

## ABSENCE OF POLLEN DISCOUNTING IN A GENOTYPE OF *IPOMOEA PURPUREA* EXHIBITING INCREASED SELFING

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**Abstract.**—Throughout southeastern North America, the annual morning glory *Ipomoea purpurea* exhibits a polymorphism at a locus that influences the intensity of floral pigmentation. Previous studies have shown that when rare, the homozygous white genotype has a greater selfing rate than the homozygous dark genotype. In the absence of pollen discounting (a reduction in transmission of pollen to other plants by genotypes that exhibit increased selfing) and inbreeding depression, this increased selfing rate should favor the white allele. Experiments reported here confirm that the white genotype has elevated selfing rates when rare but indicate pollen discounting is not associated with elevated selfing. Rather, white genotypes contribute more pollen to the outcross pollen pool. The disparity between genotypes in both selfing rates and success at pollen contribution to other plants disappears at intermediate to high frequencies of the white allele. Pollinator movements are consistent with the pattern of selfing. These results suggest that elevated selfing and enhanced success at pollen donation contribute to maintenance of the white allele in natural populations of morning glories.

**Key words.**—*Bombus*, *Ipomoea*, outcross rates, pollen discounting, polymorphism, selfing, selfing rates.

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Fisher (1941) demonstrated theoretically that in the absence of inbreeding depression a gene that increases the rate of selfing of an individual that carries it will increase to fixation in a population. However, several authors have shown that this tendency toward fixation can be retarded or even prevented by pollen discounting, the association of increased selfing with a reduction in transmission of pollen to other plants (Wells 1979; Lloyd 1979; Holsinger et al. 1984). One mechanism that can, at least in theory, produce such an association is pollination by insects or other animal vectors. Because reduced visitation may cause less pollen to be removed by pollinators and transmitted to other plants (e.g., Stanton et al. 1986), increased selfing achieved by decreased visitation by pollinators and a concomitant reduction in the deposition of foreign pollen on the stigma may lead to pollen discounting. The extent to which such discounting influences the evolution of floral characteristics, however, is unknown.

Throughout southeastern North America, the annual morning glory *Ipomoea purpurea* exhibits a polymorphism at a locus that influences the intensity of floral pigmentation (the “W” locus of Ennos and Clegg 1983). The alleles, designated “W” and “w,” are codominant, with the genotypes WW, Ww, and ww producing darkly pigmented, lightly pigmented, and white flowers,

respectively. In a study of 17 populations from North Carolina, South Carolina, and Georgia, the w allele ranged in frequency between 0 and 0.43, with a mean of approximately 0.11 (Epperson and Clegg 1986).

Previous studies have indicated that variation in floral pigmentation associated with this polymorphism affects the mating system of *I. purpurea*. When white-flowered plants (ww genotype) are in the minority, they are visited by pollinators (primarily bumblebees) less frequently than are pigmented flowers (Ww and WW genotypes; Brown and Clegg 1984; Epperson and Clegg 1987a). Presumably as a consequence of this less frequent visitation, whites also exhibit greater selfing rates when rare (Epperson and Clegg 1987a). These effects are frequency dependent, however, because when whites are common, the rate at which they are visited by pollinators and their selfing rate do not differ from those of darks (Epperson and Clegg 1987a).

Pear (1983) has provided evidence suggesting that inbreeding depression is minimal in *I. purpurea*. Consequently, the evolutionary consequences of differences among the W-locus genotypes in selfing rates may depend largely upon the degree of pollen discounting that is associated with increased selfing by whites. In particular, in the absence of complete pollen discounting, differences in visitation and selfing rates should

contribute a net advantage to the *w* allele when it is rare, helping to maintain it in a population. By contrast, if pollen discounting is extensive, there may be little or no net selection favoring the *w* allele when rare, and hence effects of the *W* locus on other fitness components are more likely to explain protection of the *w* allele.

The experiments reported here were designed to assess the role of pollen discounting in contributing to the maintenance of the *W*-locus polymorphism. In particular, we attempted first to confirm prior results that white-flowered plants when rare self more than dark-flowered plants and that this difference in selfing rate is accompanied by greater bee visitation to darks. We then attempted to determine whether increased selfing by whites is accompanied by pollen discounting by estimating the outcrossing rate of and proportion of outcrossed seed pollinated by each genotype.

#### MATERIALS AND METHODS

*Experimental Arrays.*—The general design of the experiment reported here is similar to that of Epperson and Clegg (1987a). On each of 16 d between June 25 and August 1, 1991, an experimental array consisting either of 16 dark (*WW*) and 4 white (*ww*) plants (dark-majority arrays) or 16 white and 4 dark plants (white-majority arrays) was set up in an old agricultural field with a native population of *Ipomoea purpurea* (the same field used by Simms and Rausher 1987, 1989; Rausher and Simms 1989; and Rausher and Fry 1993). The field had been disked in late May, then mowed in late June to prevent flowering by native morning glories during the experiment. In addition, native morning glories within a radius of about 500 m from the field were removed or prevented from flowering to minimize the possibility of pollen flow from other sources into the experimental arrays.

On the afternoon of the day before an array was to be run, the plants were transported from greenhouses at Duke University to the experimental field and were placed randomly at positions on a 5 × 4 grid with a spacing of 1 m between plants in each direction. All but one flower bud were removed from each plant. The plants were watered and left overnight in the field. Beginning about 7:00 the following morning, an observer recorded bee visits to plants in the experimental array (see below) until about 11:00 A.M. The flowers were then individually

tagged, and the plants were transported back to the greenhouses to allow seed to develop. As the tagged seed capsules matured, they were collected for later scoring of seed genotypes. A total of 230 and 217 seeds were scored from dark-majority and white-majority arrays, respectively.

To produce the experimental plants, crosses were performed between 14 pairs of dark and white field-collected plants (great grandparents). From each pair, one offspring (grandparent) that was also heterozygous at an esterase marker locus was allowed to self. The resulting offspring (potential parents) were screened for genotype at the *W* locus and at the marker locus. From each original great-grandparental pair, three parents of each double-homozygote genotype were allowed to self, and three seeds were collected from each parental plant. This series of crosses thus generated a pool of 126 (= 14 × 3 × 3) plants of each *W*-locus × esterase double homozygote genotype combination in which the background genetic variation was randomized. Plants for the experimental arrays were selected from this pool by choosing, for both darks and whites, equal numbers of the two marker homozygotes from among those plants having at least one flower that would open the following day.

*Bee Visitation.*—One species of bumble bee, *Bombus pennsylvanicus*, accounted for more than 98% of all observed pollinators in the experimental arrays. Individual bees visiting plants in the arrays were followed for up to 10 min or until they left the array. Using a portable computer, an observer recorded the identity of each flower visited, as well as the time the bee landed on the flower.

*Estimation of Outcrossing and Discounting Rates.*—For a given array type, two different parameters were estimated and compared for each intensity genotype using a maximum-likelihood analysis. One parameter, *t*, is the outcrossing rate, that is, the proportion of seeds set that result from outcrossing. The second parameter, *θ*, is the proportion of outcrossed seed produced by a given genotype that were pollinated by plants of the majority intensity genotype. Thus, in dark-majority arrays, *θ* is the proportion of outcrossed seed that was pollinated by dark plants. A value of *θ* greater than the proportion of potential dark pollen donors in those arrays (i. e., 15/19 = 0.789) would indicate the occurrence of pollen discounting, because it would mean that white plants are contributing less pollen to the outcross pool than expected.

TABLE 1. Probability ( $\gamma_{ij}$ ) of observing a particular offspring genotype for a seed produced by a particular maternal genotype.  $t$  is the probability of outcrossing.  $\theta$  is the probability that outcross pollen carries the majority allele. In genotype designations, M and m stand for the majority and minority alleles, respectively. In subscripts, M and m stand for majority and minority intensity genotype, respectively.

Maternal genotype ( $i$ )	Offspring genotype ( $j$ )								
	MMFF (1)	MMFS (2)	MMSS (3)	MmFF (4)	MmFS (5)	MmSS (6)	mmFF (7)	mmFS (8)	mmSS (9)
MMFF (1)	$(1 - t_M) + \frac{7}{15}t_M\theta_M$	$\frac{8}{15}t_M\theta_M$	0	$\frac{1}{2}t_M(1 - \theta_M)$	$\frac{1}{2}t_M(1 - \theta_M)$	0	0	0	0
MMSS (2)	0	$\frac{8}{15}t_M\theta_M$	$(1 - t_M) + \frac{7}{15}t_M\theta_M$	0	$\frac{1}{2}t_M(1 - \theta_M)$	$\frac{1}{2}t_M(1 - \theta_M)$	0	0	0
mmFF (3)	0	0	0	$\frac{1}{2}t_m(1 - \theta_m)$	$\frac{1}{2}t_m(1 - \theta_m)$	0	$(1 - t_m) + \frac{2}{3}t_m\theta_m$	$\frac{2}{3}t_m(1 - \theta_m)$	0
mmSS (4)	0	0	0	0	$\frac{1}{2}t_m(1 - \theta_m)$	$\frac{1}{2}t_m(1 - \theta_m)$	0	$\frac{2}{3}t_m(1 - \theta_m)$	$(1 - t_m) + \frac{1}{3}t_m\theta_m$

The probabilities,  $\gamma_{ij}$ , that maternal plants of genotype  $i$  produce offspring of genotype  $j$ , expressed in terms of  $t$  and  $\theta$ , are listed in table 1. In this model, the probability of selfing,  $1 - t$ , includes all selfing events. Under a standard mixed-mating model, there is some finite probability (equal to  $1/N$ , where  $N$  is population size) that pollen from the outcross pollen pool will be self pollen. It therefore might be desirable, in modeling a mixed mating system, to delete these selfing events from the estimate of  $1 - t$ . This can be done by changing the values  $7/15$ ,  $8/15$ ,  $1/3$ , and  $2/3$  in table 1 to  $1/2$ . Although we have also analyzed this modified model, the results are qualitatively and quantitatively very similar to those obtained by the model portrayed in table 1 and lead to the same conclusions. We therefore do not also report the results from the modified model.

The most general version of the probability model allows dark and white plants to differ in both  $t$  and  $\theta$ . Consequently, these parameters are subscripted to indicate whether they correspond to the majority or the minority genotype. The overall likelihood of observing the data is then

$$L = C \prod_{i,j} (\gamma_{ij})^{N_{ij}},$$

where  $C$  is a constant and  $N_{ij}$  is the number of seeds of genotype  $j$  produced by maternal plants of genotype  $i$ . Estimates of the  $t$ s and  $\theta$ s and their standard errors were obtained from this likelihood model by implementing standard maximum-likelihood techniques (Edwards 1972) on Mathematica software (Wolfram 1991).

Tests of whether majority and minority genotypes differed in outcrossing rates and  $\theta$  were performed by comparing the likelihood,  $L_U$ , of the four-parameter (unrestricted) model with the likelihood,  $L_R$ , of a restricted model in which one or both of the restrictions  $t_M = t_m$  and  $\theta_m = \frac{1}{15}\theta_M$  were present, using the standard log-likelihood ratio statistic

$$\chi^2 = 2 \ln \left[ \frac{L_U}{L_R} \right],$$

which has an approximately chi-square distribution with degrees of freedom equal to the number of restrictions (Hocking 1985).  $\theta_m = \frac{1}{15}\theta_M$  is the appropriate restriction, rather than  $\theta_m = \theta_M$ , because under random outcrossing the expectation that a foreign pollen grain landing on the stigma of a plant of the majority genotype carries the majority allele is  $15/19$ , whereas the same probability for a plant of the minority genotype is  $16/19$ . Consequently, the expectation for plants of the minority genotype is  $[16/19] \div [15/19] = 16/15$  times the expectation for the majority genotype. In addition, hypotheses about whether parameters  $t$  or  $\theta$  are equal to particular values were tested using a similar log-likelihood ratio statistic, in which the restricted likelihood was calculated using the hypothesized value of the parameter(s).

## RESULTS

### Bees Visitation

A total of 1520 visits by bees were observed during the study. For neither the dark-majority

TABLE 2. Bumblebee visitation to white and dark morphs in dark-majority and white-majority arrays. Expected visits to white flowers equal the proportion of white plants in the plot multiplied by the total number of visits to the plot.

Date (mo/day)	All bees				Bees observed before 9:00 A.M.			
	Total no. of visits	Visits to white flowers			Total no. of visits	Visits to white flowers		
		Expected	Observed	$\chi^2$		Expected	Observed	$\chi^2$
Dark-majority arrays								
6/25	78	15.6	6	7.48**	46	9.2	3	5.22*
6/26	120	24.0	21	0.47	68	13.6	13	0.03
7/03	48	9.6	11	0.26	22	4.4	5	0.10
7/10	146	29.2	30	0.03	101	20.2	16	1.09
7/17	146	29.2	28	0.06	112	22.4	20	0.32
7/19	108	21.6	19	0.39	77	15.4	12	0.94
7/24	70	14.0	10	1.43	62	12.4	7	2.94
8/01	112	22.4	24	0.14	80	16.0	17	0.78
Pooled	828	165.6	149	2.08	568	113.6	93	4.67*
White-majority arrays								
6/27	12	9.6	8	1.33	12	9.6	8	1.33
6/28	94	75.2	68	3.45	75	60.0	57	0.75
7/02	12	9.6	10	0.08	12	9.6	10	0.83
7/12	70	56.0	52	1.43	70	56.0	52	1.43
7/16	184	147.2	154	1.57	112	89.6	94	1.08
7/18	133	106.4	100	1.92	109	87.2	80	2.97
7/26	88	68.8	67	0.24	69	55.2	54	0.13
7/31	103	82.4	84	0.16	64	51.2	50	0.14
Pooled	692	555.2	543	1.34	523	418.4	405	2.15

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

nor the white-majority arrays was there any strong indication that bees visited the intensity genotypes other than randomly when data from all bees were analyzed (table 2). For dark-majority arrays, on 1 of 8 d bees significantly undervisited white-flowered plants, but on the remaining days the two genotypes were visited in proportion to their representation in the arrays. When pooled over days, there is also no indication that bees preferred one intensity genotype. For white-majority arrays, deviations from random visits were not significant on any of 8 days, nor for the data when pooled over days.

Epperson and Clegg's (1987a) detection of undervisitation of whites was based on observations of bees for a 2-h period after plants were exposed to pollinators. To make our analysis more comparable to theirs, we reanalyzed our data on bee visitation, including only those bees on which observation was begun before 9:00 A.M. Because bees began visiting our arrays between 6:30 A.M. and 7:00 A.M., this restricted data set included only bees that visited the experimental plants within 2–2½ h of the beginning of pollinator visitation, a period similar to that of Epperson and Clegg's.

For the dark-majority arrays, there is an indication that during the first part of the morning, bees undervisited white plants. Bees on which observation was begun before 9:00 A.M. exhibited significant undervisitation of white flowers (table 2). By contrast, bees on which observation was begun after 9:00 A.M. exhibited a nonsignificant tendency to overvisit white flowers (table 2, by subtraction). The difference between these two periods approaches significance ( $G = 3.15$ ,  $df = 1$ ,  $P \approx 0.08$ ), and on 7 of 8 d the proportion of visits to darks was less after 9:00 A.M. than before 9:00 A.M. ( $P = 0.035$ ). For white-majority arrays, there was no evidence of nonrandom visitation for bees before 9:00 A.M. (table 2,  $G = 2.15$ ,  $df = 1$ ,  $P > 0.10$ ).

Also for the dark-majority arrays, there is a suggestion that whether a flower visited is a white or a dark one depends upon whether the previous flower visited was white or dark (table 3). In particular, a bee currently on a white flower has a greater probability of visiting a dark flower than does a bee currently on a dark flower. With a standard contingency test of homogeneity of transition probabilities, this effect approaches significance ( $G = 3.38$ ,  $P \approx 0.075$ ). However,

TABLE 3. Observed frequencies of the four possible types of successive visits by bumblebees to flowers in the experimental arrays. Data pooled over all dates for each type of array.

First visit	Second visit					
	Dark-majority arrays			White-majority arrays		
	Dark	White	Proportion dark	Dark	White	Proportion white
All bees						
Dark	458	113	0.802	25	107	0.811
White	113	17	0.869	112	348	0.756
Bees observed before 9:00 A.M.						
Dark	322	74	0.813	18	81	0.811
White	77	6	0.928	82	268	0.766

some difference of this type is expected just because of the small size of the arrays. When a bee is on a dark-flowered plant, the proportion of darks among plants to which the bee could move is  $1/9$ . By contrast, when a bee is on a white-flowered plant, the proportion of darks among plants to which the bee could move is  $1/9$ . Thus, even if bees move randomly between plants, there should be a weak association between current flower intensity and previous flower intensity. When observed frequencies of transitions are tested against these expected frequencies, there is no indication that bees move nonrandomly between plants ( $\chi^2 = 1.268, P > 0.5$ ). For those bees on which observation was begun before 9:00 A.M., there is a suggestion that bees currently visiting a white flower are more likely to visit a dark flower next than are bees currently visiting a dark flower (table 3), but this trend is not quite

TABLE 4. Estimates of outcrossing rates ( $t$ ) and proportion of outcrossed seed pollinated by the majority genotype ( $\theta$ ). Standard errors of estimates are in parentheses. Subscripts M and m refer to estimates for majority and minority genotypes, respectively. Expected values for  $\theta$  calculated as proportion of majority-genotype plants among potential outcross pollen donors.

Parameter	Dark-majority arrays		White-majority arrays	
	Expected	Observed	Expected	Observed
$t_M$	—	0.964 (0.065)	—	0.674 (0.063)
$t_m$	—	0.685 (0.081)	—	0.711 (0.078)
$\theta_M$	0.789	0.829 (0.031)	0.842	0.793 (0.041)
$\theta_m$	0.842	0.595 (0.092)	0.789	0.812 (0.082)

TABLE 5. Log-likelihood ratio statistics ( $2 \ln[L_U/L_R]$ ) for pairwise comparison of models with different numbers of restrictions. Statistics have an approximate  $\chi^2$  distribution with degrees of freedom equal to the difference in number of constraints in the model.

Restrictions on $L_U$	Restrictions on $L_R$	Dark-majority arrays	White-majority arrays
none	$t_M = t_m$	7.40**	0.14
none	$\theta_m = 1/15 \theta_M$	10.54***	0.14
none	$t_M = t_m$ $\theta_m = 1/15 \theta_M$	20.57***	0.26
$t_M = t_m$	$t_M = t_m$ $\theta_m = 1/15 \theta_M$	13.18***	0.12
$\theta_m = 1/15 \theta_M$	$t_M = t_m$ $\theta_m = 1/15 \theta_M$	10.04***	0.12

\*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

significant ( $\chi^2 = 5.91, P \approx 0.052$ ). In the white-majority arrays, there is no evidence that transition probabilities are heterogeneous for bees on dark versus white flowers (all bees,  $\chi^2 = 3.99, P > 0.10$ ; bees observed before 9:00 A.M.,  $\chi^2 = 1.62, P > 0.25$ ).

*Outcrossing and Discounting Rates*

In the white-majority arrays, white and dark plants exhibited outcrossing rates (table 4) that did not differ significantly (table 5). Moreover, the proportion of outcross pollen carrying the dark allele did not differ from expectation based on the relative numbers of dark and white plants in the arrays (tables 5, 6). In other words, dark and white plants made similar per capita contributions to the outcross pollen pool.

In the dark-majority arrays, the outcrossing rate was considerably higher in dark plants than in white plants (table 4). The significance of this difference was evaluated in two ways: (1) by a log-likelihood ratio statistic comparing a model in which all four parameters were free to vary with a model in which  $t_M$  was constrained to be equal to  $t_m$ , and (2) by a log-likelihood ratio statistic comparing two models that both included the constraint  $\theta_m = 1/15 \theta_M$ , but that differed in whether the constraint  $t_M = t_m$  was included. In both cases, the addition of the  $t_M = t_m$  constraint significantly decreased the likelihood associated with the model (table 5), that is, dark plants had significantly greater outcrossing rates than white plants.

In the dark-majority arrays, the proportion of pollen fertilizing outcrossed seeds that carried

the dark allele differed significantly for dark and white plants (table 5; significance of  $\theta_m = 1/15\theta_M$  restriction). The estimated proportion of pollen carrying the dark allele in outcrossed seeds of dark plants was approximately equal to the proportion expected based on the relative number of plants of each color type (0.829 estimated versus 0.789 expected). However, the proportion of dark pollen in outcrossed seeds of white plants in the same arrays was considerably less than expected (0.595 estimated versus 0.842 expected). The deviations from expectation were highly significant for white plants but not for dark plants (table 6). It thus appears that white plants were more successful than dark plants on a per capita basis at pollinating other white plants, whereas the two genotypes were equally successful at pollinating dark plants.

#### DISCUSSION

The pattern of selfing exhibited by our experiments is similar to that observed by Epperson and Clegg (1987a) in two important ways: (1) when white genotypes are in the minority, they exhibit higher selfing rates than darks, and (2) this difference disappears when whites are in the majority, that is, the disparity in selfing rates among whites and darks is frequency dependent. Our ability to repeat the results of Epperson and Clegg at a different locality using plants of independent origin suggests that these two properties are general characteristics of the breeding system of *Ipomoea purpurea*, at least when grown in small arrays of the type employed here.

Although whites, when rare, selfed more frequently than darks, our experiments indicate that pollen discounting did not occur. The failure to find pollen discounting suggests that increased selfing by whites when rare may contribute to protection of the white allele against elimination from *I. purpurea* populations. As demonstrated by Fisher (1941), in the absence of pollen discounting and inbreeding depression, a gene that enhances selfing should increase in frequency when rare and spread to fixation. Our demonstration of the lack of pollen discounting, coupled with Pear's (1983) failure to detect inbreeding depression in *I. purpurea*, suggests that the process envisioned by Fisher may be operating in natural populations of morning glories in which the white allele is at low frequency. This inference must be viewed as tentative, however, because Pear's estimates of inbreeding depression were obtained from potted plants grown in a

TABLE 6. Log-likelihood ratio statistics for tests of hypotheses that contribution by the majority genotype to outcross pollen pool fertilizing seeds of majority ( $\theta_M$ ) or minority ( $\theta_m$ ) genotype is proportional to representation of the majority genotype in set of potential outcross pollen donors. Statistics have an approximate  $\chi^2$  distribution with 1 degree of freedom.

Arrays	$\theta_M$ unconstrained versus $\mu_M = 15/19$	$\theta_m$ unconstrained versus $\mu_m = 16/19$
Dark-majority	1.43	9.38***
White-majority	0.01	0.14

\*  $P < 0.001$ .

greenhouse. It is possible that plants grown under field conditions would exhibit inbreeding depression that is not expressed under presumably more benign greenhouse conditions.

Rather than pollen discounting, our experiments indicated that white-flowered plants were more successful at transmitting pollen to other white plants than were dark-flowered plants, giving white an overall transmission advantage in the outcross pollen pool. This observation is consistent with the finding by Schoen and Clegg (1985) that white plants contributed more pollen than dark plants to the outcross pollen pool in experiments in which whites and darks were equally abundant. The greater outcrossing success of whites when rare (our experiments) and at intermediate frequencies (Schoen and Clegg 1985) should also contribute to an increase in the frequency of the white allele when rare.

The observed frequency dependence of disparity in both selfing rates and outcrossing success among whites and darks, however, suggests that the white allele will usually not be carried to fixation as envisioned by Fisher. Our experiments indicate that selfing rates become equal before the frequency of the white allele reaches 0.8, and the experiments of Epperson and Clegg (1987a) indicate that this occurs even before a frequency of 0.5 is reached. At whatever frequency equality of selfing rates is achieved, the transmission advantage favoring further increase in the frequency of the white allele is eliminated. Moreover, our experiments indicate that at some frequency of the white allele below 0.8 the outcross transmission advantage associated with white plants also disappears. Nevertheless, this frequency dependence cannot by itself explain the widespread persistence of W-locus polymorphism. If no other processes are operating, then

at moderate to high frequencies of the *w* allele the lack of difference in selfing and outcross transmission rates would render variation at the *W* locus neutral. Genetic drift would thus tend to eliminate the dark allele. In general, the range of gene frequencies expected in natural populations would be on the order of 0.5 to 1.0 for the white allele, with a mean approaching 1.0. Because observed frequencies range from 0.0 to approximately 0.5, with a mean of approximately 0.1, however (Epperson and Clegg 1986), it is clear that some other process is opposing fixation of the white allele by drift. This reasoning suggests that selection favoring the dark allele at other stages of the life cycle is likely occurring, although differential survival and differential fecundity do not appear to contribute to such selection (Rausher and Fry, 1993).

Epperson and Clegg (1987a) found clear evidence that white flowers were undervisited in dark-majority arrays. Such undervisitation would explain the increased selfing of whites, because fewer visits would mean less movement of outcross pollen onto the stigmas of white flowers. Our observations of pollinator behavior are more equivocal but are consistent with those of Epperson and Clegg. Although data from all bees observed provided no evidence of undervisitation of whites, data from bees observed early in the morning (before 9:00 A.M.) did provide such evidence. It appears that there may have been a shift in bee behavior during the morning. Such a trend would be consistent with bees having become less selective as the supply of nectar in the arrays became scarce. Moreover, some evidence indicates that precedence of only a few minutes in deposition gives pollen a strong competitive advantage in *I. purpurea* (Epperson and Clegg 1987b). It is thus likely that the behavior of bumblebees early in the morning governs the pattern of pollen transfer, because virtually all plants are visited well before 9:00 A.M. on most days.

We had expected that fewer visits to whites in the dark-majority arrays would mean not only that less foreign pollen would be brought to white plants, resulting in a reduction in outcrossing rate, but also that less pollen would be exported, resulting in lower success at pollen donation, that is, in pollen discounting. One possible explanation for our failure to detect discounting is that the number of pollen grains imported and the number exported by a flower are related in different ways to the number of visits that flower

receives. For example, Young and Stanton (1990) show that the amount of pollen removed from wild radish (*Raphanus sativa*) plateaus quickly as the number of bee visits increases, whereas the number of pollen grains deposited on the stigma increases linearly with number of visits. If a similar pattern occurs in *I. purpurea*, that is, if a single visit removes most pollen from a flower, but deposits only a few pollen grains, then even if whites are visited somewhat less frequently than darks, all plants of both intensity genotypes will have had their outcross pollen deposited on bees very early in the morning, because most flowers will be visited at least once by that time. By contrast, it would not be until later in the morning, after many more visits, that all pollen grains destined to fertilize ovules would be deposited by bees. If the difference between time of removal of most pollen and time of deposition of most pollen is great, then for most of the morning bees will be carrying pollen with dark and white alleles in proportion to the numbers of dark and light plants in the array. There would still be a small degree of pollen discounting arising from the very earliest pollinations when bees are disproportionately carrying pollen from dark plants. However, this small amount of discounting would be difficult to detect in the experiment reported here and probably would be insufficient to counteract the transmission advantage associated with increased selfing by whites (Wells 1979; Holsinger et al. 1984). Pollen dynamics of this sort would also yield no pollen discounting in the white-majority arrays, because rates of both pollen removal and deposition for the two genotypes would be equal because visitation rates are equal. A second possible explanation for our failure to observe pollen discounting is that the increased selfing of whites when rare is not caused by undervisitation by bees but by some other mechanism that has no expected link with pollen export.

We are unable to suggest a simple hypothesis about pollen dynamics that can explain why white plants export more successful pollen when they are in the minority but not when they are in the majority. Greater per-flower production of pollen could explain the former but should lead to greater export of pollen in the white-majority arrays as well. Moreover, the trend in successive bee visits would lead to the opposite expectation: the enhanced probability of a bee on a dark flower moving to a white flower, compared with the probability for a bee on a white flower, suggests

that outcrossed white flowers should be disproportionately pollinated by darks rather than by whites. A solution to this puzzle, as well as determination of the reason for lack of pollen discounting, must await further examination of the dynamics of pollen transfer in morning glories.

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