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MODES OF SELF-FERTILIZATION IN *MIMULUS GUTTATUS* (SCROPHULARIACEAE): A FIELD EXPERIMENT[1]

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We inferred Lloyd's modes of selfing in a natural population of the common monkeyflower, *Mimulus guttatus*. Estimates were obtained using floral manipulations combined with seed counts and isozyme analyses of selfing rates. Of the 25% selfing estimated from isozyme markers, about one-half was competing, about one-third was geitonogamous, and at least one-fifth (perhaps twice this) was due to biparental inbreeding. Estimates of prior and delayed selfing were small and did not significantly differ from zero. These results were obtained using plants with the characteristic pair of open flowers at an inflorescence node. The second-opening flower showed twice the rate of selfing, presumably because of protogynous-based geitonogamy differences. Solitary-flowered plants, which have smaller flowers but no geitonogamy, showed about 50% selfing, consisting of about equal components of competing selfing and biparental inbreeding. While geitonogamy and biparental inbreeding might be unavoidable by-products of adaptations for outcrossing, competing selfing is subject to more direct natural selection and warrants adaptive explanations.

Partial self-fertilization, wherein a certain fraction of progeny are produced by self-pollination as opposed to outcrossing, is a common reproductive strategy in angiosperms (Barrett and Eckert, 1990). While self-fertilization provides assured seed reproduction without pollinators (Darwin, 1862), it can entail the cost of inbreeding depression (Charlesworth and Charlesworth, 1987). Intermediate selfing rates may be advantageous under some patterns of inbreeding depression (Maynard-Smith, 1977; Latta and Ritland, in press), population structure (Holsinger, 1986), or biparental inbreeding (Uyenoyama, 1986). Functional factors, including unavoidable selfing as a by-product of adaptations for outcrossing, may also favor intermediate selfing rates (Lloyd, 1992).

The evolutionary and adaptive basis of selfing may be better understood if we identify the specific "mode" of selfing (Lloyd, 1979) practiced by a species. Lloyd and Schoen (1992) distinguished six modes of self-pollination. The two most well-recognized modes are cleistogamy, or selfing occurring in closed flowers structurally specialized for self-fertilization; and geitonogamy, or selfing caused by transfer of pollen between flowers of the same plant. Three additional modes of selfing involve transfer of pollen *within* a flower, without the participation of pollinators. These modes are "prior selfing," or selfing preceding an outcrossing opportunity; "competing selfing," or selfing coinciding with an outcrossing opportunity; and "delayed selfing," or selfing occurring after an outcrossing opportunity. These three modes are all components of "autonomous selfing," and contrast with the sixth mode, termed "facilitated selfing," which is selfing caused by the action of pollinator vectors (see Lloyd and Schoen, 1992).

Inferences about modes of selfing have been classically based upon comparative and correlative studies. Active experimentation, particularly in the field, with treatments designed to elucidate the modes of selfing, has been conspicuously absent. In addition, the use of genetic markers to measure the selfing rate after performing floral manipulations is only very recent (Dudash and Ritland, 1991; Kohn and Barrett, 1992; Schoen and Lloyd, 1992).

Mimulus guttatus Fischer ex DC (Scrophulariaceae), also known as yellow monkeyflower, is an herbaceous annual and perennial plant that occurs throughout western North America in wet, semi-dry meadows and along small streams. This species is at the center of an actively evolving species complex (Vickery, 1964, 1978). Pennell (1951) recognized 21 species in this complex, while Campbell (1950) recognized four species, classifying the others as varieties of *M. guttatus*. Regardless, *M. guttatus* has at least three, independently derived selfing relatives: *M. platycalyx, M. micranthus,* and *M. laciniatus* (Ritland and Ritland, 1989).

Mimulus guttatus produces pairs of showy zygomorphic yellow flowers in sequential progression up flowering stems; in most populations, only one pair of flowers is open at any one time on the flowering stem. The corolla is strongly bilabiate; its throat, usually exserted, opens with a zone of trichomes. The four filaments are all antheriferous, didynamous, and epipetalous. The stigma of *M. guttatus* is described as being "sensitive." Upon touch, the two stigmatic lobes clasp together within about 6 sec (Kerner, 1985; Ritland and Ritland, 1989). The flower is bee-pollinated (Kiang, 1972), but Macnair, Macnair, and Martin (1989) also reported that various Diptera and certain species of butterflies contribute to pollination. The selfing rate of *M. guttatus* averages 52% (range 21%–75%) over 14 populations (Ritland, 1990a).

Greenhouse studies (Dole, 1990, 1992) have indicated the presence of delayed selfing in this species, but a field study (Dudash and Ritland, 1991) found no evidence of its presence. The other modes of selfing have not been studied in this species. The large, showy flower of *M. guttatus* and previous estimates of outcrossing in the species clearly rule out the presence of cleistogamy. Furthermore, facilitated selfing seems unlikely, since in species with sensitive stigmas, stigma contact of the pollinator

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probably precedes pollen contact (Lloyd and Schoen, 1992).

The purpose of our study was therefore to estimate the proportion of four potential modes of autonomous self-pollination in *M. guttatus*: prior, geitonogamous, competing, and delayed. We used a more detailed experimental design than Dudash and Ritland (1991) to study these additional modes of selfing. The experiment was undertaken in a natural field setting with techniques chosen to minimize disturbance to plants and their pollinators. Because we studied selfing in a natural population with isozyme markers, we also estimate the extent that biparental inbreeding (mating between relatives) contributes to the measured selfing rate. Identification of the modes of selfing should help illuminate factors underlying the evolution of mixed-mating in *M. guttatus*.

MATERIALS AND METHODS

The study site was located in Washington Park, located 1 km SW of Anacortes, Washington. This site was a semi-wet meadow, approximately 35 m × 15 m in total. The meadow was surrounded on three sides (east, west, and south) by a band of conifer and deciduous forest, while the north side was delimited by a hiking trail. The terrain had a gentle slope, and five large patches (at least 500 plants in each patch) of *M. guttatus* could be identified: a north patch, a southwest patch, a southeast patch, and two central south patches.

Several manipulations of the flower, described below, were designed to isolate components of selfing. For all treatments, the initial step consisted of gently stimulating the stigmatic lobes with a clean toothpick to prevent the occurrence of accidental selfing. Specific treatments were then applied and corolla widths also measured, then plants left to produce seeds. Corollas were tagged with numbered white tape at the base of their peduncles, and plants flagged with small kabob sticks flagged with colored tape. All treatments took place simultaneously, so that variations of weather did not confound treatments. All plants had a single inflorescence with only the two flowers at a single node open during the experiment. The experiment was performed in early May 1992. Capsules containing seed were collected after 3½ wk, brought back to Toronto, the seed counted, and selfing rates estimated from germinated seed through isozyme markers.

To estimate seed number, a small container with a 7 cm × 7.5 cm rectangle drawn at the bottom was designed. Seven smaller squares of 1 cm × 1 cm were randomly drawn within the larger rectangle, each capsule individually crushed, and seeds spread evenly within the larger rectangle with a pasteur pipette. The total number of seeds within the smaller squares was counted and multiplied by a conversion factor. To establish the validity of the estimation procedure, seed number in each of 20 capsules was estimated as described above, then recounted exactly. We found the standard deviation of these estimated seed counts to be within 10% of their true values.

For isozyme electrophoresis, seeds were germinated and cotyledons extracted with a *Mimulus* grinding buffer composed of the following ingredients: 0.05 M Na_2HPO_4 at pH 7.0, one drop of Tween-80 per 10 mL of grinding buffer, 4.15 mg BSA per 10 mL of grinding buffer, 0.023 M DIECA, 0.013 M DTT, 4.57 mM EDTA at pH 7.0, 0.82 M sucrose, and 5.25 mM PVP-40. Using 10% starch/10% sucrose gels (1:20 dilution) and an electrode buffer of 0.04 M citric acid monohydrate with N-(3-aminopropyl)-morpholine added to pH 6.1, the following four polymorphic enzyme systems were assayed: diaphorase (DIA), phosphoglucoisomerase (PGI), malate dehydrogenase (MDH), 6-phosphogluconic dehydrogenase (6PGD, 2 loci). In addition, using 11% starch/5% sucrose gels and an electrode buffer of 0.2 M boric acid with 0.04 M lithium hydroxide at pH 8.3 (gel buffer: 0.03 M anhydrous citric acid, 0.2 M Tris-HCl, 1% electrode buffer), the following two polymorphic enzyme systems: glutamate oxaloacetate transaminase (GOT, 2 loci) and triose phosphate isomerase (TPI).

Among all experimental treatments and controls, 4,070 seedlings were assayed and scored for the eight polymorphic isozyme loci. Specific sample sizes are given in the tables of results; for a given treatment, the number of families was usually 20 (or slightly below, due to loss of plants to herbivores or unknown causes), and the number of progeny per family was usually 24–30 (giving total sample sizes of ca. 300), but occasionally more progeny per family were assayed if the number of families was less than 20 due to failures of seed set.

Data were grouped by families, and multilocus outcrossing rates were found with two programs, MLT and ML2T (Ritland, 1990b). MLT uses maximum likelihood to estimate selfing rate and pollen gene frequencies with progeny arrays censused for several unlinked marker loci. ML2T is a modification of MLT. It estimates selfing rates for two groups, and assumes pollen frequencies are the same among groups. This program is appropriate for experimental manipulations involving one treatment and one control. The bootstrap method (Ritland, 1990b) was used to determine standard errors of selfing rates and their differences.

Prior selfing—To determine the extent of selfing before corolla unfurling, 40 parental plants in the north patch were chosen wherein on each plant, a pair of flowers at a node was at the "furled" stage, ready to open the following day. Two experiments were then conducted: 1) on each of 20 plants, one corolla was removed at this furled stage, the other flower serving as an unmanipulated control; 2) on the other 20 plants, the stigma was removed on the unfurled flower, the other flower again serving as a control.

Components of competing selfing—To determine the extent of geitonogamy (mating between flowers of the same plant) and autonomous selfing (mating within a flower), 30 randomly chosen plants with one pair of furled flowers at a node were chosen. Plants were located in the southeast, southwest, and one of the central south patches. Two experiments were then conducted: 1) in 15 plants, the anthers of one corolla were removed prior to corolla unfurling, and the other flower left unmanipulated as a control; 2) on the other 15 plants, both flowers were left unmanipulated, but the day of corolla unfurling was noted (in *M. guttatus,* anthesis of the pair of flowers at a node is not strictly synchronous; often their openings are staggered by about 1 d). In these plants, the first opened flower was tagged "Day 1" and the flower that opened the fol-

TABLE 1. Gene frequencies at loci used for estimation of selfing rates. All loci were diallelic except mdh-4, which was triallelic.

Locus	Gene frequency (SE)
6pgd-1	0.947 (0.024)
6pgd-2	0.749 (0.048)
pgi-3	0.742 (0.052)
mdh-4 (allele 1)	0.697 (0.049)
mdh-4 (allele 2)	0.268 (0.046)
tpi-2	0.681 (0.043)
got-2	0.946 (0.019)
got-3	0.610 (0.041)
dia-2	0.325 (0.059)

lowing day was tagged "Day 2." These treatments do not distinguish competing selfing from facilitated selfing, but the latter is assumed not to be present based upon observations of floral biology (see Discussion).

Biparental inbreeding—Biparental inbreeding was indirectly estimated as the difference between single-locus and multilocus estimates of selfing. A positive difference indicates a minimum value of biparental inbreeding (Ritland and Jain, 1981). The bootstrap method (Ritland, 1990b) was used to assign the confidence interval upon this difference of selfing rates.

Delayed selfing—To determine the extent of selfing occurring during corolla abscission, 40 randomly chosen parental plants in the north and central south patches were chosen. Each plant had one pair of flowers at a node in the furled stage. Two experiments were performed: 1) in 20 plants, the corolla was removed after the second day of flowering, the other unmanipulated flower serving as a control; 2) in the other 20 plants, the stigma was removed from one flower after the second day, and the other flower left undisturbed. The treatments of both experiments were designed to prevent mating after the second day.

Components of selfing in solitary flowered plants—In plants producing just one flower, we estimated rates of biparental inbreeding and autonomous selfing as follows. Thirty solitary flowered plants were located in the southwest and central south patches. On 15 of these plants, anthers were removed before the corolla unfurling. The control for this treatment consisted of the remaining 15 undisturbed solitary flowered plants. The difference of selfing between treatment and control represents autonomous selfing, while the selfing rate of treated plants represents biparental inbreeding.

TABLE 2. Estimates of prior selfing. In experiment A, the stigma was removed prior to opening; in experiment B, the corolla (including anthers) was removed. In each experiment, the second flower at the node was the unmanipulated control.

	No. parents	No. progeny assayed	Seeds per capsule (SE)	Selfing rate (SE)
A. Stigma removal experiment				
Treated	20	0	0	—
Control	20	0	384 (65)	—
Treatment effect			−384 (65)	—
B. Corolla removal experiment				
Treated	20	178	12 (6)	0.141 (0.074)
Control	20	461	331 (52)	0.209 (0.084)
Treatment effect			−319 (52)	−0.067 (0.127)

TABLE 3. Estimates of competing selfing. In experiment A, the anthers were removed on the treated flower and left alone on the control flower. Components of competing selfing were obtained by comparing their selfing rates with selfing rates of flowers at undisturbed nodes. In experiment B, flowers at the same node that opened on staggered days were noted, and the selfing rates of these flowers estimated.

	No. parents	No. progeny assayed	Seeds per capsule (SE)	Selfing rate (SE)
A. Anther removal experiment				
Treated	14	310	108 (21)	0.135 (0.095)
Control	14	322	170 (30)	0.172 (0.087)
Undisturbed	26	594	243 (30)	0.251 (0.094)
Autonomous selfing (undisturbed *minus* treated)				0.116 (0.067)
Geitonogamy (undisturbed *minus* control)				0.079 (0.064)
B. Effect of asynchrony				
Open day 1	13	282	242 (47)	0.163 (0.108)
Open day 2	13	312	244 (36)	0.339 (0.079)
Difference			2 (60)	−0.176 (0.098)

RESULTS

Table 1 gives the gene frequencies, averaged over experiments, at isozyme loci used to estimate selfing rates. On average, most frequencies were in the range that provide good information about selfing ($0.10 < P < 0.90$). However, due to microsite variations in gene frequency, not all loci were sufficiently variable in most experiments; usually five or six loci were used for a given experiment.

Table 2 gives estimates of the extent of prior selfing. The elimination of mating after the furled corolla stage had a very significant impact on seed production: flowers that had their stigma removed at the furled stage produced no seeds, while flowers that had their corolla removed produced only 12 seeds on average, compared to 331 for the unmanipulated flowers (Table 2, A). Thus, most or all matings take place after the opening of the corolla. Among the few seeds produced, the selfing rate was 0.14, compared to the selfing rate of 0.21 for unmanipulated flowers (Table 2, B). These rates do not significantly differ, further ruling out prior selfing.

Table 3, part A, gives estimates of the two components of competing selfing (geitonogamy and autonomous selfing). Undisturbed plants had a selfing rate of 25.1%. When autonomous selfing was excluded by removal of anthers, the selfing rate decreased from 25.1% to 13.5%, indicating 11.6% selfing due to stigmas receiving pollen from the same flower. Examination of the distribution of bootstrap estimates shows this difference is marginally significant ($P < 0.10$). When geitonogamy was excluded by removal of anthers on the opposite flower at a node, the selfing rate significantly decreased from 25.1% to 17.2%. This indicates a selfing rate of 7.9% due to stigmas receiving pollen from other flowers on the same plant. Removal of anthers reduced seed set by about 50% (243 to 108, Table 3, A), while removal of anthers on the other flower of a

TABLE 4. Estimates of the minimum selfing due to biparental inbreeding, by comparison of single-locus outcrossing rate with multilocus outcrossing rate.

No. of parents	85
No. of progeny	2,344
t_m (SE)	0.726 (0.037)
t_s (SE)	0.669 (0.042)
$t_m - t_s$(SE)	0.057 (0.015)

node reduced seed set by about 30% (243 to 170, Table 3, A). These differences are in accord with the observed changes of selfing; reduction in seed set accompanies a reduced selfing rate, indicating removal of a mating component.

Table 3, B, shows that the selfing rate exhibited by a flower depends greatly upon whether it opens before vs. after the opposite flower of that node. Flowers that open second, as opposed to first, show a significant 17.6% higher rate of selfing. This is presumably due to differences in the rate of geitonogamy, as discussed below. Nevertheless, the seed set of first vs. second flowers does not differ (Table 3, B).

An estimate of the minimum value of biparental inbreeding is given in Table 4. Again, all control flowers were pooled for this analysis, giving a total of 85 control flowers with 2,344 progeny used for the estimate. Subtraction of the single-locus outcrossing rate from the multilocus outcrossing rate gives a minimum estimate of biparental inbreeding of 5.7%.

Table 5 gives estimates of matings that occur after the second day in the life of the flower, after which delayed selfing can potentially occur. Removal of the corolla after 2 d of flowering did not result in significantly different selfing rates shown in the seed progeny (Table 5, A). Likewise, removal of the stigma after 2 d also did not appreciably affect the selfing rate (Table 5, B). Thus, there is no change of the selfing rate before vs. after 2 d, and delayed selfing is evidently not present. In both experiments, unmanipulated flowers produced three to five times more seeds than the treated flowers (Table 6), indicating that two-thirds to four-fifths of the matings took place after 2 d.

TABLE 5. Estimates of delayed selfing, detected by restricting mates after the second day in the life of the flower. In experiment A, the corolla was removed from one of the two flowers at a node. In experiment B, the stigma was removed on one of the two flowers at a node. In both experiments, the second flower at the node served as an unmanipulated control.

	No. parents	No. progeny assayed	Seeds per capsule (SE)	Selfing rate (SE)
	A. Corolla removed			
Treated	15	334	117 (35)	0.243 (0.093)
Control	15	337	337 (54)	0.140 (0.089)
Treatment effect			−220 (65)	0.103 (0.106)
	B. Stigma removed			
Treated	14	255	43 (14)	0.322 (0.096)
Control	14	290	225 (40)	0.255 (0.120)
Treatment effect			−182 (42)	0.067 (0.120)

TABLE 6. Estimates of autonomous selfing in solitary-flowered plants. Treated plants had anthers removed prior to corolla unfurling; controls were undisturbed.

	No. parents	No. progeny assayed	Seeds per capsule (SE)	Selfing rate (SE)
Treated	12	329	104 (22)	0.287 (0.097)
Control	12	340	116 (28)	0.641 (0.107)
Treatment effect			12 (36)	−0.353 (0.138)

Table 6 provides estimates of selfing modes in solitary flowered plants, wherein geitonogamy cannot occur. The selfing rate of unmanipulated plants was 0.641, while the selfing rate of flowers with anthers removed was reduced to 0.287. This change of 35.3% is the fraction of autonomous selfing, but is only representative of solitary flowered plants. The seed set was not affected by this treatment (Table 6). The biparental inbreeding rate equals the selfing rate of treated plants (0.287). Solitary-flowered plants also had a significantly smaller flowers; the mean corolla width for these plants was 1.71 cm (0.10 SE), while the mean corolla width for plants with more than one flower was 2.36 cm (0.05 SE).

DISCUSSION

From the floral manipulations performed in this study, we can infer that among *Mimulus guttatus* plants having the characteristic pair of open flowers, about one-half of the observed 25% selfing is due to autonomous selfing (pollination by anthers of the same flower), about one-third of the 25% selfing is due to geitonogamy (mating to the opposite flower at the node), and at least one-fifth of the 25% selfing is due to biparental inbreeding (mating among relatives). Selfing rate varied greatly between the two flowers of a node, with the second-opening flower showing twice the rate of selfing. Despite their absence of geitonogamy, solitary-flowered plants show roughly twice the selfing rate of plants with more flowers. About half of this selfing was inferred to be autonomous selfing, and about half due to biparental inbreeding.

Potential artifacts—Artifacts may have been introduced by our manipulations, primarily by removal of signals or rewards for pollinators (Schoen and Lloyd, 1992). Our manipulations have involved either removal of the stigma or the corolla, to which the anthers are attached. We cannot conceive of any role the stigma of *Mimulus* plays in pollinator attraction, but its removal may interfere with fertilization by developing pollen tubes and hence prevent the success of recent matings. Removal of the corolla was assumed by us to prevent further pollinator visitation and male function. However, small amounts of further seed set indicate that stigmas receive some pollen (Table 2), either by the act of corolla removal, by wind transport of pollen, or by the action of crawling insects carrying pollen.

In *Mimulus guttatus,* a feature of the floral biology we have used is the presence of paired flowers. Their flowering times are closely synchronized, and unless the plant has multiple inflorescences or has cloned rhizomatically, their

flowering period does not overlap those of other flowers on the same plant. The study population did not contain many multibranched plants (less than 1%), and was annual and hence did not contain rhizomatous clones. This is characteristic of populations in shallow soiled wet meadows in many West Coast states and British Columbia, and the proportions of selfing modes inferred in our study are probably representative of these populations.

However, populations along streams and perennial seeps have large proportions of multibranched plants and are often perennials with clusters of cloned plants. The results from our study thus cannot be extended to these populations. For example, a high selfing rate of 60% occurred in one such population (Wreck Beach, University of British Columbia, Ritland and Ganders, 1987). Mating among members of the same clone is probably responsible for this increased selfing, which further study needs to document.

Statistical power—One drawback emphasized by this study is the relatively low statistical power to detect differences of mating system among experimental treatments. Treatments may create differences of selfing on the order of 5%, yet unless sample sizes are unreasonably large, the standard errors of selfing estimates are of the same magnitude, precluding detection of treatment effect. One avenue to increase the precision of estimates is to use additional marker loci for selfing estimates, either through the use of more highly polymorphic populations, or additional marker loci.

A second avenue, only used in designed garden experiments (e.g., Kohn and Barrett, 1992), is to preferentially use parents that are homozygous for polymorphic marker loci. The information about mating events in the progeny of such parents is several times that of heterozygous parents (Ritland, unpublished data). In a field experiment, it would be most practical to conduct treatments on a large number of parents, then through a two-stage assay procedure, assay progeny arrays of only the more homozygous parents. However, one would have to document that the level of homozygosity does not influence the mating system.

Competing or facilitated selfing?—If autogamous selfing is mediated by insects, it is termed "facilitated." If it occurs in the absence of pollinators, it is termed "competing." Lloyd (1992) states that "facilitated selfing is almost impossible to eliminate entirely unless there is a mechanism that ensures that stigma contacts in a flower strictly precede pollen contacts, as in . . . species with sensitive stigmas." Because of the sensitive stigma of *M. guttatus* and its position above the anthers, we have assumed that facilitated selfing is not present in *M. guttatus.* However, this mechanism may not be perfect, and a future study is needed to test the presence of facilitated selfing in *M. guttatus,* perhaps by excluding pollinators during flowering (see Schoen and Lloyd, 1992).

Prior selfing—Both seed set and selfing rate data (Table 2) demonstrate that this mode of selfing does not contribute to self-fertilization in *M. guttatus.* While this is totally expected for a protogynous species such as *M. guttatus,* our observations suggest that three selfing relatives, *M. micranthus, M. platycalyx,* and *M. laciniatus,* could employ prior selfing, so that prior selfing could not be unambiguously ruled out prior to our study.

Biparental inbreeding—Among nonsolitary flowered plants, biparental inbreeding apparently caused at least 0.057/0.251 or about 20% of the observed selfing. The quantity 0.057 was obtained by subtraction of the single-locus estimate from the multilocus estimate of selfing. However, computer simulations indicate that with six loci, this will underestimate the true biparental inbreeding rate by up to 50% (Ritland, unpublished data). This is because the multilocus estimate is also affected by biparental inbreeding, with bias removed quite slowly with additional marker loci (the single-locus estimate accurately captures all apparent selfing due to biparental inbreeding, providing there is no correlation of biparental inbreeding with parental F; Ritland, 1984). Thus, the proportion of selfing due to biparental inbreeding probably lies within the 20%–40% range, which is closer to the 50% proportion of biparental inbreeding directly estimated in the solitary flowered plants (Table 6).

Although biparental inbreeding is a very significant portion of the total inbreeding practiced by *M. guttatus,* it cannot at present be estimated separately from true selfing in an unbiased way. The method of Waller and Knight (1989) for estimating the "biparental genetic correlation between truely outcrossed mates" (termed m_b) also underestimates biparental inbreeding. They had to assume no biparental inbreeding when estimating the multilocus (actual) selfing rate (Waller and Knight, 1989, p. 865), which is essentially the assumption we used that lead to the bias. More explicit models and methods are needed for estimating biparental inbreeding in partially selfing populations.

Delayed selfing—Delayed selfing in *M. guttatus* is thought to occur when the corolla drops after anthesis, dragging the stamens across the stigma (Griffiths, Carey, and Ganders, 1981; Dole, 1990, 1992). There was no evidence for delayed selfing occurring through "corolla dragging" in this population of *M. guttatus.* We tested this in two experiments, one involving corolla removal, the other involving stigma removal. In both, the resulting selfing rates shown by seed progeny were actually slightly higher in the treated flowers (the reverse expectation), compared to the control flowers. Thus, there was no increase in the selfing rates through the life span of the flower (if anything, there was a decrease). Because it is clearly impossible to affect self-pollination by the act of stigma removal, and because the same results were obtained in both treatments, we also conclude that the act of corolla removal does not cause any accidental selfing.

These results are in accord with Dudash and Ritland (1991) but conflict with Dole (1990, 1992). Dole found that "corolla dragging" was an important mechanism used for selfing in a different population of *M. guttatus.* By gluing corollas onto calyxes (preventing their abscission) in a pollinator-free greenhouse, he found that those flowers set fewer seed than undisturbed flowers, attributing it to "corolla dragging." Dole's conflicting results could either be caused by the artificial conditions of his treatments and the greenhouse environment, or as he suggests, by

the potential for populations of *M. guttatus* to vary in the degree of delayed self-pollination through corolla abscission.

Among-flower variation of selfing—An intriguing new facet of the mating system of *M. guttatus* we discovered was the difference of selfing rate between the two flowers of a node: the second-opening flower on a node had twice the selfing rate of the first. There are two possible explanations for this. First, there could be a decrease in the frequency of visitation by the pollinators when two flowers are open. However, we found no significant difference in the mean number of seeds produced by both flowers, as would be expected if the second-opening flower incurred reduced visitation. A second explanation is that there is microtemporal variation in gender over the span of 1–2 d. The flower of *M. guttatus* exhibits protogynous characteristics, wherein the stigma matures before the stamens. If flowers are predominantly female in function for the first 1–2 d, then predominantly male in function for the remaining time, then geitonogamous selfing would be more frequent in the second-opening flower.

Temporal differences in the functioning of male and female organs of flowering plants are a common feature of monoecious and andromonoecious taxa as reported by Thomson and Barrett (1981). Temporal variation of gender in hermaphroditic flowers is much harder to establish since estimated values for functional gender are based on the total production of male and female flowers during the flowering season (Lloyd, 1980). Confirmation of temporal gender variation in individual hermaphroditic flowers of *M. guttatus* would require studies of pollen removal, transport, and fertilization rates. In addition, floral developmental studies of *M. guttatus* would help substantiate this hypothesis.

Solitary-flowered plants—The selfing rate of solitary-flowered plants was twice that of plants with more flowers, and their corolla size 25% smaller. In these plants, it is not known if the stigma-anther separation is less, predisposing them to more autonomous competing selfing, or if pollinator visitation is lower because of lower attraction and rewards and/or the absence of a second flower. Further study is needed.

Solitary flowered plants also produced fewer seeds per capsule compared to multiflowered plants (Table 6 vs. Tables 2–5). This is in accord with the suggested genetic basis of Vickery (1978), who observed a wide variation in seed set within populations of *M. guttatus* complex and suggested a genetic basis for this variation. The genetic basis expressed itself as a general size relationship in which tall, vigorous plants with large flowers produced more pollen and ovules, and hence more seeds, than small plants.

Conclusion—We note that no previous experimental attempts have been made to determine the relative importance of the three modes of selfing—prior, competing, and delayed—in any species (Lloyd and Schoen, 1992), in part because of the lack of appropriate procedures for assessing their importance (Schoen and Lloyd, 1992). This study has illustrated how these new procedures for studying self-fertilization involve a combination of floral manipulation, often unique to the floral biology of the study organism (Schoen and Lloyd, 1992), counts of seed production, and marker-based estimation of mating system (Ritland, 1990b).

Geitonogamy is probably a by-product of adaptations for outcrossing (Lloyd, 1992), and biparental inbreeding is an unavoidable consequence of population structure. By contrast, the presence of significant levels of competing selfing in *M. guttatus* warrants an adaptive explanation, given the high levels of viability inbreeding depression measured in this species (Dole and Ritland, 1993) and the sensitive stigmas of *Mimulus* (Ritland and Ritland, 1989). If this mode of selfing is under direct genetic control, through the degree of protogyny and the structural placements of stigmas and anthers, then the fitness consequences of partial selfing must be beneficial to the overall fitness of plants practicing partial self-fertilization. Indeed, Table 3A indicates that the seed set in control flowers is almost 60% greater than that of treated flowers (where competing selfing was prevented). This fecundity advantage of partial selfing, if a general feature of *M. guttatus,* may be sufficient to overcome a large disadvantage in viability inbreeding depression.

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