posited to result in joint maximization of both outcrossing and seed set (Becerra and Lloyd 1992). Cryptic self-incompatibility is a similar strategy in which the success of self-pollen tubes decreases under competition with outcross pollen tubes in the same pistil (Bowman 1987). All of these strategies promote outcrossing while providing reproductive assurance through selfing if outcrossing fails. In such "best of both worlds" strategies, it is assumed that outcrossing yields advantages over selfing, although this is not often demonstrated, perhaps as a result of the difficulties (described above) in estimating lifetime fitness of selfed versus outcrossed progeny. "Best of both worlds" strategies are often invoked to explain experimental results (Cruden and Lyon 1989; Becerra and Lloyd 1992), but they require more theoretical and empirical attention (Goodwillie et al. 2005).

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