RESEARCH ARTICLE

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Losing one's touch: Evolution of the touch-sensitive stigma in the *Mimulus guttatus* species complex[1]

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PREMISE OF THE STUDY: The stigmas of several species are touch sensitive and respond to pressure by closing. Previous research suggests that stigma closure could prevent self pollination within a flower during a pollinator's visit or enhance male function by increasing pollen export. Both factors could be favored in outcrossers, and neither would be beneficial in selfers.

METHODS: We investigated variation in stigma-closing and the duration of closure in annual and perennial populations of the variable species *Mimulus guttatus* and whether four closely related selfing species (*M. cupriphilus*, *M. laciniatus*, *M. nasutus*, and *M. pardalis*) have lost their touch sensitivity. We grew plants in a controlled environment and performed experiments with and without the addition of pollen to the stigma.

KEY RESULTS: In *M. guttatus*, the speed of stigma-closing was rapid and unaffected by the deposition of pollen. Populations varied significantly in closing speed, which may reflect their geographic location. For annual populations only, anther–stigma separation significantly affected closing speed. Also, stigmas that closed quickly stayed closed longer, and stigmas that received pollen remained closed longer. Finally, in the selfing species, stigma-closing was more variable; some populations have entirely lost the ability to respond to touch.

CONCLUSIONS: We discuss our results in the context of traits that promote outcrossing and traits that are under selection during the evolution of selfing. This is the first characterization of variation in touch responses across multiple populations within a species and the first to demonstrate the loss of touch sensitivity in selfing lineages.

KEY WORDS herkogamy; mating system; *Mimulus*; Phrymaceae; pollination; outcrossing; touch-sensitive stigma; selfing

For hermaphroditic plants, avoiding selfing is a common problem, and various contrivances have evolved to limit the transfer of pollen within and between flowers of the same plant (Barrett, 2002). Commonly, these involve spatial (herkogamy) or temporal (dichogamy) separation of the female and male sexual organs. In addition to the well-recognized problem of avoiding selfing, floral traits may also evolve to promote pollen dispersal and increase fitness through male outcrossed siring success. Thus, a second challenge for hermaphroditic plants is to avoid interference between female and male parts within the same flower and the loss of mating opportunities that may result (Lloyd and Webb, 1986; Webb and Lloyd, 1986).

One of the more curious mechanisms to limit selfing is the rapid touch response that occurs in the stigmas of several flowering plant families, where the two stigma lobes are sensitive to pressure and close together when contacted by an insect. The curiosity of irritable stigma lobes drew commentary by Linnaeus, Sprengel, and Darwin, among others (von Sachs, 1890). Darwin acknowledges "Mr." Kitchener for ingeniously explaining the use of these movements, namely, to prevent the self-fertilization of the flower (Darwin, 1876; although the author is "Mrs." Kitchener: Kitchener, 1874). Because the bilobed stigma closes rapidly in response to touch by a pollinator, it reduces the transfer of self-pollen from anthers to stigma within a flower during a pollinator's visit. Although the anti-selfing explanation is the most common interpretation for touch-sensitive stigmas (Newcombe, 1922), it has also been characterized as an example of movement herkogamy (spatial separation of anthers and stigma) to avoid interference between female and male organs (Webb and Lloyd, 1986). Fetscher (2001) provided empirical support for this idea with an elegant experiment that demonstrated that in the bush monkey flower, *Mimulus aurantiacus*, stigma closure facilitates greater pollen export, by removing interference between female and male parts, and only minimally prevents intrafloral selfing.

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Despite the genetic benefits of outcrossing, the evolution of selfing is a common transition (~20%) in flowering plants (Barrett and Harder, 1996). The evolution of selfing often entails a reduction in flower size and the loss of various outcrossing and interference–avoidance mechanisms. The characteristic floral features that evolve in selfing lineages are often described as the “selfing syndrome” (Ornduff, 1969; Sicard and Lenhard, 2011). In addition to an overall reduction in floral display (Goodwillie et al., 2010), transitions to selfing can involve a breakdown of genetic self-incompatibility mechanisms (e.g., Busch et al., 2010), the loss of heterostyly (e.g., Barrett et al., 1989), a decrease in dichogamy (e.g., Kalisz et al., 2011), and a reduction in the physical separation of anthers and stigmas within flowers (e.g., Herlihy and Eckert, 2007). One would predict that selfing lineages that arise from ancestors with touch-sensitive stigmas should lose their irritability, although to our knowledge, this has never been demonstrated.

In this study, we first characterized the variation in the touch-sensitive stigma within the predominantly outcrossing, self-compatible, species *Mimulus guttatus*. Although in the same genus, the mechanisms proposed by Fetscher (2001) for the bush monkey flower (*M. arauntiacus*) may not be analagous in the yellow monkey flower (*M. guttatus*). In *M. guttatus*, the stigma often reopens, even after receiving pollen, so the trait does not provide the complete switch from female function to male function as it does in *M. arauntiacus*. Furthermore, Fetscher and Kohn (1999) suggested that the speed of stigma closure may not be under direct selection, but rather be governed by physiological constraints. Unlike *M. arauntiacus*, that is hummingbird-pollinated (Fetscher et al., 2002), *M. guttatus* is pollinated by a variety of generalist bees. Because bees spend more time foraging at a flower, rapid closure may indeed function to reduce self pollination within a visit, as well as to minimize intrafloral interference between female and male function.

Here we investigated correlations between floral morphology and speed of closing and duration of closure, with or without the application of pollen, in the highly variable *Mimulus guttatus* species complex. We were interested in three main questions: (1) What are the influences on speed of stigma closure and the duration of closure? (2) Are these components of touch sensitivity correlated with floral morphology? (3) Has touch sensitivity changed in four independently derived selfing species within the *M. guttatus* species complex? For these questions, we used a collection of 10 annual and 11 perennial populations of *M. guttatus* and two populations each of four closely related selfing species (*M. cupriphilus*, *M. laciniatus*, *M. nasutus*, and *M. pardalis*). This design allowed us to examine how touch sensitivity has evolved with changes in floral morphology, life history, and mating system.

MATERIALS AND METHODS

Study system—The yellow monkey flowers of the *Mimulus guttatus* species complex (sect. *Simiolus*, Phrymaceae) are a phenotypically diverse, yet broadly interfertile group of wildflowers with their center of diversity in western North America (Vickery, 1978; Beardsley et al., 2004). The group comprises species with a variety of life history and developmental and physiological traits that enable them to occupy a broad range of habitats, ranging from coastal sand dunes to montane meadows, serpentine barrens and copper mine tailings (Wu et al., 2008, and references therein).

We assembled a collection of 29 different populations that span the geographic range, life history, and mating system of species in the *M. guttatus* species complex (Fig. 1; Appendix S1, see online Supplemental Data with online version of this article). These included populations from coastal perennial, inland perennial, and annual populations of *Mimulus guttatus* DC (Pennell, 1947; Twyford and Friedman, 2015): the self-fertilizing copper-tolerant *M. cupriphilus* Macnair; granite-endemic and high-altitude selfing *M. laciniatus* Gray; the selfing, serpentine *M. pardalis* Pennell; and the self-fertilizing *M. nasutus* Greene. The classification of *M. guttatus* as either annual or perennial rests largely on the degree of investment in vegetative structures. Annual plants are diminutive, with small leaves and thin stems, and have fibrous roots. Perennial plants have much larger leaves, produce stolons, and have thick stems. In the field, annuals die in summer due to lack of water, while perennials persist. These distinctions have been well characterized elsewhere (Friedman et al., 2015).

Experimental design—From each *M. guttatus* population, we germinated seed from 3 to 6 open-pollinated maternal families, with between 2 and 10 individuals from each family, for a total of 242 plants. For the other 4 species, we used 2 populations from each species, with 4 maternal families, and 4 individuals from each family, for a total of 128 plants. Seed were sown in 6-cm pots filled with moist Fafard 4P potting mix (Sun Gro Horticulture, Agawam, Massachusetts, USA), and stratified in the dark at 4°C for 1 wk. Pots

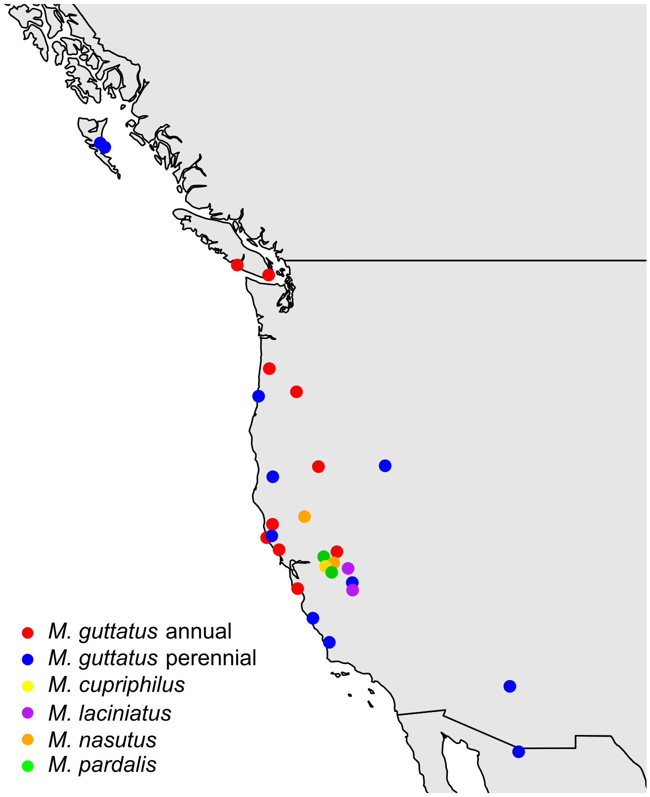

FIGURE 1 Map of locations of populations used in this study. Population points are color-coded to indicate whether they are annual or perennial *Mimulus guttatus* or from the selfing species *M. cupriphilus*, *M. laciniatus*, *M. nasutus*, or *M. pardalis*.

were then moved into a walk-in growth chamber at Syracuse University, where they were grown with 16-h days, 21°C/18°C temperature, and 55% relative humidity. Because the touch-sensitive response can be dependent on flower age and moisture level, we imposed several careful provisions. Plants were monitored daily, and flowers were tagged the day they first opened. Touch experiments on a given flower were conducted the following day (i.e., on 1-d-old flowers). We recorded the position of the flower (the floral node) and the total number of flowers on the plant. In addition, to ensure even hydration of plants, pots were bottom-watered for 1 h at 09:00 hours every day, and touch experiments were conducted between 11:00 and 12:00 hours.

For each trial, we recorded the time it took for the stigma to close (seconds; measured as the time it took for the stigma lobes to touch) and the duration of closure (seconds; measured as the time until the stigma lobes had fully opened again). The same two people conducted all trials on *M. guttatus*—one person (K. S. Hart) applied the touch, and a second person (M. C. den Bakker) used a stopwatch to record speeds. In addition, all trials were videorecorded using a Nikon D3100 camera mounted on a tripod. For 30 random trials, we compared the speed of stigma closure using the stopwatch vs. playback of the video and determined that the live measurements were sufficiently accurate (absolute value of difference ranged from 0.01 to 0.68 s). To examine the difference between mechanical touch and application of pollen, we conducted two experimental treatments on separate flowers of each plant, with their order randomized. The first treatment (mechanical touch) involved sweeping a clean wooden toothpick across the stigmatic lobes. The second treatment (pollen application) involved first loading the wooden toothpick with fresh conspecific pollen, and then sweeping it across the stigmatic lobes. The pollen was collected from a random set of 10 donors that included a mix of populations from the same species. Fresh pollen was collected every 20 min (up to 5 flowers were assayed during this interval). Because the stigmas of the selfing species often had self-pollen on the stigma by the time the flower was open, we included an additional set of observations for these species. We dissected floral buds at the stage just before protrusion of the corolla and examined them for stigma movement using a dissecting microscope.

For each plant, we measured a suite of floral characters, including corolla length and width, style length, stamen length of the short and long stamens, stigmata width, and anther–stigma separation. Corolla length and width were measured to the nearest millimeter; the other measurements were made using a dissecting microscope and recorded to the nearest millimeter with 2 decimal places.

Data analyses—For all *M. guttatus* plants, we analyzed speed of closure and duration of closure with general linear models (mixed procedure of SAS, release 9.4, SAS Institute, Cary, North Carolina, USA). Dependent variables were log-transformed to assure normality. Analyses accounted for the split plot design, where life history (annual vs. perennial) was the whole plot, and treatment (mechanical touch vs. pollen application) was the split-plot. Both life history and treatment were fixed effects; population (nested within life history) and maternal family (nested within population and life history) were included as random factors. In addition, analyses considered potential covariates including flower position, the total number of flowers on the plant, and the latitude and longitude of the source population. We also included the various floral morphology measures to test whether they affect stigma movement, as would be expected if they evolve in a coordinated manner. The analysis of duration of closure included speed of closure as an independent variable to specifically examine this association. All analyses initially assessed the effects of all factors and covariates and relevant interactions. Terms were excluded from the model by backward elimination ($\alpha = 0.05$) if they did not explain a significant proportion of the variation in the dependent variable by themselves and they were not involved in a more complicated, significant interaction. We used restricted maximum likelihood (REML) to estimate the variance components of random effects, and their significance was calculated from hierarchical log-likelihood ratio tests. Denominator degrees of freedom for F tests of the general linear models were calculated using the approximation of Kenward and Roger (1997), which can result in fractional degrees of freedom. All plotted values are adjusted to account for the other components in the model.

We next examined whether the selfing species were as touch responsive as *M. guttatus* by first testing the proportion of stigmas that showed any movement. We classified movement into three categories: no movement, some movement, and stigma closed. We used an ordinal logistic regression and included species and population nested within species as random factors. We analyzed stigma movement in open flowers and in flower buds separately. Because a large fraction of plants showed no stigmatic movement, we then considered only plants that showed any movement and tested for species and population differences in the time to close (seconds). We used a general linear model and included species and population nested within species as random factors.

RESULTS

Variation in stigma movement within Mimulus guttatus—For most *M. guttatus* plants, stigmas were responsive to touch. Across all populations, the mean (±SE) time to close was 6.62 ± 1.09 s, and the duration of closure was 7 min 39 ± 11.68 s. However, for 14 individuals (from 10 different populations), some of the stigmas did not respond to touch and never closed. In addition, stigmas took longer than 40 min (2400 s) to reopen from 20 individuals (from 15 different populations), in all but 1 case after the application of pollen. Because we stopped recording reopening time at 40 min, these flowers were excluded from analyses of reopening time.

Overall, populations differed significantly in their speed of stigma closing and for how long they stayed closed (Table 1). However, the effect of life history was not significant (i.e., annual and perennial populations did not differ in any consistent ways) for either stigma closing or duration of closure. Moreover, the speed of closing was unaffected by whether the toothpick was empty or contained pollen (treatment main effect: $F_{1,555} = 0.18$, $P = 0.67$), suggesting that mechanical touch alone is enough to induce closure. In contrast, the duration of closure was significantly affected by whether pollen was deposited (treatment main effect: $F_{1,561} = 79.43$, $P < 0.0001$). Furthermore, there is a strong negative relationship between closing time and duration of closure ($F_{1,535} = 45.44$, $P < 0.0001$); the quicker a stigma closes, the longer it stays closed; and the slower it is to close, the more quickly it opens back up (Fig. 2). This pattern is consistent across all populations and both life histories. The longitudinal location of populations explained a significant proportion of the variation in the time to close of stigmas (Table 1, Fig. 3), with more inland populations closing more slowly. Neither latitudinal location of population, the number of flowers open on a plant, nor

TABLE 1. Summary of general linear models of the influences on speed of stigma closing and duration of stigma closure for flowers of *Mimulus guttatus*.

Source of variation	Speed of stigma closing	Duration of stigma closure
Treatment	$F_{1,555} = 0.18$	$F_{1,561} = 79.43$ ***
Life history	$F_{1,54.3} = 3.23$	$F_{1,18.8} = 0.15$
Population (Life history)	$\chi^2 = 13.7$ **	$\chi^2 = 16.9$ ***
Family (Population)	$\chi^2 = 19.1$ ***	$\chi^2 = 0.9$
Longitude	$F_{1,14.2} = 4.66$ *	
Anther–stigma separation	$F_{1,53.7} = 5.34$ *	
Anther–stigma × Life history	$F_{1,53.6} = 4.65$ *	
Speed of stigma closing		$F_{1,535} = 45.44$ ***

Notes: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

the position of the flower had any effect on closing speed or duration of closure.

Relations between floral morphology and touch sensitivity—Many of the flower measurements were highly positively correlated with each other, and the strength of the correlations was similar across both the annual and perennial populations. However, anther–stigma separation was only weakly correlated with style length and was uncorrelated with all other floral measures in both annuals and perennials, and stigma width was only correlated with other floral measurements in annual populations (online Appendix S2). When we analyzed the impact of floral morphology on stigma movement, only anther–stigma separation had a significant effect. The degree of anther–stigma separation had a significant effect on stigma closing time (main effect: $F_{1,\,53.7} = 5.34$, $P < 0.05$) and a significant interaction with life history (interaction effect: $F_{1,\,53.6} = 4.65$, $P < 0.05$). Examination of this interaction revealed that for annuals only, the lower the anther–stigma separation, the slower the stigma closes (Fig. 4, see figure legend for partial regression coefficients). For perennial populations, there is no relationship between anther–stigma separation and closing speed. There is no effect of any flower morphological measurements on the duration of closure.

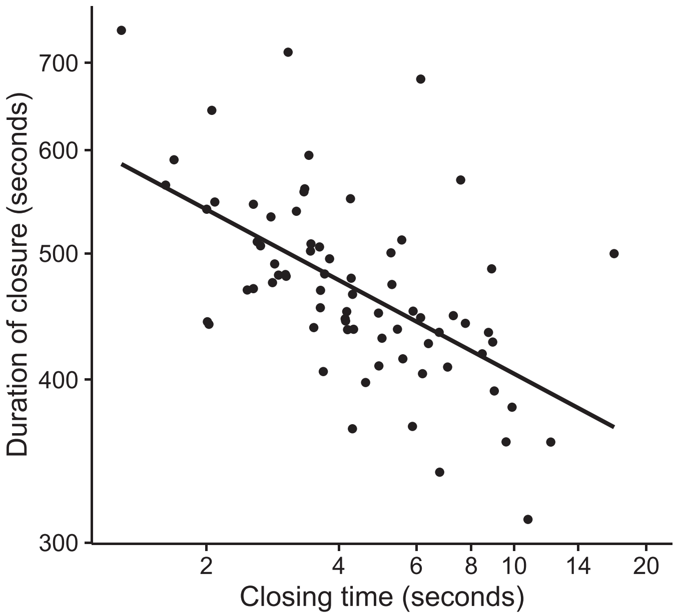

FIGURE 2 Relationship between closing time of stigma and the amount of time the stigma stays closed (duration of closure) for *M. guttatus*. Family means are plotted. The fitted line depicts the partial regression from the full general linear model ($b \pm \mathrm{SE} = 0.17 \pm 0.02$, $t_{535} = -6.74$, $P < 0.0001$). See Table 1 for details of overall analysis. Both axes show back-transformed values on a logarithmic scale.

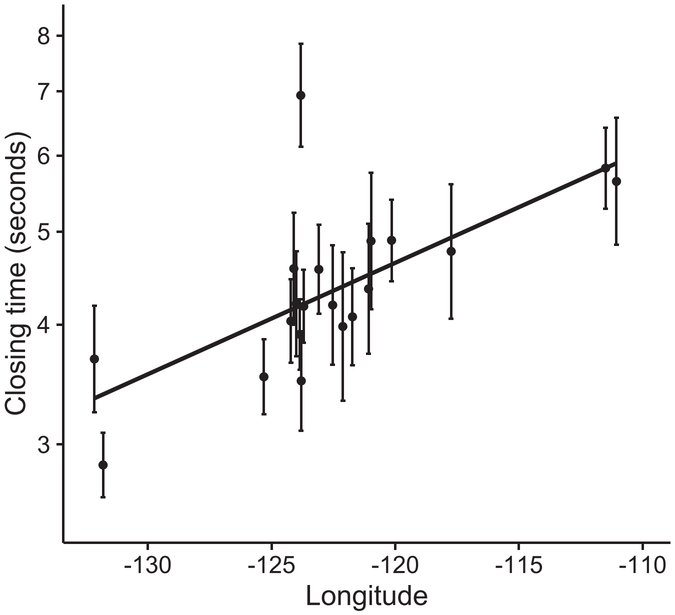

FIGURE 3 Relationship between longitudinal position of a population and its mean ± SE stigma closing time for *M. guttatus*. Population means are plotted. The fitted line depicts the partial regression from the full general linear model ($b \pm \mathrm{SE} = 0.013 \pm 0.006$, $t_{14.1} = 2.15$, $P < 0.05$). See Table 1 for details of overall analysis. The *y*-axis shows back-transformed values on a logarithmic scale.

Touch sensitivity in four selfing species—Unlike *M. guttatus*, where almost all stigmas responded to touch, the selfing species were more variable in whether stigmas responded to touch with movement. Across the four species (two populations each), the proportion of stigmas that closed ranged from 0 to 55% (Fig. 5A). Because many of the stigmas had self pollen on them by the time the flower opened, we also dissected buds just before opening and examined touch sensitivity at this stage. The results were very similar, with 0–33% of stigmas responding to touch, except for a population of *M. laciniatus*, WLF, where 80% closed (Fig. 5B). Species, and populations within species, responded differently to touch (logistic regression based on three categories of response, Species effect: $\chi^2 = 31.38$, $P < 0.0001$; population nested within species effect: $\chi^2 = 18.71$, $P < 0.001$). The species with the greatest population differences was *M. laciniatus* (Fig. 5), where one population was substantially more responsive to touch (WLF) than the other (PET). For the fraction of stigmas that did respond to touch, we examined the time it took for the stigma to close. Overall, the stigmas of the selfing species took longer to close (mean ± SE: *M. cupriphilus* 12.85 ± 2.37 s; *M. laciniatus* 10.06 ± 2.01 s; *M. pardalis* 9.71 ± 2.21 s; *M. nasutus* N/A no stigmas closed) than in *M. guttatus*. Neither the effect of species, nor the effect of population within species, was significant on the time to close (data not shown). However, we had limited ability to detect differences, because our sample size here was much reduced as a small proportion of stigmas closed at all.

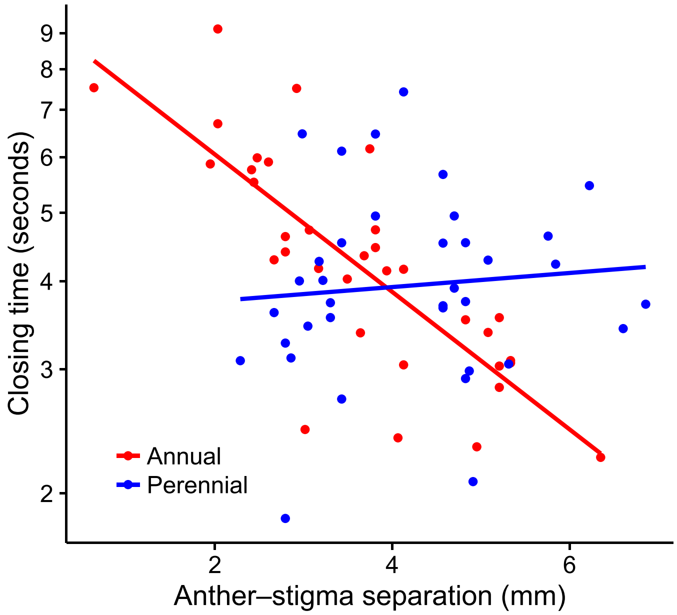

FIGURE 4 Relationship between anther–stigma separation and the closing time of the stigma, for annual and perennial populations of *M. guttatus*. Family means are plotted. The fitted line depicts the partial regression from the full general linear model (annual $b \pm \mathrm{SE} = -0.088 \pm 0.026$, $t_{50.8} = -3.08$, $P < 0.05$; perennial $b \pm \mathrm{SE} = -0.003 \pm 0.027$, $t_{52.8} = -0.10$, $P > 0.9$). See Table 1 for details of overall analysis. The *y*-axis shows back-transformed values on a logarithmic scale.

DISCUSSION

Here we showed that the speed of stigma closing and duration of closure are variable in the outcrossing plant *Mimulus guttatus*. Almost all plants had stigmas that closed upon touch, and most reopened after several minutes. We also showed that stigmas that closed more rapidly stayed closed longer, suggesting a functional relation between these features. Interestingly, in our experiments, the speed of closure was not influenced by the application of pollen. However, stigmas stayed closed for significantly longer if they received pollen vs. mechanical touch alone. Furthermore, we showed that in all four selfing species (close relatives of *M. guttatus*), stigmas are less touch-responsive, with populations varying in whether they have lost the characteristic completely.

Populations differed significantly in both their speed of closure and the duration they stayed closed. We explored whether latitude or longitude of the populations correlated with these differences. Only longitude explained a significant amount of variation in the speed of stigma closure (Table 1; Fig. 3). Of course, longitude is a surrogate for any number of differences between populations that might vary clinally. We suspected the significant effect of longitude might reflect precipitation because water potential is known to affect other touch-sensitive responses that rely on cellular turgor pressure changes (Braam, 2005). However, none of the climate variables we tested, including precipitation, were associated with stigma closure. The population differences might reflect differences in pollinator abundance or community, or their foraging behavior.

Speed of response to touch sensitivity was variable in *M. guttatus*, but the ability to respond was ubiquitous. In contrast, many individuals in the selfing species appear to have lost their touch sensitivity. Whether plants received mechanical pressure or the application of pollen, they responded with the same rapidity of closing, similar to that found in *Oroxylum indicum* (Sritongchuay et al., 2009), but unlike *Campsis radicans* (Yang et al., 2004) or *Ipomopsis aggregata* (Waser and Fugate, 1986). In the latter two species, plants that received pollen closed more rapidly. Nonetheless, despite no difference in closing speed in our experiments, stigmas that received pollen remained closed for longer. The longer duration of closure with pollen appears to be ubiquitous among the various species that have been investigated. Overall, this result suggests that in *M. guttatus* the initial closing is a constitutive reaction to any mechanical pressure ("thigmotropism"), but then there is a biochemical or physiological interaction with the pollen (Heslop-Harrison and Heslop-Harrison, 1985; Edlund et al., 2004), so that stigmas open up differently depending on the presence of pollen. In some systems, where the amount of pollen was manipulated, the duration of closure (or whether stigmas reopen at all) depends on the quantity of pollen received (Waser and Fugate, 1986; Richardson, 2004; Yang et al., 2004), but in others the effect of pollen load did not alter the probability of stigmas reopening (Fetscher and Kohn, 1999; Sritongchuay et al., 2009).

Our experiments were not designed to distinguish between whether stigma closure acts to prevent the deposition of self pollen or to limit interference between stamens and stigma. Both of these mechanisms could be at play in the rapid closing time of stigmas, as they would prevent a bee from depositing self pollen on the stigma within a single flower visit, and they would also move the stigma out of the way for the bee as it exits the flower with pollen. However, the selective mechanism on extended duration of closure is less intuitive. In species in which stigmas remain closed after pollination, this closure provides a switch to exclusively male function. In *M. guttatus*, we have found that stigmas generally reopen, so it only provides an interval of male function, and a window in which selfing is prevented. Why might selection favor stigmas that remain closed for a short, but substantial, period of time (average in our study for plants that received pollen: 9 min)? At this point, we can only speculate, but there are several possible costs and benefits of extended closure. The most obvious cost is lost pollination opportunities for female function if another pollinator visits the flower while the stigma is closed, a possibility since sequential pollination has been shown in *M. guttatus* (Dudash and Ritland, 1991). One potential benefit is enhanced pollen export due to reduced anther–stigma interference. In *Mimulus arauntiacus*, researchers found that lost mating opportunities for female function were more than compensated for by male function though increased pollen export (Fetscher, 2001). In addition to this male function benefit, extended closure might also limit geitonogamous pollination if bees forage locally between flowers on the same plant, and visit the same flower twice. The closed stigma lobes might also provide a more favorable environment for pollen tube germination.

Previous research on *Mimulus guttatus* has shown that even with herkogamy and touch-sensitive stigmas, populations have an average of ~25% selfing, although this varies considerably by location and year (Ritland and Ganders, 1987; Dudash and Ritland, 1991; Willis, 1993). Despite strong inbreeding depression, self-pollination will be favorable if flowers have not been pollinated by the end of their life span. Dole (1990) suggests that as a flower ages and the corolla abscises, it drags the anthers over the stigma so that unfertilized ovules may be self-pollinated. Dudash and Ritland (1991) do not find evidence for corolla-dragging induced selfing and

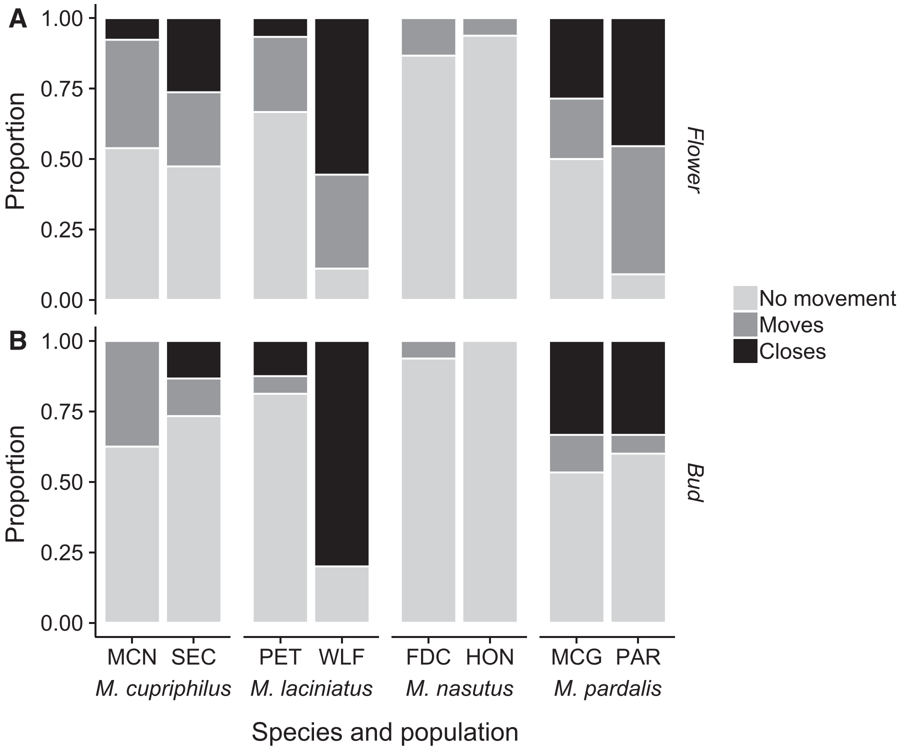

FIGURE 5 Proportion of stigmas that responded to touch for four selfing species (*M. cupriphilus*, *M. laciniatus*, *M. nasutus*, *M. pardalis*), each with two populations. Movement was scored categorically as no movement (light gray), some movement (dark gray), and stigma closed (black). Top panel shows stigmas that were tested in open flowers; bottom panel shows stigmas that were tested in dissected buds.

instead show that the amount of selfing is consistent across the life span of a flower. They suggest that selfing occurs during a pollinator visit or by mating with related plants nearby. The former would imply that touch-sensitive stigmas do not completely prevent facilitated selfing. A study by Leclerc-Potvin and Ritland (1994) showed that biparental inbreeding may account for 20–40% of the selfing experienced by a plant and that geitonogamous selfing between flowers on the same plant might also be common. Neither of these forms of selfing can be prevented by touch-sensitive stigmas, a limitation that was first pointed out by Burck (1902).

Surprisingly, we found that most of the measures of flower size were uncorrelated with touch sensitivity. The only morphological measurement that was associated was anther–stigma separation. The closer the anthers and stigmas were, the slower the stigma closed. A reduction in anther–stigma separation has previously been characterized as a key trait associated with the transition to selfing in *M. guttatus* lineages (Dole, 1992; Bodbyl Roels and Kelly, 2011; Fishman et al., 2002). A reduction in anther–stigma separation facilitates direct contact between the female and male organs so that selfing can be achieved autonomously (Dole, 1992). That we found this trait also to be associated with stigma-closing speed further supports the evidence that stigma closing functions to prevent selfing. Because we designed our experiment to identify population differences, we did not have the statistical power within families to determine whether this association between anther–stigma separation and closing time represented a genetic correlation. Nonetheless, we expect that if selection favors selfing and a reduction in anther–stigma separation (Bodbyl Roels and Kelly, 2011), then mutations that hamper stigma closing might also be favored.

The loss of touch sensitivity in selfing lineages adds to the suite of traits that are involved in a generalized selfing syndrome (Ornduff, 1969). Either function of touch sensitivity—preventing selfing or avoiding pollen–stigma interference—would be superfluous for selfing taxa. The loss of touch sensitivity in the selfing species suggests that stigma closure does not act to facilitate pollen germination, as this would still be necessary in selfers. We found species and populations were variable in whether they had lost touch sensitivity completely. This variation suggests that selection is relaxed in terms of maintaining the trait, but not strong enough to remove it altogether. To better understand the loss of touch sensitivity in selfing lineages, further studies could employ a QTL mapping approach. Because these species are all interfertile, a mapping population using crosses between *M. guttatus* and one or more of the selfing species could be used to identify the genomic basis of touch sensitivity.

The maintenance of touch-sensitive stigmas in the predominantly outcrossing *M. guttatus* suggests that this trait is under selection. The significant association between closing speed and geographic location suggests that selection is variable and might depend on other aspects of the biotic or abiotic environment. Interestingly, we found a negative association between anther–stigma separation and closing speed in annual populations only. The four selfing species we investigated are each derived from annual ancestors and have much reduced anther–stigma separation. In these species, the ability to respond to touch is variable and, in some cases, lost entirely. We suggest that the variation in touch response seen within *Mimulus guttatus* and its close relatives could be used to ask whether touch sensitivity correlates with outcrossing success in a natural pollination environment.

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