Multiple Paternity and Self-Fertilization in Relation to Floral Age in Mimulus guttatus (Scrophulariaceae)

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MULTIPLE PATERNITY AND SELF-FERTILIZATION IN RELATION TO FLORAL AGE IN MIMULUS GUTTATUS (SCROPHULARIACEAE)[1]

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To gain some understanding of the mechanisms responsible for the intermediate levels of both multiple paternity and selfing observed previously in the common monkeyflower, *Mimulus guttatus,* we performed a field experiment to assess the role of 1) multiple paternity through sequential pollinator visits and 2) selfing through corolla abscission. In *M. guttatus,* flowers remain open for several days; then, in the process of corolla abscission anthers drag past the stigma. We predicted that multiple visitation by pollinators over this period should increase the degree of multiple paternity of sibling arrays, and that corolla dragging may be the primary cause of the observed selfing. In three northern California populations corollas were removed after being open for 1 day (preventing further mating), and the type of matings resulting was compared to matings from adjacent flowers with undisturbed corollas. On average, seed set was twice as great in flowers with unmanipulated corollas, indicating that about half of all matings in the lifespan of a *M. guttatus* flower occur after 1 day. Pollen supplementation revealed that pollen was limiting in one population. Electrophoretic assay of progeny showed multiple paternity was greater in unmanipulated flowers in the two populations that were not pollen limited, suggesting that sequential pollination may be an important source of multiple paternity. However, no difference was detected in the selfing rate between the two treatments, suggesting that corolla dragging was not a source of selfing even under pollen-limited conditions.

If we are to understand the selective forces governing the evolution of plant mating systems, experimental studies involving manipulative changes of the mating system at a fine scale are needed. In plant population biology, two of the major parameters of the mating system currently of interest are the selfing rate and the multiplicity of paternity (or correlation of paternity) among outcrossed siblings. The selfing rate and the correlation of paternity can be readily estimated by quantifying allozyme variation among progeny within a fruit via starch gel electrophoresis. In the common monkeyflower, *Mimulus guttatus,* intermediate levels of selfing (ca. 20%–40%, Ritland, 1990) and correlated paternity (ca. 20%–40%, Ritland, 1989) are characteristic. In this field study, we investigate some factors that may modulate such mixed-mating systems.

Multiple paternity can occur by the depo-

sition of a mixed-pollen load by a single pollinator (Marshall and Ellstrand, 1985; Epperson and Clegg, 1987), by sequential visits by several pollinators each carrying pollen from a single male, or by sequential visits by several pollinators each carrying mixed-pollen loads. The extent and mechanism(s) responsible for multiple paternity have potential implications for sexual selection (Haldane, 1924; Hamilton, 1964; Janzen, 1977; Kress, 1981; Queller, 1983; Ellstrand, 1984), offspring quality (Marshall and Ellstrand, 1986; Marshall and Whittaker, 1989; but contrast Snow, 1990), mating system estimation (Schoen and Clegg, 1986; Ritland, 1989), and measurement of gene flow (Epperson and Clegg, 1987). The mechanism(s) responsible for multiple paternity may also help explain the observed variation among taxa in the lifespan of individual flowers, renew the importance of variation in pollinator visitation rates throughout the season, and the time interval between pollinator visits to a given flower. Thus, the mechanism(s) responsible for multiple paternity within a fruit could ultimately contribute to variation in reproductive fitness among individuals within a population.

Previous studies of multiple paternity have found it to be predominantly caused by deposition of mixed-pollen loads by the first pollinator visit (*Raphanus sativus,* Marshall and Ellstrand, 1985; *Ipomoea purpurea,* Epperson

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and Clegg, 1987), which places importance on "pollen primacy" (i.e., the fertilization rate advantage of earlier deposited pollen). However, both of these species produce relatively few seeds per fruit (less than ten), and in species with many seeds per fruit, sequential pollination may play a larger role in determining the paternity of seeds. Pistils of *M. guttatus* contain close to 1,000 ovules (Ritland and Ritland, 1989), of which 10%–50% ultimately develop into seeds, indicating a potential role of sequential pollination in this species.

A variety of floral mechanisms may be associated with self-fertilization (e.g., Kerner, 1895; Lloyd, 1979). Lloyd (1979) coined the term "delayed selfing" as the condition wherein during the first part of a flower's lifespan, predominant outcrossing occurs to the available mate pool. Then, if not all ovules are fertilized, those remaining are self-fertilized. In *M. guttatus*, this delayed selfing mechanism has been postulated to be corolla abscission (Ritland, 1989; Dole, 1990). Self-pollination may occur as the anthers are dragged over the bilabiate stigma because the stamens are attached to the corolla tube. The considerable amount of selfing previously documented in *M. guttatus* (Kiang, 1972; Ritland and Ganders, 1987; Ritland, 1990) may be due at least in part to this delayed selfing mechanism, besides pollinator behavior, and individual flowering phenology.

Thus, we address two primary questions in an experimental field study involving three populations of *M. guttatus*: 1) does multiple paternity increase as flowers age, as expected with sequential pollinator visitation; and 2) does the selfing rate increase as flowers age, as expected from corolla abscission? We also estimate the level of pollen limitation in each study population by comparing the seed produced by supplemental outcrossed pollinations to normal, open-pollination. We expect that in pollen-limited populations multiple paternity and levels of outcrossing may be lower, whereas the delayed selfing mechanism may play a greater role in seed production. Few studies have linked pollen limitation to mating system parameters, particularly in a species with many-seeded fruits.

MATERIALS AND METHODS

Mimulus guttatus (Scrophulariaceae), the common yellow monkeyflower, has large, bee-pollinated flowers and is a herbaceous annual (this study) or perennial that occurs throughout western North America in wet meadows and small streams. It produces one pair of flowers at each node of the flowering stem, which open at similar times. Individuals commonly produce at least two dozen flowers. This inflorescence architecture is ideally suited for experiments involving two treatments, as many factors such as resource availability and chance of pollinator visitation are assumed to be similar for flowers at the same node. In May 1988, three California populations were chosen for study: 1) Guenoc Winery (GW) 5 m SE of Middletown in Napa County; 2) Hough Springs (HS) in Lake County; and 3) the S slope of Tulloch Reserve (TR) in Tuolumne County. *Mimulus guttatus* is abundant at these sites with populations of hundreds to thousands of flowering individuals in 1988. The experiments in each population were conducted over a ca. 100-m² area.

Mimulus guttatus is described as having a "sensitive" stigma, as upon touch, the two stigmatic lobes clasp together within about 6 sec (Kerner, 1895; Ritland and Ritland, 1989). One of us (Dudash) found that following hand-pollination in the growth chamber, the bilobed stigmas did not reopen or took at least twice as long to reopen as stigmas touched with a paint brush without pollen. One possibility is that the stigmas that did reopen received insufficient pollen. It is also likely that following a pollinator's visit there is a period of time where visits by other pollinators would not be successful in depositing additional pollen grains onto the stigma because the stigmatic lobes are still closed.

Pollen limitation field study—At least 50 randomly chosen plants in each population were marked with numbered bird bands and green flags. Pairs of flower buds at the same node that were about to open were tagged with tape on their peduncles and randomly assigned two alternative pollination treatments: 1) hand-outcrossing, wherein supplemental pollen was applied to the stigma from a single donor greater than a meter away; and 2) open-pollination, wherein the flower was left alone and subject to natural pollination. Sample sizes are given in Table 1. The pollen-dependent stigma behavior described earlier was observed in only the Hough Springs field site (*N* = 8 flowers), so we applied additional pollen from a single donor to those reopened stigmas. This study was performed in an area adjacent to the early-late study because natural pollination of early-late plants might have been disturbed by our activity of pollen supplementation.

Capsules were harvested after 3 wk, and seed number per capsule was recorded in the lab. Virtually all hand- and open-pollinated flowers

TABLE 1. *Mean seed set per capsule following open-pollination vs. hand-pollination, for three populations of* M. guttatus, *with standard errors. The t-test was based on paired comparisons within N plants*

Population	N	Open (SE)	Hand (SE)	t	P[a]
Guenoc Winery	47	203 (24)	207 (22)	0.22	ns
Hough Springs	73	197 (19)	179 (16)	1.23	ns
Tulloch Reserve	53	179 (35)	509 (35)	6.41	<0.001

[a] ns = nonsignificant, $P > 0.05$.

produced fruits, but a small number of herbivore-damaged fruits were not included in the seed counts. No transformations were needed, and paired t-tests were performed on the data using Bonferonni's correction for multiple t-tests (Snedecor and Cochran, 1980).

"Early-late" field study—In each population, another set of approximately 60 plants was randomly chosen. On each plant, two flower buds at the same node position, about to open, were marked with tape on their peduncles and covered with dialysis tubing in the evening to control at what time they were available to pollinators the following day. The next morning the dialysis tubing was removed from the flowers, thus allowing them to undergo natural pollination for 24 hr, whereupon one randomly chosen corolla was removed from each plant. Corolla removal involved cutting the corolla with fine scissors and "unpeeling" the corolla while remaining below the stigma throughout the procedure. In *M. guttatus,* emasculation is via corolla removal. Therefore, corolla removal prevented further self-pollination and outcrossing because pollinators cannot land (Vickery, 1978). Corolla removal in this manner did not affect future seed and fruit formation. A study in a pollinator-free glasshouse demonstrated virtually no seed set following corolla removal as described above. Thus, contamination of self-pollen grains via this treatment is unlikely in our field study.

Initially, all flowers involved in this study had their stigmatic lobes open. To avoid the possibility that the act of corolla removal caused some selfing, 5 plants were excluded from the study when their stigmas closed immediately following corolla removal, and additional plants were randomly chosen. In *M. guttatus,* the two stigma lobes clasp shut upon touch, and are thought to reduce selfing as bees back out of the flower (Kerner, 1895; Ritland and Ritland, 1989). For our purpose, the absence of stigma closure suggests that self-pollen grains did not land on the stigma during corolla removal. Flowers treated in this manner were termed "early."

The other flower at this node was allowed to abscise naturally. Corolla abscission commonly occurs after day 4 in the lifespan of a flower; however, it may occur sooner if environmental conditions become severe (i.e., a heavy rain). The physiological basis of corolla abscission in *M. guttatus* is not known, although in another member of the Scrophulariaceae, *Digitalis,* corolla abscission is a function of the rate of ethylene production (Stead and Moore, 1979, 1983). Flowers treated in this manner were termed "late."

After 3 wk, the seed capsules produced from the early and late treatments were harvested and seeds were counted in the lab. A small number of herbivore-damaged fruits were not included in the seed counts (sample sizes are in Table 2). No transformations were necessary, and paired t-tests were performed using Bonferonni's simultaneous confidence intervals for multiple t-tests (Snedecor and Cochran, 1980).

Mating system estimation—Collected seeds from the early-late study were germinated, and extracts for starch gel electrophoresis were obtained from 3–4 wk-old seedlings using the grinding buffer of Ritland and Ganders (1987). Fifteen randomly chosen seedlings per capsule, two capsules (early and late treatments) and ca. 30 families per population, were electro-

TABLE 2. *Average seed produced under the "early" and "late" corolla removal treatments, with standard errors. The t-test was based on paired comparisons within N plants*

Population	N	Early (SE)	Late (SE)	t	P
Guenoc Winery	55	572 (53)	785 (51)	3.94	<0.001
Hough Springs	57	125 (26)	267 (40)	4.36	<0.001
Tulloch Reserve[a]	56	129 (43)	356 (59)	3.07	0.025

[a] Pollen limited.

TABLE 3. *Gene frequencies at loci used for mating system estimation, by population (averaged over early and late pollen pools; "—" denotes monomorphic loci excluded from analysis)*

Locus	Allele	Population		
		Guenoc Winery	Hough Springs	Tulloch Reserve
idh	1	0.62	0.88	0.35
	2	0.29	0.12	0.64
	3	0.09	0.00	0.01
6pgd	1	0.84	—	0.75
	2	0.15	—	0.22
	3	0.01	—	0.03
pgi	1	0.23	0.35	0.47
	2	0.63	0.33	0.37
	3	0.14	0.32	0.06
tpi	1	0.92	0.42	—
	2	0.08	0.58	—
dia-1	1	0.76	0.89	0.78
	2	0.23	0.11	0.21
	3	0.01	0.00	0.01
dia-2	1	0.39	—	0.43
	2	0.61	—	0.57

phoresed in the morpholine-citrate gel buffer (pH 6.1) of Ritland and Ganders (1987). The sample sizes are given in Table 4. Five enzyme systems were stained for six polymorphic enzyme loci: isocitrate dehydrogenase (idh), 6-phosphoglucoisomerase (6pgd), phosphoglucoisomerase (pgi), triphosphoglucoisomerase (tpi), and diaphorase (dia-1 and dia-2).

Data were grouped by families, and maximum-likelihood estimates were obtained for parameters of the correlated matings model (Ritland, 1989), separately for capsules from the early and late treatments. Estimates were obtained as a minimum variance average over loci, and the estimation procedure is given in Ritland (1989). The statistical distribution of estimates was obtained via the bootstrap method (Efron and Gong, 1983), with the progeny array as the unit of resampling. To make paired comparisons, estimates for early and late treatments were computed for two bootstrap replicates having the same bootstrap sample of parents, but with early and late progeny, respectively. After estimating the parameters separately for the early and late fruit, we performed t-tests on the 50 bootstrap estimates for each parameter.

The correlated matings model specifies the relatedness between sibs with three parameters: 1) the population selfing rate s; 2) the correlation of selfing within sibships r_s (equivalently, the normalized variance of selfing among sibships); and 3) the correlation of outcrossed paternity within sibships r_p (equivalently, the fraction of sib-pairs sharing the same male parent). All three parameters can take

values from zero to one. The correlation of selfing, if ignored, can bias estimates of the correlation of paternity (Ritland, 1989).

The correlation of paternity r_p is an index of multiple paternity; it equals zero when sibs share no fathers, and equals unity when sibs all share the same father. There are two distinct advantages of using this measure of paternity over such measures as the minimum number of fathers (e.g., Ellstrand, 1984; Devlin and Ellstrand, 1990). First, the expected estimate of r_p is unaffected by both the level of polymorphism at marker loci and the size of the sibling array; as few as one locus and two progeny per family can be used (although a great number of arrays would be needed). Thus, one can make valid comparisons of multiple paternity between populations that differ in polymorphism or sampling methods. Secondly, r_p with s and r_s specify the covariance between sibs for quantitative characters, and hence can be directly related to quantitative genetic models of sexual selection.

RESULTS

Both the Guenoc Winery population and the Hough Springs population showed no pollen limitation, as capsules produced about 200 seed regardless of whether additional pollen was artificially applied (Table 1). By contrast, the Tulloch Reserve population showed significant pollen limitation, with hand-pollinated flowers producing 150% more seed that naturally pollinated flowers (Table 1).

In all three populations, flowers that were allowed to retain their corollas (the "late" treatment) produced significantly more seed than flowers that had their corollas removed after 1 day (the "early" treatment, Table 2). These data also indicate that the proportion of successful matings practiced after the first day of flowering are $(785 - 572)/785 = 28\%$, $(267 - 125)/267 = 53\%$, and $(356 - 129)/356 = 64\%$ in the Guenoc, Hough, and Tulloch populations, respectively. This proportion has an average of 48% over all three populations. Interestingly, the pollen-limited Tulloch population showed the greatest proportion of late matings.

The allele frequencies at the isozyme loci used for mating system estimation are given in Table 3. Because the correlated matings model has more parameters than traditional models of the mating system, several polymorphic loci are desirable to estimate its parameters (Ritland, 1989); indeed, most loci had three alleles, and each population had at least four polymorphic loci. All three populations

TABLE 4. *Estimates of the correlation of paternity within capsules under the "early" and "late" corolla removal treatments, and the difference between treatments, with standard errors (SE of difference found by paired comparison of bootstrap replicates; see text)*

Population	No. of families[a]	Early r_p (SE)	Late r_p (SE)	Late–early Dr_p (SE)
Guenoc Winery	32	0.39 (0.13)	0.24 (0.10)	−0.15 (0.12)
Hough Springs	22	0.43 (0.18)	0.30 (0.10)	−0.13 (0.11)
Tulloch Reserve[b]	21	0.34 (0.17)	0.36 (0.12)	0.02 (0.20)
Mean	75	0.39 (0.09)	0.30 (0.06)	−0.09 (0.09)

[a] 30 progeny per family, divided evenly between treatments, were assayed.
[b] Pollen limited.

have gene diversities higher than typical for *M. guttatus*, making these populations especially appropriate for such fine-structure analyses of the mating system.

The correlation of paternity decreased from the "early" to the "late" treatments in the Guenoc and Hough populations that were not found to be pollen limited. However, this decrease was not statistically significant, as shown by the standard errors for their difference (Table 4). The pollen-limited Tulloch population demonstrated no difference in correlation of paternity between the treatments.

The selfing rate did not change between the "early" and "late" treatments, averaging about 27% (Table 5). In contrast to the paternity correlation, the lack of significant differences in selfing rate was not due to large standard errors. In fact, the differences between treatments were remarkably small, averaging 2% over all populations, especially considering that at 27% selfing there is much latitude for variation of selfing rate. The pollen-limited Tulloch population showed no difference in selfing rate from the other populations.

The within-capsule correlation of selfing significantly increased by 32% from "early" to "late" treatments in only the Guenoc population (Table 6), although this mating system parameter suffers from the largest standard error (compare Tables 4, 5, 6). Smaller but nonsignificant increases were observed in the other two populations, so the average increase over the three populations was nonsignificant at 0.14.

DISCUSSION

Experimental studies of plant mating systems can reveal underlying mechanisms governing their evolution. The monitoring and manipulation of floral changes, combined with isozyme estimates of the mating system, offers a new approach to experimental plant population biology. However, this field study has illustrated the difficulties of such an approach, owing to our limited statistical power for mating system estimation given our sample sizes. Nevertheless, important inferences can be drawn from this first field study of changes in the mating system throughout the lifespan of individual flowers.

Multiple paternity and sequential pollination—The significant excess of seed produced by "late" flowers over "early" flowers (Table 2) is firm evidence for sequential pollination in *M. guttatus*. Roughly half of the matings occurred after 1 day in the lifespan of a flower. Furthermore, the decrease in the paternity correlation, or equivalently the increase in multiple paternity (Table 4), although not significant, suggests that subsequent pollinations can occur with different males than those encountered in the first day of flowering.

To make further inferences about the dynamics of paternity through the floral lifespan, it is useful to relate the correlation of paternity to an "effective number of mates." If outcrossing occurs randomly to a limited pool of n

TABLE 5. *Estimates of the selfing rates of flowers under the "early" and "late" corolla removal treatments, and the difference between treatments, with standard errors (sample sizes are the same as Table 4)*

Population	Early s (SE)	Late s (SE)	Late–early Ds (SE)
Guenoc Winery	0.34 (0.06)	0.31 (0.06)	−0.03 (0.06)
Hough Springs	0.20 (0.09)	0.20 (0.08)	0.00 (0.07)
Tulloch Reserve[a]	0.30 (0.06)	0.26 (0.07)	−0.04 (0.05)
Mean	0.28 (0.04)	0.26 (0.04)	−0.02 (0.03)

[a] Pollen limited.

TABLE 6. *Estimates of the correlation of selfing within capsules under the "early" and "late" corolla removal treatments, and the difference between treatments, with standard errors (sample sizes are the same as Table 4)*

Population	Early r_s (SE)	Late r_s (SE)	Late–early Dr_s (SE)
Guenoc Winery	0.17 (0.16)	0.45 (0.17)	0.32 (0.20)[b]
Hough Springs	0.50 (0.27)	0.54 (0.25)	0.04 (0.19)
Tulloch Reserve[a]	0.23 (0.36)	0.33 (0.15)	0.10 (0.27)
Mean	0.30 (0.08)	0.44 (0.04)	0.14 (0.10)

[a] Pollen limited.
[b] $P < 0.05$.

males, the expected paternity correlation equals $1/n$, and thus the effective number of mates equals $1/r_p$ (Ritland, 1989). From Table 4, the effective number of mates per capsule in *M. guttatus* averaged 2.56 for flowers allowed to mate for only 1 day, and increased to 3.33 for flowers that mated for their natural lifespan. Thus, by remaining open for more than 1 day, flowers increased the number of mates by roughly $(3.33 - 2.56)/2.56 = 30\%$. From Table 2, over the same period, seed set increased by ca. 100%. If we compare the effective number of mates in the pollen-limited and nonlimited populations we find a 6% and 52% increase in the late fruits, respectively. These results demonstrate that pollen limitation may influence the potential for multiple paternity within a fruit. Fewer pollinations take place in pollen-limited populations; thus, the potential for multiple paternity should be lower in such populations, as we found.

Our results differ from those of Marshall and Ellstrand (1985) and Epperson and Clegg (1987), who studied *Raphanus sativus* and *Ipomoea purpurea*, respectively. In controlled greenhouse studies, they found sequential pollination did not play a role in increasing the number of males siring the seed within a fruit. Both groups suggested that pollen carryover was responsible for multiple paternity within fruits, as observed in paternity analysis of naturally pollinated plants. Because multiple paternity in *M. guttatus* was high even in flowers that mated for only 1 day (2.56 mates on average), we also believe pollen carryover is important for determining the level of multiple paternity in *M. guttatus* capsules. However, the 30% increase in mate number over the remainder of flowering also points to an important role for sequential pollination in determining multiple paternity within a fruit of *M. guttatus*.

Our conflicting results may be resulting from the unique suite of traits associated with the reproductive biology of *M. guttatus*. This member of the Scrophulariaceae can produce hundreds of seeds per capsule, and individual

flowers remain open for several days (Dole, 1990; this study). Further studies quantifying changes in correlation of paternity throughout the lifespan of individual flowers of other species capable of producing many-seeded fruits are needed.

Other studies have documented among-population variation of pollination limitation (Heithaus, Stashko, and Anderson, 1982; Galen, 1985; Campbell, 1987), but have not linked its presence to quantitative estimates of the mating system. Pollination limitation varied in the same population of *Sabatia angularis* from year to year (Dudash, 1987), and for this reason it seems pertinent to examine pollination limitation jointly with other aspects of reproductive biology. Additional estimates of pollen limitation throughout the flowering season would provide further insight to the overall importance of individual variation in pollination limitation (e.g., Dudash, 1987; Zimmerman and Pyke, 1988). Our finding of pollen limitation is also of consequence for mate choice theory, because when mates are scarce, a female may not have the choice of providing resources to some seeds and/or fruits and not others (Willson and Burley, 1983).

Self-fertilization rates—Selfing rates did not increase as expected through the lifespan of the flower (i.e., "late" treatment selfing rates were not higher than "early" treatments in Table 5). Thus, there was no evidence for "delayed selfing," and in particular, we found no evidence for the mechanism of "corolla dragging" as an agent of selfing in these populations of *M. guttatus*. One would expect corolla dragging to at least be evident in pollen-limited populations, but even the population that was pollen limited (Tulloch Reserve) did not show any change of selfing rate with floral age.

It is surprising that even after 1 day of flowering, 28% selfing was observed. We can largely rule out geitonogamy as a cause of this selfing, because during the early-late experiment, we also counted the total number of flowers open on each treated plant. We then computed es-

timates of selfing for each plant from the iso-
zyme data, and regressed these family esti-
mates of selfing upon number of flowers open.
No relationship was found, suggesting geito-
nogamy was not the major cause of the 28%
selfing observed after 1 day of flowering. Thus,
it seems likely that selfing either occurs when
the pollinator visits an individual flower, de-
spite the presence of the stigmatic closure
mechanism and/or many supposedly out-
crossed pollination events lead to matings be-
tween related individuals. It would be infor-
mative to study the way in which a bumblebee
"works" a *M. guttatus* flower and determine
genetic relatedness via isozymes among neigh-
boring plants to help explain the observed self-
ing rates.

Our finding that corolla dragging seems un-
important for selfing in *M. guttatus* is in con-
trast to Dole's (1990) recent experiments. Dole
glued corollas onto calyxes (preventing their
abscission) in a pollinator-free greenhouse, and
found that such manipulated flowers set fewer
seed than flowers with undisturbed corollas.
Since this seed could only be produced by self-
ing, he concluded this was evidence for a role
of corolla dragging in causing selfing. However,
his study was performed on another population
of *M. guttatus* and the stigma closure/reopen-
ing phenomena that we observed may not be
universal. We also did not observe the stigma
curling as flowers age noted by Dole (1990).
Dole (1990) goes on to suggest that populations
of *M. guttatus* may vary in their potential for
delayed self-pollination via corolla abscission,
as we found.

Variation of selfing—The correlation of self-
ing within capsules increased significantly
through the floral lifespan in the Guenoc pop-
ulation (Table 6). Interestingly, this increase of
correlation occurred despite no change of av-
erage selfing rate (Table 5). This indicates that
the self-fertilization events are repartitioned
among flowers during their lifespan: in the later
days of flowering, some flowers shift to greater
outcrossing while others appear to shift to
greater selfing. Perhaps the apparent shift to a
greater correlation of selfing within capsules is
because this parameter is detecting different
levels of local population substructuring be-
tween the early and late estimates (Ritland,
1989). Corolla dragging does not explain the
significant increase in correlation of selfing
within a fruit in our Guenoc population of *M.
guttatus* because there was not a concurrent
increase in the selfing rate between the early
and late treatment.

The results of this study call for further ex-

amination of the topic of multiple paternity.
The frequency of sequential pollination affects
the potential for increasing female fitness
through multiple siring of seed, female choice,
and male competition episodes staggered
throughout the lifespan of a flower (Mulcahy,
Curtis, and Snow, 1983), and the resulting con-
sequences in terms of progeny fitness. The time
between sequential episodes of pollination may
influence the strength and nature (inhibitive or
stimulative) of competition between pollen
grains from different episodes. Additionally,
there may be physiological differences between
flowers in the early and latter stages of their
floral lifespan that may influence their mating
capabilities. This study also demonstrates how
manipulative studies of the mating system pro-
vide vital information about the factors that
influence the potential for self-fertilization. This
knowledge is critical to the development of
testable models of mating system evolution.

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