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First-pollination primacy and pollen selection in the morning glory, *Ipomoea purpurea*

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The relationship between pollination order and the transmission of male genes to seed was investigated by performing a large number of genetically marked, double and mixed artificial pollinations in the common morning glory, *Ipomoea purpurea*. Second pollinations were substantially less effective than first pollinations, even when applied immediately afterwards. When delayed 30 and 60 minutes, effectiveness was reduced further to about 14 and 7 per cent of fertilisations, respectively. After 120 minutes, effectiveness decreased to less than 2 per cent. Among a set of mixed pollinations, pollen from some individuals suffered a strong disadvantage in competitive ability. This disadvantage is partially genetic.

The primacy of first pollinations, and the presence of strong pollen competition, have implications for studies of pollination biology and genetic transmission in natural populations of *Ipomoea purpurea* and other plant species. Specifically, sequential pollination ineffectiveness, and strong competition within pollen loads, should reduce the numbers of paternal parents and thus increase the numbers of full sibs among outcross seed within capsules. This represents a substantial violation of one of the critical assumptions in the mixed mating model and could influence the genetic structure of *Ipomoea* populations. In addition, these data indicate that gametophytic selection may be an important component of natural selection in *Ipomoea*. The mechanisms underlying the sequential and competitive effects probably include pollen-stigma interactions, differential pollen production, viability differences, or pollen tube growth rate differences, and possibly embryo abortion.

These experiments also establish that outcrossing rates vary among individuals in natural populations, due to genetic variation in the distance between anthers and the stigmatic surface.

INTRODUCTION

It has become widely recognised that the mating system plays a critical role in the dynamics of genetic transmission in populations (Allard, 1975; Clegg, 1980). Numerous studies have revealed complex mating system phenomena which cause substantial deviations from the assumptions made in earlier, simpler population genetic models (reviewed by Clegg and Epperson, 1985). These factors influence the structure of genetic variability in populations and hence the course of evolution.

Many mating system studies have estimated such parameters as inbreeding coefficients, rates of gene flow, and outcrossing rates based on observations of either mating patterns or progeny arrays (Clegg, 1980; Brown and Allard, 1970). Several assumptions are made in these estimation procedures. For example, in insect pollinated plants, genetic transmission or fertilisation of ovules, has been assumed to correspond with pollinator visits. There presently is a large descriptive literature where pollinator efficacy is used to infer such aspects of reproductive biology as outcrossing rates and gene flow (Baker and Hurd, 1968; Stucky and Beckmann, 1982; Cruden, 1977, Faegri and van der Pijl, 1979). These inferences are usually based on species constancy, and the mechanical efficacy of intraspecies and self pollen deposition onto stigmas by pollinators, which is not necessarily coincident with genetic transmission to progeny. In addition, consanguinity of the seeds on a plant due to shared paternal parentage may increase the level of inbreeding beyond the expected levels in isolation by distance models (Wright, 1946). Finally, observations of both pollinator flight distances and dispersal of dyed pollen have been used to estimate gene flow, neighbourhood

size, population subdivision, inbreeding, and other aspects of plant population genetic structure (*e.g.*, Levin and Kerster, 1968, 1969; Kerster and Levin, 1968; Schaal, 1980; Ennos and Clegg, 1982; Thompson and Plowright, 1980). Both methods may be biased if: (a) flight distances vary substantially over time and sequential pollinator visits vary in fertilisation effectiveness; or (b) if pollen competitive ability varies with dispersal distance *e.g.*, increased incompatibility of pollen on stigmas of related nearby individuals.

Multiple paternal parentage from repeated and/or mixed pollinations in plants is usually assumed when estimates of mating system parameters are based on progeny arrays. The standard model for estimating outcrossing rates assumes that the outcross pollen parents are uncorrelated (Brown and Allard, 1970; Clegg *et al.*, 1978). However, positive correlations of paternal parentage can cause large biases in the estimates (Schoen and Clegg, 1984). Sequential pollination ineffectiveness in fertilisation and strong competition within pollen loads are two mechanisms which increase the correlation of paternal parentage of seed within fruits.

The experiments reported in this paper were motivated by studies on artificial populations of *Ipomoea purpurea* which suggested that pollen from the first insect pollinator visit contributed disproportionately to fertilisations (Epperson, unpublished data). In this study, visitation patterns of the primary pollinators, *Bombus pennsylvanicus* and *B. impatiens*, to genetically marked plants were compared to genotypic arrays of progeny. The advantage of the first pollination was apparent even though second pollinations often occurred within 20 minutes, and the average number of visits per flower was as high as 5·0 during the course of each experiment, from 0900–1100 hour. Furthermore, in mixed populations, white flowering plants had higher male fitness than did coloured flowering plants (Schoen and Clegg, 1985).

In the present study, these phenomena were investigated by performing artificial pollinations (from 0900–1100 hour). The results indicate that the effectiveness of second pollinations is lower than first pollinations, even when the second pollinations occur immediately after the first pollination. Further reductions are obtained when the second pollination is delayed 30, 60, and 120 minutes. Competition between white flowering and coloured flowering pollen donors within mixed pollinations was also measured, and pollen from some coloured flowering plants suffered large competitive disadvantages.

MATERIALS AND METHODS

Flower colour types

Ennos and Clegg (1983) showed that at least three genetic loci determine flower colour phenotype in *I. purpurea*. The locus W/w controls the distribution and degree of pigmentation. The homozygous genotype ww is white flowered, while the heterozygote Ww is partially pigmented and the alternative homozygote WW has a fully pigmented corolla. The parent plants employed in these experiments were either ww (referred to as white below) or WW (referred to as coloured below).

Methods of crossing

Each morning, flowers used as maternal parents were trimmed and emasculated at 0700 hour, about 1–2 hours before anther dehiscence. Each pollination was performed by rubbing one, at least partially dehisced, anther on the stigmatic surface until most of the pollen was removed. Little pollen was lost in the process, and generally a single pollination resulted in fairly complete coverage of the stigmatic surface.

Treatments

Ten families each consisting of five selfsib individuals were used in the tests. Each replicate of the experiment consisted of the five treatments listed below:

Treatment 1: One anther each of the two pollen donor families was simultaneously applied to the stigma at 0900 hour.
Treatment 2: One anther from the first donor was applied at 0900 h, and the second anther was applied immediately afterwards.
Treatment 3, 4, 5: As in treatment 2, except the second anther was applied at 0930, 1000, 1100 hour, respectively.

In almost every replication, the same individuals from each of the three families (maternal and 2 paternals) were used for all five treatments. Occasionally a self-sib was substituted in some treatments due to insufficient numbers of flowers. However, in the analysis of gametic competition in Treatment 1 presented below, replications are indexed only by the families represented (not by specific individuals). This procedure is justified by the expected large genetic correlation between the individuals of a family of self-sibs.

Experimental design

The design of the crosses consisted of five replications of each pair of pollen donors, where one donor was from one of four white flowering families (families 6–9), and one was from one of five coloured flowering families (families 1–5). This resulted in $5 \cdot 4 \cdot 5 = 100$ replications. In total, 499 crosses were included in the analysis. Maternal plants were chosen mainly by daily availability; but, overall, approximately equal numbers of coloured and white maternal phenotypes were used. The white family # 10 was used only as an occasional maternal plant.

The experiments were performed under greenhouse conditions from 25 March through 8 April, 1984. The dates of pollinations are unbalanced with combinations, but probably are not a factor in this study. Temperature is known to affect rates of pollen tube growth (*e.g.*, Buchholz and Blakeslee, 1927); however, the ambient temperature in the greenhouse varied only a few degrees centigrade from day to day.

Identification of progeny parentage

Only one polymorphic allozyme marker locus was available, an esterase locus with two alleles, S and F (Ennos and Clegg, 1983). All individuals in coloured families were homozygous for the S allele of esterase and individuals in white families were homozygous FF. The first pollen donor (FIRSTFAM) in the treatments was always the opposite colour type from the family of the maternal plant, MATFAM. For example, if MATFAM was white, then FIRSTFAM was coloured and the second pollen donor family (SECFAM) was white. In Treatment 1, FIRSTFAM specifies the family of the pollen type opposite the MATFAM, and in Treatments 2–5, FIRSTFAM specifies the family used in the first pollination. Thus, fertilisation by FIRSTFAM pollen always resulted in progeny seedlings that were heterozygotes for esterase and fertilisation by SECFAM pollen or MATFAM self pollen resulted in homozygotes. Seedling esterase genotypes were scored employing standard electrophoretic procedures. In total, over 98 per cent of the seed were successfully germinated and genotyped.

Statistical procedures

The relative competitive ability of outcross pollen from different families can be measured in Treatment 1. However, because substantial self-fertilisation occurred, the basic data (fraction of heterozygotes for esterase) must be transformed in order to account for rates of outcrossing. The transformed data were independent of the MATFAM used and are indexed only by the two nonordered pollen donor families, not by FIRSTFAM and SECFAM. This resulted in a balanced design of five replicates of each combination of white and coloured pollen donor families.

Anther stigma distances

The distance between the tallest and/or nearest anther and the top of the stigmatic surface was measured for at least 10 flowers on all 45 individuals from families 1–9 on days immediately following cessation of the pollinations. On an individual flower, this distance was zero if any anther was in contact with the stigma. Flowers in which one or more anthers were actually taller than the stigma always contained at least one anther in contact with the stigma, and were accordingly scored as zero. There was remarkable homogeneity of individual mean anther stigma distances within families, which supports results by Ennos (1981) indicating that the heritability of anther stigma proximity is quite high.

RESULTS

Effectiveness of second pollinations

The average fraction of esterase heterozygotes ($\bar{h}$) for the five treatments is presented in fig. 1. $\bar{h}$ steadily increased from 0·361 for Treatment 1 to 0·459 in Treatment 2, almost doubling to 0·685 in Treatment 5. $\bar{h}$ for Treatment 2 is considerably larger than for Treatment 1. Apart from the effect of pollination order, there is a strong negative effect of the time delay on the rate of success of pollen from the second pollinations. The linear regression equation of the fraction of heterozygotes per capsule, h, on time delay in minutes, θ, for Treatments 2–5, is $h = 0{\cdot}513 + 0{\cdot}0017\theta$. The slope is significant at the 0·0001 level. Fig. 1 indicates that the effect is nonlinear over the range $0 < \theta < 120$, and begins to reach a plateau at about $\theta = 60$. This means that no further decrease in effectiveness of the second pollination occurs when delayed longer than about 1 hour.

$\bar{h}$ for Treatment 1 is much smaller than the expected value of 0·5, assuming 100 per cent outcrossing and equal frequencies of donor types. This indicates that some self-fertilisation occurred in

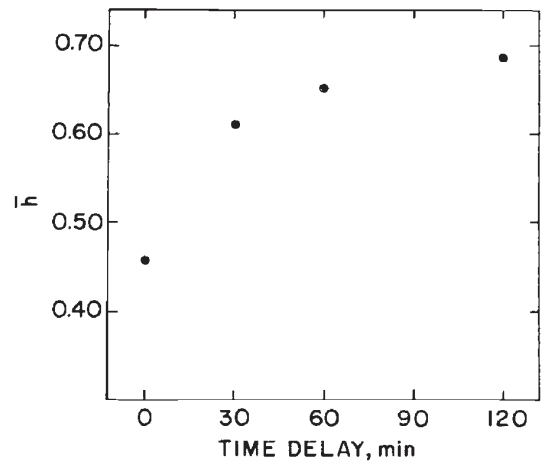

Figure 1 Average fraction of heterozygoes ($\bar{h}$) in capsules as a function of the time delay of the second pollination in Treatments 2-5. $\bar{h}$ was 0·36 in Treatment 1.

the experimental materials prior to emasculation. To convert the results into proportions of outcross fertilisations from each of the outcross pollen donors, we use the fact that $h = tq$ for each cross. Here q is the frequency of fertilisations by the first pollen donor, and t is the rate of outcrossing.

An estimate of t for each cross for Treatments 1-4 can be obtained from the results of Treatment 5, which indicate that all outcross progeny are identifiable (*i.e.*, all outcross fertilisations are by the first donor). To demonstrate that this must be nearly true, we note that the average h increased nearly two-fold for Treatment 1 to Treatment 5. We can assume that overall $t_5 \geqq t_i$ for $i = 1, 2, 3, 4$, because less outcross pollen is available early to compete with pre-emasculation, self-pollen loads. In Treatment 1, the mean, $\bar{q}$, should be very near 0·5, because any competition between pollen donors should cancel out as each family was used as the first and second donor equally. Thus the two-fold increase in $\bar{h}_5$ over $\bar{h}_1$ implies that $\bar{q}_5 \approx 1{\cdot}0$. For each replication, h_5 is an estimate (independent of q_1) of t_5 and an upper bound for t_i, $i = 1, 2, 3, 4$. Because there is significant variation in mean h_5 among maternal families, the estimate of $t\,(t_m)$ for a given replication was taken as the mean $\bar{h}_5$ for all crosses in which the same MATFAM was used. Thus, we estimate each q_i in a cross (in Treatments $i = 1, 2, 3, 4$) as follows:

$$\hat{q}_i = \frac{h_i}{t_m}.$$

The mean values of $\hat{q}_i$ are $\hat{q}_1 = 0{\cdot}51$; $\hat{q}_2 = 0{\cdot}67$; $\hat{q}_3 = 0{\cdot}86$; and $\hat{q}_4 = 0.93$. Estimates of q_5 are not completely independent of t_m because the cross being considered was used as a small part of the data used to estimate t_m. Nonetheless, the estimate is $\hat{q}_5 = 0{\cdot}98$, which accords with the initial assumption that each q_5 is 1·0, and mean q_1 is about 0·5. If t is smaller in Treatments 1-4 than in Treatment 5, the $\hat{q}_i$ are lower bounds where $i = 1, 2, 3, 4$.

The number of seeds per capsule did not vary substantially among treatments. Overall, 90 per cent of the capsules contained 4-6 seed. Capsules from Treatment 1 contained slightly fewer seeds than those from Treatment 2. A regression analysis of the number of seeds per capsule on time delay for Treatments 2-5 was not significant, however, regression of the total average number of seeds on time delay was significant. In this case, the equation was $y = 5{\cdot}28 - 0{\cdot}00169\theta$ where θ is the time delay in min. The effect is small, suggesting that a sufficient amount of pollen was available from the first pollination to ensure comparable seed set.

Effects of anther-stigma distance

The regression of average outcrossing rate estimates (t_m) of MATFAMs on mean effective anther stigma distance (ASD) was significant in Treatment 5 (tables 1 and 2). ASD is apparently more critical among coloured MATFAMs (small group mean ASD) than among white MATFAMs (large group mean ASD). In Treatment 1, the average fraction of heterozygotes is similarly related to ASD. In this case, the regression of h_1 based on all 9 MATFAMs is confounded with the competitive advantages of white pollen (see below). Despite the fact that as a group coloured MATFAMs have smaller ASDs, the frequency of heterozygotes is large because the selective advantage of white

Table 1 Mean anther-stigma distance (ASD), and average fraction of heterozygotes per capsule ($\bar{h}$) in treatments 1 and 5, by maternal families (MATFAMs)

		$\bar{h}$	
MATFAMs	ASD	Treatment 1	Treatment 5
1	0·694 (0·262)*	0·526	0·845
2	0·820 (0·642)	0·806	1·000
3	0·036 (0·081)	0·290	0·540
4	0·020 (0·045)	0·223	0·407
5	0·020 (0·045)	0·708	0·786
6	1·186 (0·833)	0·298	0·598
7	0·740 (0·867)	0·161	0·553
8	1·924 (0·185)	0·346	0·833
9	1·230 (0·641)	0·288	0·933

* Standard error of individual means in parentheses.

Table 2 Regression of average fraction of heterozygotes on mean anther-stigma distance, x

Treatment	MATFAMs	Regression	Prob.	R-Square
1	1–9	$0{\cdot}40-0{\cdot}05x$	0·40	0·007
1	6–9	$0{\cdot}08+0{\cdot}15x$	0·17	0·04
1	1–5	$0{\cdot}38+0{\cdot}28x$	0·05	0·07
1	1–4	$0{\cdot}27+0{\cdot}50x$	0·0003	0·28
5	1–9	$0{\cdot}57+0{\cdot}16x$	0·01	0·06
5	6–9	$0{\cdot}41+0{\cdot}25x$	0·11	0·06
5	1–5	$0{\cdot}54+0{\cdot}47x$	0·002	0·17
5	1–4	$0{\cdot}42+0{\cdot}64x$	≤0·0001	0·33

pollen is expressed as an increase in h, whereas with white MATFAMs the advantage is expressed as a decrease in h. Consequently, the regression of h on ASD is significant only within either white MATFAMs, or coloured MATFAMs.

MATFAM 5 was an outlier among coloured MATFAMs in both Treatments 1 and 5. Despite its small ASD a high value of h is obtained, suggesting that as a maternal parent, self-pollen was available to the stigma, but self-fertilisation was partially ineffective. Seed set in MATFAM 5 was only about 3·7 in Treatment 1, and 4·6 in Treatment 5 compared to 5·5 for all MATFAMs. This could be due to selection against selfed embryos in MATFAM 5, which would bias the estimate of h upward. Family 5 was also a poor pollen donor (see below).

Competition in mixed pollinations

The fraction of esterase heterozygotes for combinations of pollen donors in Treatment 1 may be used to measure competition between outcross pollen for successful fertilisation unconfounded with the effects of time delays or pollination order. As discussed in the Materials and Methods section, the data must be transformed to account for selfing. The method uses the estimate of average t, t_m, in Treatment 5 for each MATFAM. Briefly, this procedure is justified by the fact that the same combinations of individuals were used in Treatments 1 and 5 for almost all replications, and by the lack of pollen donor effects or MATFAM by pollen donor interactions in Treatment 5 (results not shown) and interaction effects between pollen donors and effects of MATFAM in the results of ANOVA for Treatment 1 (see table 3). Additionally, the estimates of average t for MATFAMs in Treatment 5 show a strong correlation with anther stigma distance. These results indicate that outcrossing rates for individual capsules are a function of MATFAM and are independent of the combination of pollen donors.

The transformation of the raw data does not affect the variance-covariance structure of competitive effects because the error in using estimates of t enters the linear model as part of the error term.

$$\hat{d} = 2h - t_m \quad \text{if MATFAM is coloured}$$

$$\hat{d} = t_m - 2h \quad \text{if MATFAM is white.}$$

$\hat{d}$ is an estimate of $d = p'_j - q'_j$.

Here $\acute{p} \equiv$ proportion of progeny fertilised by the white pollen parent among the outcross progeny of a capsule in Treatment 1. Thus, $\acute{p} + \acute{q} = t_m$ and $\acute{q} = qt_m$.

The results of a two-way ANOVA with interactions is given in table 3. The mean $\hat{d} = 0{\cdot}23$ indicates that overall white pollen is favoured. The white flowering parents are not significantly different in effect, but there are significant differences between the effectiveness of coloured flowering pollen donors. Family 1 is equivalent to the whites (*i.e.*, $d \approx 0{\cdot}0$); Families 2 and 3 are slightly less effective, and Families 4 and 5 are substantially less effective. Two-way ANOVA was conducted for a different transformation which simply divides h by t_m to estimate q. This analysis does not enjoy the same advantages as the one described above. However, the hierarchy of competitive ability among families observed above for Treatment 1 was maintained for this analysis in Treatments 1–4. Competitive effects were diminished as time delays increased, with no effect in Treatment 5 (Results not shown).

The results obtained from all methods show a lack of interaction between the parent pollen families in the transformed data, indicating that the first order effect of coloured flowering pollen families is consistent over white flowering pollen parents.

The results of Treatment 1 indicate a strong competitive deficiency for the coloured flowering Families 4 and 5. The two progenitors of these two families were themselves half-sibs. All other pro-

Table 3 (a) Two-way ANOVA with interactions tables for the effects of different families of coloured and white pollen donors, (b) family means based on a transformation of the fraction of heterozygotes per capsule in Treatment 1. q is an estimate of the frequency of fertilisations by coloured pollen among outcrosses; d is an estimate of $\acute{p} - \acute{q}$, where $\acute{q} = qt$, $\acute{p} = t - \acute{q}$, and t is the outcrossing rate. N is the number of capsules

a. ANOVA Table		F	Prob.
White donor		0·03	0·992
Coloured donor		0·56	0·010
White by coloured donor interactions		0·88	0·568
b. Family		N	Mean d
White	6	25	0·24
	7	25	0·20
	8	25	0·23
	9	25	0·24
	Total Means		0·23
Coloured	1	20	0·015
	2	20	0·089
	3	20	0·083
	4	20	0·36
	5	20	0·58
	Total Means		0·23

genitors of the families were genetically uncorrelated, except the pairs 7, 9 and 6, 8, where in each case the two progenitors were obtained from the same open-pollinated plant. In order to determine if the competitive disadvantage is genetic, we may examine the performance of specific individuals within Families 4 or 5. The results indicate little difference between individuals within Families 4 or 5 in performance as outcross competitors with white pollen in Treatment 1. Table 4 lists the results of a two-way ANOVA with or without accounting for the actual white families used. As in the comparison between families, there was little overall difference among white competitors. In Family 5, one plant, 5-2, was quite a good competitor, two plants, 5-1 and 5-5, were near the average for Family 5, and two plants, 5-3 and 5-4, were particularly poor. It appears that the deficiency is partially genetic because 4 of 5 sibs are poor pollen donors. In Family 4, one plant, 4-2, was a particularly poor competitor, and the 3 others used were moderate competitors (results on 4-2 are based on only 2 capsules however). It therefore appears that the deficiency in Family 4 also has a genetic component.

Table 4 Two-way ANOVA tables for the effect of using different individuals within Family 5, and the effect of white families, as pollen donors in Treatment 1. d, and N are defined in table 3. (b) Same as above, except that one effect is for using individuals within Family 4

a. Family 5	Type I*		Type IV†	
	F	Prob.	F	Prob.
Individual	2·28	0·13	2·32	0·13
White family	0·18	0·84	0·17	0·84
Individual	N		Mean d	
5-1	8		0·58	
5-2	3		0·15	
5-3	4		0·87	
5-4	2		0·87	
5-5	3		0·45	
b. Family 4	Type I		Type IV	
	F	Prob.	F	Prob.
Individual	0·19	0·90	0·80	0·52
White family	0·90	0·47	0·84	0·50
Individual	N		Mean d	
4-1	9		0·30	
4-2	2		0·72	
4-3	4		0·34	
4-4	5		0·35	
4-5	0		—	

* Type I does not correct for unbalanced design.
† Type IV does correct for unbalanced design.

DISCUSSION

Effects of sequential pollination on mating system

The effectiveness of repeated pollinations has considerable impact on several key areas in the transmission genetics of plant populations. These areas include mating system estimation and the measurement of gene flow. The results of this study suggest that the first pollination controls the mating structure in *I. purpurea*. Even non-delayed second pollinations suffer a significant disadvantage. The second pollination ($p = 0{\cdot}33$) is about one-half as effective as the first ($q = 0{\cdot}67$). The closer proximity in Treatment 2 of pollen from the first donor (which results in heterozygotes), to the stigmatic surfaces results in higher rates of successful fertilisation. A delay of 30 minutes renders subsequent pollinations largely ineffective ($q = 0{\cdot}88$; $p = 0{\cdot}14$).

Results of sequential pollination studies on other species are varied. In a similar artificial

pollination study on witloof chicory (*Cichorium intybus*), Eenink (1982) found that second pollinations delayed 60 minutes or more were ineffective. Similarly, Marshall and [illegible]id (1985) found that 30 minute delays result in ineffective second pollinations in *Raphanus sativa*. In contrast, second pollinations delayed 1 or 2 days were more effective than first pollinations in apple (Visser and Verhaegh, 1980) and pear (Visser and Marcucci, 1983). In these studies, the first pollination apparently "paves the way" for the second. Repeated visits of bees to pear blossoms also increased seed set (Panov and Petkov, 1975). The contrast to the results in *I. purpurea* may be due, in part, to the ephemeral nature of morning glory flowers.

In natural populations of *I. purpurea*, the effectiveness of subsequent pollinations depends on (a) the amount of pollen deposited during each pollinator visit; (b) the cumulative pollen load on a stigma and amount of stigmatic surface remaining exposed after pollinations; (c) the length of time between pollinations or the number of visits per morning; and (d) whether or not pollen loads deposited by bees decrease as the morning proceeds, *e.g.*, after most flowers have received several visits and the anthers have been stripped of most of their pollen.

Regarding the first issue, bumblebees are very effective pollinators of *Ipomoea* species. In foraging for nectar, their ventral, abdominal, thoratic surfaces, and femurs and proboscises become coated with *Ipomoea* pollen, and they rarely clean themselves and never remove pollen to their scopae. Their intrafloral behaviour ensures pollen deposition onto the stigma and they exhibit considerable constancy to *Ipomoea* species (Brown and Clegg, 1984; Stucky and Beckmann, 1982; Austin, 1978). Spears (1983) found that a single visit of *B. pennsylvanicus* to flowers of *Ipomoea trichocarpa* in natural populations increased seed set from an autopollination rate of about 0·6 seeds per capsule to 2·5. These values correspond closely to the per ovule rate of seed set estimated under autopollination (Ennos, 1981) and the rates observed in this study respectively. Spears (1983) observed no further increase in seed set in flowers receiving more than one visit. If single pollinations are as effective in *I. purpurea*, then the direct effects of time delay and pollination order observed in this study should also occur in natural populations.

In experimental populations of *I. purpurea* stigmas are well covered after a few pollinator visits. Application of two anthers (about 300 pollen grains) also resulted in fairly complete coverage of the stigma (Methods and Materials). In natural populations of *I. pandurata*, Stucky and Beckmann (1982) found daily pollen loads on stigmas to range from 124 to 428, but it is not clear how many visits by bumblebees had occurred. These loads are comparable to pollen from 1 to 3 anthers of *I. purpurea*.

Other evidence suggests that the first few pollinations predominate in populations. The results of one experiment in which the sequence of all pollinator visits to flowers of genetically marked plants was compared to progeny arrays, suggest that the first pollination predominates in fertilisation even when subsequent pollination visits followed within 20 minutes (Epperson, unpublished data). In addition, most anthers in a flower were almost devoid of pollen after a few visits. Thus, pollen loads on bees should decrease as the morning progresses.

The frequency of pollinator visits in populations of *I. purpurea* ranges from 1 to 20 visits per flower during a 2 hour period (average waiting time ranges from 2 hours to 6 minutes) on different days (Brown and Clegg, 1984; Epperson, unpublished data). The multiple visits per morning observed in some populations, combined with reduced effectiveness of late pollinations, means that many pollinations are ineffective in *Ipomoea*. If this is true of other plants, then patterns of pollinator visitation may be used to infer genetic transmission and gene flow only if the earliest pollinations, and the sequence of pollinations are included. For example, studies of gene flow based on deposition of marked (usually dyed) pollen must also account for the time of deposition. Pollinator behaviour may vary with the hour (in experimental populations which received relatively few visits, bees tended to fly slightly greater distances later in the day). Consequently, measures of gene flow based only on the number of visits and mean distance travelled may be biased.

Ineffective subsequent pollinations also increase the consanguinity of seeds within a capsule. Consequently, if seed dispersal is limited and there is an increased probability of pollination with geographic proximity, then the next generation should have a higher level of inbreeding and population subdivision than expected based on the isolation by distance model.

Consanguinity of progeny within a capsule also affects estimates of outcrossing rates based on progeny arrays. The simplest and most common estimation procedure assumes that the pollen parents of a progeny array from a single maternal plant are uncorrelated *i.e.*, that the progeny within an array are either half-sibs or self-sibs, not full-sibs.

Violations of this assumption can strongly bias estimates of t (Schoen and Clegg, 1984). Where ineffective subsequent pollinations are suspect, it is more appropriate to use models which include correlations of the paternal parentage, such as the estimation model of Schoen and Clegg (1984) which assumes that all progeny within a family share the same pollen parent.

Mechanisms of ineffective second pollination

The effect of pollination order may be simply due to the closer proximity of the initial (FIRSTFAM) pollen load to the stigmatic surface. Pollen which is not in contact with the stigmatic surface may fail to germinate. This is supported by the fact that pollen germination failure is the primary mechanism of interspecific sterility and self-sterility in many *Ipomoea* species (Martin, 1970*a*, *b*), and is apparently not due to deficiencies in signals originating within the pollen grains themselves, in at least some of the crosses. For example, Guries (1978) found that cross fertility among four species of *Ipomoea*, including *I. purpurea*, was not improved by adding irradiated compatible pollen *i.e.*, the mentor pollen technique. Furthermore, pollen of several *Ipomoea* species are difficult to germinate *in vitro* (Martin and Ortiz, 1966). Germination of *Ipomoea* pollen may require substances secreted by stigmas (Fujise, 1964). Sood *et al.* (1982) found that self-incompatibility can be overcome by the application of one component of stigmatory secretion, auxin, in *Ipomoea cairica*.

Regardless of the direct effects of pollination order, the results indicate that there are additional effects of time delay. The frequency of fertilisations by the second pollen donor decreased from 0·33 in Treatment 2, to 0·14, 0·07, 0·02 approximately with time delays of 30, 60, and 120 minutes respectively. The added effect of time delay may simply be due to an advantage or "head start" in the race of pollen tube growth, for the first pollen.

In the sweet potato (*Ipomoea batatas*), pollen grains begin to germinate within minutes, and pollen tubes begin reaching ovules within 4 hours (Martin and Cabanillas, 1966; Kokubu *et al.*, 1982). If pollen tube growth rates are comparable in *I. purpurea*, then the first pollen will have grown much closer to the ovules during the time delays. Considering the numbers of pollen grains per pollination utilised in this study, a 2 hour delay would ensure that most fertilisations are by the first pollination, even if there is substantial variation in tube growth rates within pollinations.

The effect of time delay is probably not caused merely by changes in conditions on the stigma, if *I. purpurea* reacts as sweet potato does. In the sweet potato the appearance of stigmas and the exudate contents of stigmas does not change for several hours after pollination, and pollen grains continue to germinate up to 3 to 4 hours after pollination (Martin and Cabanillas, 1966; Martin and Telek, 1971). There were no obvious changes in the appearance of stigmas by noon in these studies. In our previous experiences with *I. purpurea*, artificial pollinations have been successful when executed as late as 1200 hour.

Gametic competition in mixed pollen loads

The competitive ability of pollen was greatly reduced in members of some coloured flower families. Pollen in Family 4 is about one-half as effective as its competitors, and Family 5 is about one-eighth as effective. The analysis of competitive effects within each of these two families indicates that the deficiency is partially genetic. The deficiency cannot be entirely accounted for by genotype at the colour loci because pollen from individuals in Family 1 competes quite well. But the fact that Families 2 and 3 are also below average competitors suggests that the colour loci could play some role in the competitive response. This would also account for unexpected results in artificial populations. Specifically, Schoen and Clegg (1985) observed that although there were fewer pollinator visits to white flowers, male reproductive success was higher for white flowers than for dark-coloured flowers, contrary to theoretical expectations.

Regardless of the genetic mechanism underlying the competitive effect, these differences in competitive ability affect genetic transmission. The fact that some individual plants or flowers may have non-functional or partially functional pollen increases the likelihood that other plants predominate as male parents. This results in increased numbers of full-sibs within capsules and reinforces the sequential pollination effect.

The possible mechanisms accounting for differences in competive ability in Treatment 1 include production of inviable pollen, late anther dehiscence, reduced germination, and retarded pollen tube growth rates. Pollen grain production does vary considerably between anthers on a single flower, and may vary among individuals (Epperson, unpublished data). Differences in competitive ability must be due to gamete competition not embryo competition, because the average seed set

whenever Families 4 or 5 were used as pollen donors was approximately 5·5 out of a maximum of six seeds per capsule, and 98 per cent of the seeds were germinated and genotyped.

Effects of anther-stigma distance on outcrossing rate

Ennos (1981) established that autopollination rates are correlated with anther-stigma distance, and that anther-stigma distance is highly heritable. The results of this study extend the work of Ennos (1981) by establishing that anther-stigma distance affects the rate of outcrossing. Consequently, substantial genetic variation for anther-stigma distance and outcrossing must also occur in natural populations of morning glories (see Clegg and Epperson, 1985 for review of genetic variation in outcrossing rates).

Taken in their entirety, these investigations show that the transmission of male genes in plant populations is more complex than pollinator behaviour might suggest. Competition between pollen deposited simultaneously or sequentially on stigmas interacts with pollinator behaviour in shaping the pattern of male transmission. These factors in turn combine to influence inbreeding rates and population subdivision, and thus affect the direction and efficiency of evolution in plant populations.

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