

# Drought alters the expression of mating system traits in two species of *Clarkia*

Kathleen M. Kay · Devon A. Picklum

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**Abstract** Variation in mating system traits can have important consequences for plant populations by affecting reproductive assurance, the expression of inbreeding depression, and the colonization of and persistence in new or altered habitats. Environmental stressors, such as drought, have been hypothesized to induce higher rates of self-fertilization, yet this hypothesis has rarely been tested. Here we measure the response of two sister species of self-compatible annual herbs from contrasting habitats, *Clarkia breweri* and *C. concinna*, to an experimentally imposed greenhouse drought treatment. We find that the species differ in their baseline per-flower autogamy rates and the degree of spatial and temporal separation of male and female function within their flowers. Both species show a reduction in temporal separation of anthesis and stigma receptivity with the drought treatment. However, the species from the more mesic habitat, *C. concinna*, increases its low autogamy rate under drought conditions, whereas the species from the more xeric habitat, *C. breweri*, decreases its high autogamy rate under drought conditions. Neither species showed a response to drought in flower size or anther-stigma distance. Our results demonstrate that the induction of selfing under environmental stress cannot be assumed and that, in this case, the developmental timing of flower maturation is more plastic than floral morphology.

**Keywords** Autogamy · Dichogamy · Environmental stress · Herkogamy · Mating system · Self-fertilization

## Introduction

Rates of self-fertilization (selfing) vary widely among plant taxa, and evolutionary transitions to selfing, with their accompanying changes in life history, floral biology, and ecology, have occurred countless times (Stebbins 1974). Selfing may provide reproductive

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K. M. Kay (✉) · D. A. Picklum  
Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, 1156 High St.,  
Santa Cruz, CA 95064, USA  
e-mail: kmkay@ucsc.edu

assurance when pollinators, mates and/or resources for flowering are scarce (Baker 1955; Stebbins 1957). It may also facilitate the colonization of new habitats by mitigating the swamping effects of gene flow from ancestral populations or closely related species (Lewis 1973; Fishman and Wyatt 1999; Martin and Willis 2007), increasing levels of assortative mating and additive genetic variance for fitness in the new habitat, and staving off population extinction during establishment (Stebbins 1957; Grant 1981; Levin 2010). In the short term, these benefits may be counteracted by inbreeding depression, and, over the long term, by higher extinction rates caused by the loss of genetic variation and adaptive potential (Stebbins 1957; Goldberg et al. 2010).

Mixed mating, in which hermaphroditic organisms reproduce with a combination of selfing and outcrossing, also occurs in plants. Although once considered a transitional state during the evolution of selfing, there is growing empirical evidence that mixed mating is common and theoretical work predicting situations under which it may be favored and stable (reviewed in Goodwillie et al. 2005). Selfing rates for mixed mating plants may vary widely among species, populations, and individuals within populations (Vogler and Kalisz 2001; Kalisz et al. 2004; Goodwillie et al. 2005). Understanding the causes of variation in selfing rates is important because of their consequences for individual reproductive fitness and for population dynamics and evolution (Grant 1981; Charlesworth 1992).

Environmental stressors may affect the expression of mating system traits, with repercussions for plant populations on both ecological and evolutionary time scales. Environmentally-induced selfing may facilitate adaptive divergence and population persistence in novel or degraded habitats (Levin 2010), which may be especially important with rapid climate change (Nicotra et al. 2010; Levin 2012). Moreover, fluctuations in selfing rates with temporal or spatial variation in environmental conditions may facilitate the canalization of mixed mating or obligate selfing over evolutionary time (Schoen and Lloyd 1984; Lloyd 1992). The genetic load may be exposed to selection during times of high selfing, and populations could recover demographically during times of high outcrossing. This intermittent selfing could gradually purge alleles causing high inbreeding depression, if extinction is avoided in the short term (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Barrett and Charlesworth 1991; Goodwillie et al. 2005; Karron et al. 2012). Changes in the mating system expression may themselves be adaptive if environments vary across space or time and provide reliable information about optimal selfing rates (Schoen and Lloyd 1984; Via et al. 1995; Kalisz et al. 2004). Conversely, environmentally-induced changes in selfing could be a maladaptive consequence of resource limitation in stressful environments, could be caused by correlations with other traits under selection, or could be selectively neutral (van Kleunen and Fischer 2005). Nevertheless, most past research on plant mating system evolution has focused on genetically-based variation in selfing rates among species or populations, whereas variation within mixed mating populations in response to environmental stress has seldom been examined (Karron et al. 2012).

Environmental stress has been hypothesized to cause increases in selfing rates (Stebbins 1957; Levin 2010, 2012). Stress-induced self-fertilization may have been selectively favored if plants in marginal environments are pollen limited, or it may be an inevitable byproduct of resource limitation if difficult growing conditions cause changes in floral phenology, development, or morphology. These same factors are thought to promote the evolution of constitutively high selfing over evolutionary time. Highly selfing plants typically flower and set seed sooner than their outcrossing relatives due to trade-offs between time to flowering and the amount of resources needed for flowering (Mazer et al. 2004, 2010; Dudley et al. 2007; Bolmgren and Cowan 2008). Selfing is also correlated with

the production of smaller flowers with shorter lifespans that use less water resources than typical outcrossed flowers (Ashman and Schoen 1997; Runions and Geber 2000). Finally, stressful habitats may have lower plant densities due to plant mortality, smaller floral displays due to smaller plant size, and lower abundances of insect pollinators during the flowering season (e.g., Lewis 1962; Eckhart et al. 2010, 2011; Moeller et al. 2012), thus making reproductive assurance paramount. Whether plastic responses to environmental stress in mating system traits are generally in the same direction as evolutionary responses is poorly understood.

From prior studies, we know that environmental stressors can affect mating system traits in some plants (reviewed in Bradshaw 1965; Steets and Ashman 2004; Levin 2010). In certain cases, decreases in water or nutrient availability or increases in herbivory or competition have resulted in lower herkogamy (spatial separation between male and female structures), the breakdown in self-incompatibility, or the production of higher proportions of cleistogamous flowers. In other cases responses have been equivocal. We also know that environmental stress can affect the fitness consequences of selfing by modifying the expression of inbreeding depression (e.g., Steets et al. 2007; Murren and Dudash 2012). Looking forward, it will be important to know how often and in what types of plants environmental stressors increase or decrease selfing rates; the specific plant traits that mediate plastic changes in selfing rates; and, ultimately, the fitness consequences of variation in mating system expression for different types of plants in the field.

Here we examine mating system responses to limited water availability of two sister species of annual herbs from the California Coast Ranges. These species germinate with the onset of late fall rains and complete their lifecycles as the soil dries out in the late spring. We mimic natural variation in environmental stress by experimentally imposing the early onset of summer drought conditions. We use species with contrasting habitats, one that is relatively mesic but with many co-occurring plant species, and the other that is relatively xeric and with virtually no co-occurring plants. Both have a mixed mating system, and we quantify baseline rates of autogamy under benign greenhouse conditions. We quantify the species-level response to experimentally-imposed drought in selfing rates, the degree of spatial and temporal separation of male and female structures, and flower size. We aim to address the following questions: (1) Does drought stress consistently lead to higher rates of autogamous seed production? (2) Does drought stress cause a reduction in herkogamy mediated through a reduction in overall flower size? (3) Does drought stress cause a reduction in the temporal separation of male and female phases of individual flowers (dichogamy)?

## Materials and methods

### Study species

*Clarkia concinna* (Fisch. & C. A. Mey.) Greene and *Clarkia breweri* (A. Gray) Greene (Onagraceae) are sister species of annual herbs endemic to the summer-dry Mediterranean climate of the Northern and Central California Coast Ranges (Lewis 1955; Gottlieb and Weeden 1979). Both species have showy, zygomorphic, and highly protandrous flowers indicative of outcrossing, but are self-compatible (MacSwain et al. 1973). *Clarkia concinna* has four narrow, dark pink petals, typically produces numerous flowers per plant simultaneously, and is pollinated by a mix of bees, flies, and butterflies (MacSwain et al. 1973). *Clarkia breweri*, in contrast, exhibits a moth pollination syndrome, with pale pink,

funnel shaped flowers with a strong sweet odor, and typically produces one to a few flowers per plant (MacSwain et al. 1973). Flowers of both species exhibit herkogamy, with the stigma protruding beyond the anthers at the time of receptivity, but the amount of herkogamy varies among individuals and populations, both in the field and the greenhouse (DAP, unpubl. data). *Clarkia concinna* is found in mesic oak/pine woodlands throughout the Coast Ranges of northern California, while *C. breweri* is restricted to steep exposed hillsides in a few xeric canyons at the southern edge of *C. concinna*'s range. *Clarkia breweri* populations receive approximately half of the precipitation on average as populations of *C. concinna*, with an average November to May precipitation of 42.8 cm (SD 9.1 cm) and 83.6 cm (SD 26.9 cm), respectively (T. Miller, personal communication). The much larger variance in average precipitation among *C. concinna* populations is due to the much larger geographic range of *C. concinna*. Additionally, spring 2010 and 2011 field observations of natural *Clarkia* populations documented lower and more spatially and temporally variable pollinator visitation rates to *C. breweri* compared to *C. concinna* (KMK, unpubl. data).

*Clarkia* is an ideal system for these questions. In the primarily Californian genus of 41 species, all are self-compatible and there is wide variation in mating system, from primarily outcrossing to highly autogamous (Lewis 1955; Fausto et al. 2001; Mazer et al. 2010). Many species exhibit mixed mating through varying degrees of protandry, herkogamy, and delayed selfing (Lewis 1955). Autogamous selfing has evolved repeatedly in the genus, and these transitions have been accompanied by shifts to more xeric habitats, earlier flowering times, and faster life cycles (Lewis and Lewis 1955; Moore and Lewis 1965; Sytsma and Gottlieb 1986; Gottlieb and Ford 1996; Runions and Geber 2000; Fausto et al. 2001; Mazer et al. 2010). Additionally, two independent shifts in mating system from outcrossing to primarily self-fertilizing have occurred within the *C. concinna* lineage in geographically peripheral regions (subspecies *C. concinna automixa* and *C. concinna raichei*; Allen et al. 1991). Thus *Clarkia* shows the repeated evolution of autogamy associated with the colonization of novel, drier habitats, providing likely scenarios for environmentally based variation in selfing rates to play an important role.

We collected *C. concinna* seeds in 2008 from a wild population in Napa County near Conn dam (WGS 1984 coordinate system: 38.48143 N, 122.36430 W, elevation 112 m). We collected *C. breweri* seeds from a wild population in 2008 and 2009 along San Antonio Valley Road in Santa Clara County (37.3543 N, 121.5596 W, elevation 634 m). Although we needed to collect *C. breweri* seeds over two years for sufficient sample sizes, we did not observe any significant differences in germination rates, mating system traits, or responses to the drought treatment between the cohorts. Therefore they are treated as a single population for all analyses. The seeds were stored at 4 °C in dry silica until the start of the experiment in summer 2010.

## Experimental design

We conducted a greenhouse experiment to mimic the occurrence of the early onset of summer drought. On June 10, 2010, we planted *Clarkia concinna* and *C. breweri* seeds in 3.8 cm diameter conetainers containing four parts Pro-Mix HP Mycorrhizae potting soil to one part perlite. Each conetainer received 3–6 seeds from a single family for a total of 150 families of each species. These seeds were germinated in Conviron E-15 growth chambers on a 15 °C, 10 h day and 10 °C, 14 h night schedule. The seeds and seedlings were watered with deionized water every other day. Once the seedlings had secondary leaves and were approximately 4 cm tall, we thinned the plants to one plant per conetainer. Of the

300 families planted, 110 *C. breweri* and 122 *C. concinna* families had at least one germinant, resulting in 232 total plants. In mid-August we transferred the plants to the greenhouse where they were kept between 13 and 21 °C with a 13.5 h day and daily overhead misting. Because *Clarkia* normally flower in the spring, we maintained day length throughout the remainder of the experiment by supplementing with 1000 W metal halide lights in the evenings as the natural days shortened. On September 3, we randomly assigned half of the plants of each species to a treatment group and each plant within a treatment group to one of twenty trays. At this time, we measured the height of each plant in order to evaluate preexisting differences among treatments in plant size and to use initial plant size as a potential covariate in analyses. Initial plant size did not differ between treatments for either species, nor did it affect the results. We established a step-down watering treatment over a period of 4 weeks, and the plants were given 3 weeks to acclimate to the final watering level before measurements were taken, allowing new floral bud development to occur during the desired water treatment. This step-down brought the plants from a daily watering regimen to a drought treatment of weekly overhead hand watering, and a benign treatment of overhead hand watering every 3rd day. The drought treatment plants exhibited obvious signs of water stress, such as higher leaf loss, smaller size, lower flower production and earlier senescence, but did not suffer mortality due to the treatment.

On October 22, we began observation of mating system traits. Of the 232 plants under observation, 11 *C. breweri* and 11 *C. concinna* did not produce sufficient flowers during the period of measurement and were excluded from the study. Disregarding the youngest and oldest flowers of each plant, we chose three of the most mature flowers at the bud stage. The oldest of these buds was marked for measurement, the middle for collection, and the youngest to be left undisturbed to quantify selfed seed set. Plants varied widely in the number of flowers produced and in time to first flower. Instead of selecting flowers from a standard position on the plant, we chose to standardize the amount of time the plants had experienced the watering treatments by simultaneously (over the course of a few days) selecting buds that were similar in size. We tried to avoid positional effects by not using the first or last flowers that a plant produced, and by using pre-treatment plant size and first flowering dates as covariates in our analyses, which are likely correlated with flower node position, since we chose flower buds that were at approximately the same stage of development. Pre-treatment plant size and first flowering date did not differ between treatments for either species, nor did they affect the results for mating system traits.

For the oldest of the three selected flowers, we quantified dichogamy (temporal separation between anther dehiscence and stigma receptivity), herkogamy, and flower size. For dichogamy, we designated anther dehiscence as the first day that exposed pollen was visible at the anthers. Stigmas become receptive some time after anther dehiscence, and can be seen by the splitting and curling back of the four stigma lobes. On the first day of stigma receptivity, we measured filament length and stylar exsertion for herkogamy, and left petal length and hypanthium length as proxies for flower size. Filament length was measured as the distance from the attachment point with the hypanthium to the base of the anther, and stylar exsertion was measured as the distance the style projects from the hypanthium to the base of the opening stigma lobes. Filaments are generally straight, allowing us to calculate anther-stigma distance from these measurements, yet allowing us to attribute any changes in herkogamy to changes in style and/or filament length. Flowers are zygomorphic with four petals, and the two side petals are the largest.

We used the second marked flower to obtain the dry weight as an additional measure of flower size. We removed the flower at first anther dehiscence, cut at the junction of the

hypanthium and the ovary. After drying at 60 °C for a minimum of 24 h, we weighed the flowers using a scale sensitive to 10 µg. Pollen remains on the anthers even after dehiscence unless the flowers are visited or disturbed, so these measures included the weight of pollen. By measuring flower size, we were able to determine if any change in herkogamy was associated with an overall change in flower size.

The third flower was marked and allowed to develop fruit undisturbed in order to quantify autogamous seed set. Flowers of these species collapse after blooming and remain attached to the plant for several days, potentially allowing for delayed self-fertilization. After the fruits began to ripen, we secured them with a small strip of tape at the top of the capsule to prevent the inadvertent loss of seeds. The tape allowed for unrestricted air and light passage to all but the tip of the fruit. We collected the fruits when they were ripe and counted both autogamous seed set and the number of unfertilized ovules in each fruit. Fruits that were not autogamously fertilized were often aborted, and seed set was counted as zero. 19 *C. breweri* and 28 *C. concinna* either died before setting fruit (3 plants), produced pollen sterile flowers (8 plants), or did not produce sufficient flowers during the time of measurement to set aside one to estimate the selfing rate (36 plants), and therefore were excluded from the autogamous seed set analyses. We noted a measure of plant age (days between first flowering and blooming of the flower evaluated for selfing rate) as a potential covariate in analyses of selfing rates, since senescence has been observed to affect selfing rates in other *Clarkia* (Delesalle et al. 2008).

### Statistical methods

We analyzed herkogamy, dichogamy, and all individual flower measurements (petal length, hypanthium length, and flower dry weight) separately with two-way ANOVAs for effects of species, treatment, and species by treatment interactions. A standardized measure of herkogamy was calculated as (stylar exsertion – filament length)/stylar exsertion. Dichogamy was calculated in days from anther dehiscence to stigma receptivity. A general linear model was used to analyze autogamous seed set, with effects of species, treatment, plant age, species by treatment interaction, and species by plant age interaction. Additionally, the effects of dichogamy and herkogamy on autogamous seed set within each species were examined by fitting linear regressions. All statistical analyses were performed in JMP v9.0.

### Results

*Clarkia breweri* and *C. concinna* differ in selfing rates, floral traits, and their responses to the drought treatment. Herkogamy was found to be proportionally greater in *C. breweri* than *C. concinna*, with a main effect of species ( $F_{1,206} = 40.25, p < 0.0001$ ; Fig. 1). However, no significant difference between drought and benign treatments was found ( $F_{1,206} = 2.07, p = 0.15$ ), nor was there a species by treatment interaction ( $F_{1,206} = 2.14, p = 0.15$ ). For dichogamy, a main effect of species was found ( $F_{1,206} = 732.32, p < 0.0001$ ), with *Clarkia concinna* having a much longer separation between the onset of male and female stages than *C. breweri* (Fig. 2). Additionally, plants in the drought treatment, irrespective of species, showed less temporal separation between anthesis and stigma receptivity than those in the benign treatment ( $F_{1,206} = 16.81, p < 0.0001$ ), with no species by treatment interaction ( $F_{1,206} = 1.80, p = 0.18$ ). Autogamous seed set increased with drought in *C. concinna* but decreased with drought in *C. breweri*, with total autogamous seed set greater in

*C. breweri* (main effect of species:  $F_{1,157} = 20.16$ ,  $p < 0.0001$ ; no main effect of treatment:  $F_{1,157} = 0.41$ ,  $p = 0.52$ ; species by treatment interaction:  $F_{1,157} = 5.07$ ,  $p < 0.05$ ; Fig. 3). Negative trending but nonsignificant regressions of seed set on both dichogamy and herkogamy were found within both *C. concinna* and *C. breweri*.

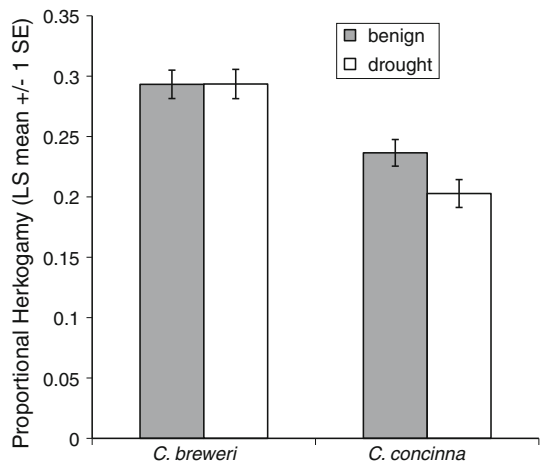
Floral size measurements included petal length, flower weight, and hypanthium length. *Clarkia breweri* had shorter petals than *C. concinna* across treatments ( $F_{1, 206} = 138.09$ ,  $p < 0.0001$ ), and we found a nonsignificant trend toward shorter petal lengths with the drought treatment across species ( $F_{1, 206} = 2.951$ ,  $p = 0.087$ ). *Clarkia breweri* had significantly heavier flowers than *C. concinna* ( $F_{1,193} = 264.76$ ,  $p < 0.0001$ ), but there was no significant treatment effect or treatment by species interaction. Hypanthium length similarly differed, with *C. breweri* having longer hypanthia than *C. concinna* ( $F_{1, 206} = 918.47$ ,  $p < 0.0001$ ). There was a trend towards increased hypanthium length with the drought treatment ( $F_{1, 206} = 3.395$ ,  $p = 0.0705$ ).

## Discussion

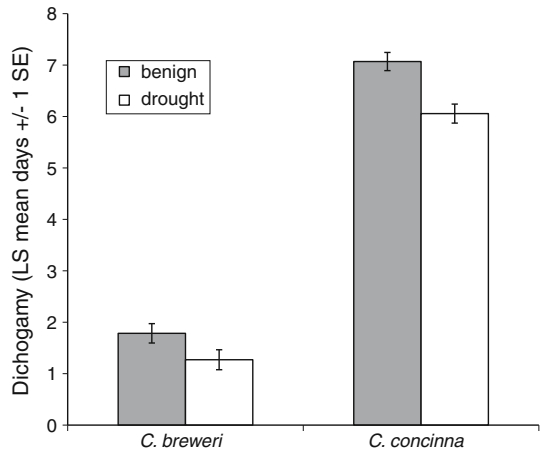
We endeavored to test the mating system responses of two closely related annual herbs to an experimentally imposed shortening of the growing season and found significant, but contrasting, responses in our study species. The species from the more mesic habitat with a later onset of summer drought, *C. concinna*, showed a significant increase in the per-flower selfing rate with the drought treatment, as predicted. In contrast, the species from the drier habitat with earlier-onset summer drought, *C. breweri*, showed a decrease in the per-flower selfing rate with the drought treatment. Its high level of autogamous seed production even under relatively benign conditions may indicate that it is already near the limit of autogamous seed production and that additional stress leads to a reduction in seed set due to resource limitation. Alternatively, it may be that autogamy decreases with drought if there is strong selection to outcross under drought conditions.

Both species exhibit substantial protandry and herkogamy, even under drought conditions, such that any selfing likely takes place after a window of opportunity for outcrossing. We observed that flowers remained on the plant after wilting, at which point any remaining

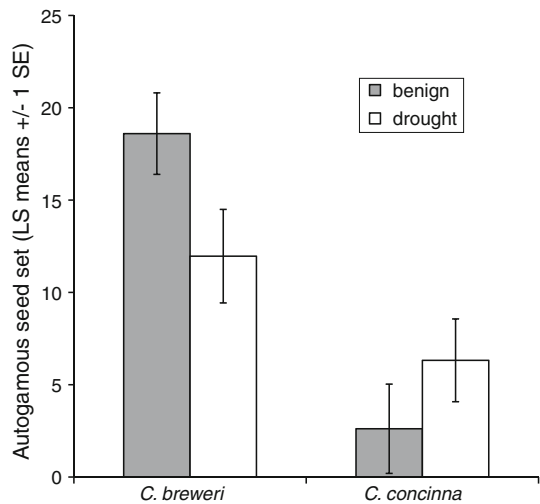
**Fig. 1** Proportional herkogamy [(stylar exsertion – filament length)/stylar exsertion] was greater for *C. breweri* than *C. concinna*, but unaffected by the drought treatment



**Fig. 2** Dichogamy, measured as the days separating the onset of anther dehiscence and stigma receptivity, was greater for *C. concinna* than *C. breweri*, and lower across species with the drought treatment



**Fig. 3** Autogamous seed set per fruit was greater in *C. breweri* than *C. concinna*. The drought treatment affected autogamy differently between the species, with an increase in *C. concinna* and a decrease in *C. breweri*



pollen could fall onto the exerted stigma. For an annual plant, delayed selfing may be the optimal mating system, since it incurs little or no cost in terms of seed or pollen discounting, and there is no need to conserve plant resources for future years of reproduction (Lloyd 1979, 1992; Kalisz et al. 1999).

*Clarkia concinna* has much greater dichogamy and likely has more reliable pollinator service than *C. breweri*. *Clarkia concinna* is effectively pollinated by a wide variety of diurnal insects, including bees, flies, moths, and butterflies (MacSwain et al. 1973) with relatively high visitation rates (KMK, unpubl. data), at least in large patches (Groom 1998). It also shows substantial inbreeding depression (Groom and Preuninger 2000). In this situation, a long delay between male and female function would reduce self-pollen interference and avoid the potential cost of unnecessary inbreeding (Lloyd and Yates 1982; Lloyd and Webb 1986).

In contrast, *C. breweri* is effectively pollinated by nocturnal hawkmoths (MacSwain et al. 1973; R. Raguso, pers. comm.), which are known to show high year-to-year variation



in abundance (Campbell et al. 1997; Sime and Baldwin 2003; Kulbaba and Worley 2008), and territorial hummingbirds, which show high spatial variation in visitation rates (KMK, unpubl. data). It may be that *C. breweri* regularly faces conditions under which selfing is advantageous, and constitutively high levels of delayed selfing allow for reproductive assurance. Alternatively, *Clarkia breweri* may exhibit higher constitutive rates of delayed selfing as a correlated response to selection for a shorter floral lifespan, since floral longevity is expected to be more costly in its drier, more open habitat and for its much larger flowers, which weigh nearly three times those of *C. concinna*. Although we did not directly measure floral lifespan in this study, the lower dichogamy in *C. breweri* may indicate a shorter overall floral lifespan, and floral longevity has been shown to be costly in other *Clarkia* (Ashman and Schoen 1997).

Dichogamy decreased with drought for both species, and may have facilitated the higher selfing rate under drought stress in *C. concinna*. Despite the separation in the timing of onset of the male and female reproductive phases, there was an observed overlap of functional genders in many flowers, and this overlap may have increased with the drought treatment, although we did not measure functional gender overlap directly by quantifying the viability of remaining pollen over time. However, when we analyzed the selfing rate across individuals of *C. concinna*, we did not see the expected negative correlation between selfing rate and dichogamy, as has been seen in other studies (Holtsford and Ellstrand 1992; Kalisz et al. 2012). Thus the increase in autogamous seed set in *C. concinna* remains somewhat unexplained, and may be a result of other mechanisms, such as changes in floral lifespan or unmeasured changes in floral morphology.

We also quantified herkogamy in our experiment, and whereas *C. breweri* exhibited larger overall herkogamy than *C. concinna*, neither species exhibited a change in herkogamy with the drought treatment. We saw a similar lack of change for flower size, which we had predicted would underlie any change in herkogamy. It may be that these factors relate more to pollinator attraction and the mechanics of pollen removal and deposition by the pollinators than to mating system per se and are thus relatively canalized and robust to environmental stress. Instead, drought stress may reduce the overall number of flowers produced, since there is predicted to be a negative trade-off between flower size and number (Cohen and Dukas 1990; Morgan 1993; Sargent et al. 2007; but see Worley and Barrett 2000), although we only observed this anecdotally and did not quantify total flower production.

Although we have documented changes in mating system expression in response to environmental stress in these species, we do not know how our results apply under realistic field conditions or whether these responses seen in the greenhouse are adaptive. In the field, selfing may result from both autogamy and geitonogamy (selfing among different flowers on the same plant), and geitonogamy may be differently affected by drought conditions. For example, if drought reduces flower production in *C. concinna*, it may reduce the opportunity for geitonogamy while at the same time increasing autogamy. Field studies of floral display, pollinator visitation, autogamy, and realized selfing rates in response to environmental variation will be necessary to separate these factors (Steets and Ashman 2004). Moreover, our seed set measures were designed to quantify the autogamous selfing rate and are not measures of fitness. In order to understand the fitness consequences of mating system plasticity, it will be necessary to replicate genotypes across environments, quantify selfed and outcrossed seed set at the whole plant level, and measure the relative fitness of selfed to outcrossed seed under realistic conditions. Whereas both species are known to exhibit inbreeding depression (Groom and Preuninger 2000;

R. Raguso, pers. comm.), we have not quantified how drought stress affects the opportunity for and costs of outcrossing in the field. We hope to address these issues in future work.

Changes in mating systems in response to environmental stress could have important consequences for plant population dynamics under rapid environmental change and for short-term population persistence and long-term evolutionary potential. Our results show that environmental stress does not necessarily lead to higher rates of autogamy, as has previously been hypothesized. Our work also suggests that for annual plants, delayed selfing may be a more important mechanism of mating system plasticity than changes in dichogamy or herkogamy because of its low associated costs. Finally, our work adds to the small but growing number of studies investigating the effects of environmental stress on the expression of mixed mating systems, which will facilitate predictions about expected responses in different types of species under climate change.

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## References

- Allen GA, Gottlieb LD, Ford VS (1991) Electrophoretic evidence for the independent origins of 2 self-pollinating subspecies of *Clarkia concinna* (Onagraceae). *Can J Bot-Revue Canadienne De Botanique* 69:2299–2301
- Ashman TL, Schoen DJ (1997) The cost of floral longevity in *Clarkia tembloriensis*: an experimental investigation. *Evolut Ecol* 11:289–300
- Baker HG (1955) Self-compatibility and establishment after “long-distance” dispersal. *Evolution* 9:347–348
- Barrett SCH, Charlesworth D (1991) Effects of a change in the level of inbreeding on the genetic load. *Nature* 352:522–524
- Bolmgren K, Cowan PD (2008) Time—size tradeoffs: a phylogenetic comparative study of flowering time, plant height and seed mass in a north-temperate flora. *Oikos* 117:424–429
- Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Adv Genet* 13:115–155
- Campbell DR, Waser NM, Melendez-Ackerman EJ (1997) Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *Am Nat* 149:295–315
- Charlesworth B (1992) Evolutionary rates in partially self-fertilizing species. *Am Nat* 140:126–148
- Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Ann Rev Ecol Syst* 18:237–268
- Cohen D, Dukas R (1990) The optimal number of female flowers and the fruits-to-flowers ratio in plants under pollination and resource limitation. *Am Nat* 135:218–241
- Delesalle VA, Mazer SJ, Paz H (2008) Temporal variation in the pollen: ovule ratios of *Clarkia* (Onagraceae) taxa with contrasting mating systems: field populations. *J Evol Biol* 21:310–323
- Dudley LS, Mazer SJ, Galusky P (2007) The joint evolution of mating system, floral traits and life history in *Clarkia* (Onagraceae): genetic constraints vs. independent evolution. *J Evol Biol* 20:2200–2218
- Eckhart VM, Singh I, Louthan AM et al (2010) Plant-soil water relations and the species border of *Clarkia xantiana* ssp. *xantiana* (Onagraceae). *Int J Plant Sci* 171:749–760
- Eckhart VM, Geber MA, Morris WF et al (2011) The geography of demography: long-term demographic studies and species distribution models reveal a species border limited by adaptation. *Am Nat* 178:S26–S43
- Fausto JA, Eckhart VM, Geber MA (2001) Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana* (Onagraceae). *Am J Bot* 88:1794–1800
- Fishman L, Wyatt R (1999) Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution* 53:1723–1733
- Goldberg EE, Kohn JR, Lande R et al (2010) Species selection maintains self-incompatibility. *Science* 330:493–495

- Goodwillie C, Kalisz S, Eckert CG (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. In Annual Review of Ecology Evolution and Systematics, Annual Review of Ecology Evolution and Systematics, pp 47–79
- Gottlieb LD, Ford VS (1996) Phylogenetic relationships among the sections of *Clarkia* (Onagraceae) inferred from the nucleotide sequences of PgiC. Syst Bot 21:45–62
- Gottlieb LD, Weeden NF (1979) Gene duplication and phylogeny in *Clarkia*. Evolution 33:1024–1039
- Grant V (1981) Plant speciation, 2nd edn. Columbia University Press, New York
- Groom MJ (1998) Allee effects limit population viability of an annual plant. Am Nat 151:487–496
- Groom MJ, Preuninger TE (2000) Inbreeding depression is not diminished in isolated subpopulations of *Clarkia concinna concinna* (Onagraceae). Evol Ecol 14:155–180
- Holtsford TP, Ellstrand NC (1992) Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). Evolution 46:216–225
- Kalisz S, Vogler D, Fails B et al (1999) The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). Am J Bot 86:1239–1247
- Kalisz S, Vogler DW, Hanley KM (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. Nature 430:884–887
- Kalisz S, Randle A, Chaffetz D et al (2012) Dichogamy correlates with outcrossing rate and defines the selfing syndrome in the mixed-mating genus *Collinsia*. Ann Bot 109:571–582
- Karron JD, Ivey CT, Mitchell RJ et al (2012) New perspectives on the evolution of plant mating systems. Ann Bot 109:493–503
- Kulbaba MW, Worley AC (2008) Floral design in *Polemonium brandegei* (Polemoniaceae): genetic and phenotypic variation under hawkmoth and hummingbird pollination. Int J Plant Sci 169:509–522
- Lande R, Schemske DW (1985) The evolution of self-fertilization and inbreeding depression in plants. I. Evolution 39:24–40
- Levin DA (2010) Environment-enhanced self-fertilization: implications for niche shifts in adjacent populations. J Ecol 98:1276–1283
- Levin DA (2012) Mating system shifts on the trailing edge. Ann Bot 109:613–620
- Lewis H (1955) The genus *Clarkia*, 20(4). University of California Press, Berkeley and Los Angeles
- Lewis H (1962) Catastrophic selection as a factor in speciation. Evolution 16:257–271
- Lewis H (1973) Origin of diploid neospecies in *Clarkia*. Am Nat 107:161–170
- Lewis H, Lewis ME (1955) The genus *Clarkia*, vol 20, no. 4. University of California Press, Berkeley
- Lloyd DG (1979) Some reproductive factors affecting the selection of self-fertilization in plants. Am Nat 113:67–79
- Lloyd DG (1992) Self-fertilization and cross-fertilization in plants. II. The selection of self-fertilization. Int J Plant Sci 153:370–380
- Lloyd DG, Webb CJ (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I Dichogamy. NZ J Bot 24:135–162
- Lloyd DG, Yates JMA (1982) Intra-sexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). Evolution 36:903–913
- MacSwain J, Raven PH, Thorp R (1973) Comparative behavior of bees and Onagraceae. IV. *Clarkia* bees of the western United States. Univ Calif Publ Entomol 70:1–80
- Martin NH, Willis JH (2007) Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. Evolution 61:68–82
- Mazer SJ, Paz H, Bell MD (2004) Life history, floral development and mating system in *Clarkia xantiana* (Onagraceae): do floral and whole-plant rates of development evolve independently. Am J Bot 91:2041–2050
- Mazer SJ, Dudley LS, Hove AA et al (2010) Physiological performance in *Clarkia* sister taxa with contrasting mating systems: do early-flowering autogamous taxa avoid water stress relative to their pollinator-dependent counterparts? Int J Plant Sci 171:1029–1047
- Moeller DA, Geber MA, Eckhart VM et al (2012) Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. Ecology 93:1036–1048
- Moore DM, Lewis H (1965) The evolution of self-pollination in *Clarkia xantiana*. Evolution 19:104–114
- Morgan M (1993) Fruit to flower ratios and trade-offs in size and number. Evol Ecol 7:219–232
- Murren CJ, Dudash MR (2012) Variation in inbreeding depression and plasticity across native and non-native field environments. Ann Bot 109:621–632
- Nicotra AB, Atkin OK, Bonser SP et al (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15:684–692
- Runions CJ, Geber MA (2000) Evolution of the self-pollinating flower in *Clarkia xantiana* (Onagraceae). I. Size and development of floral organs. Am J Bot 87:1439–1451

- Sargent RD, Goodwillie C, Kalisz S et al (2007) Phylogenetic evidence for a flower size and number trade-off. *Am J Bot* 94:2059–2062
- Schoen DJ, Lloyd DG (1984) The selection of cleistogamy and heteromorphic diaspores. *Biol J Linn Soc* 23:303–322
- Sime K, Baldwin I (2003) Opportunistic out-crossing in *Nicotiana attenuata* (Solanaceae), a predominantly self-fertilizing native tobacco. *BMC Ecol* 3:6
- Stebbins GL (1957) Self fertilization and population variability in the higher plants. *Am Nat* 91:337–354
- Stebbins GL (1974) Flowering plants: evolution above the species level. Belknap Press, Cambridge
- Steets JA, Ashman TL (2004) Herbivory alters the expression of a mixed-mating system. *Am J Bot* 91:1046–1051
- Steets JA, Wolf DE, Auld JR et al (2007) The role of natural enemies in the expression and evolution of mixed mating in hermaphroditic plants and animals. *Evolution* 61:2043–2055
- Sytsma KJ, Gottlieb LD (1986) Chloroplast DNA Evolution and Phylogenetic-Relationships in *Clarkia* (Onagraceae). *Am J Bot* 73:788–799
- van Kleunen M, Fischer M (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytol* 166:49–60
- Via S, Gomulkiewicz R, Dejong G et al (1995) Adaptive phenotypic plasticity—consensus and controversy. *Trends Ecol & Evol* 10:212–217
- Vogler DW, Kalisz S (2001) Sex among the flowers: the distribution of plant mating systems. *Evolution* 55:202–204
- Worley AC, Barrett SCH (2000) Evolution of floral display in *Eichornia paniculata* (Pontederiaceae): direct and correlated responses to selection on flower size and number. *Evolution* 54:1533–1545