

SELECTION ON A FLORAL COLOR POLYMORPHISM IN THE TALL MORNING GLORY (*IPOMOEA PURPUREA*): TRANSMISSION SUCCESS OF THE ALLELES THROUGH POLLEN

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Abstract.—The *W* locus, a codominant locus influencing floral pigment intensity in the tall morning glory, *Ipomoea purpurea*, is polymorphic throughout the southeastern United States. Previous studies suggest that this polymorphism is actively maintained by balancing selection, and that increased selfing accompanied by lack of pollen discounting (“Fisher effect”) may act to protect the white allele when it is rare. Processes that act to protect the dark allele and thus stabilize the polymorphism in conjunction with the Fisher effect have not been previously detected. The goal of this study was to determine whether any of three such processes might operate in *I. purpurea*. Estimates of breeding system parameters in a large experimental population in which the white allele was in higher than normal frequency (0.5) provided little evidence that either dark- or light-flowered plants were more successful as pollen parents than white-flowered plants. In addition, no evidence was found for a transmission bias favoring the dark allele in the ovules produced by light heterozygotes. In contrast, a strong transmission bias favoring the dark allele in pollen of heterozygotes was observed. A simple model using parameter estimates derived from this and previous studies indicates that a balance between the Fisher effect and biased transmission in heterozygote pollen could account for many properties of the polymorphism.

Key words.—Balancing selection, genetic polymorphism, pollinator behavior, segregation distortion, self-fertilization.

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A major focus of evolutionary biology over the past 50 years has been accounting for the maintenance of genetic variation in natural populations (Hartl and Clark 1989). Evidence has accumulated indicating that for certain traits, such as self-incompatibility systems (Charlesworth and Charlesworth 1979; Clark 1993; Vekemans and Slatkin 1994; Richman et al. 1995) and possibly disease resistance (Ennos 1983; Antonovics 1994), inherently frequency dependent selection maintains variation. For most traits, however, selection is not obviously frequency-dependent, and accounting for variation in these traits has been a major challenge. Because our understanding of why organisms are so genetically variable (e.g., Lewontin 1974; Wright 1977; Powell and Taylor 1979; Hedrick 1986) is at best rudimentary, detailed examination of the selective forces acting on genetic variation in a variety of organisms is required to determine which processes most commonly operate to preserve variation in nature. This report constitutes a contribution toward our long-term objective of conducting such an examination on genetic variation influencing floral characteristics in the annual morning glory, *Ipomoea purpurea*.

Four major unlinked loci influence floral pigment hue and intensity in *I. purpurea* (Ennos 1981; Ennos and Clegg 1983; Epperson and Clegg 1988). Here we concentrate on the *W* locus, which affects pigment intensity and is generally polymorphic in populations throughout the southeastern United States. Plants homozygous for the *w* allele have white flowers with pigmented rays (whites); those homozygous for the *W* allele have darkly pigmented flowers that have even more intensely pigmented rays (darks); and heterozygotes have lightly pigmented flowers with dark rays (lights). Frequencies

of the white allele are typically 0–0.4, with a mean of about 0.1, in natural populations that have been surveyed (Epperson and Clegg 1986).

Recent convergence experiments indicate that the *W* locus is subject to some form of balancing selection (Subramaniam 1994). Moreover, previous studies of the selective forces acting on the *W*-locus polymorphism have suggested a possible mechanism protecting the white allele. When white-flowered plants are in low frequency, they exhibit increased rates of self-fertilization compared to dark- and light-flowered plants (Brown and Clegg 1984; Epperson and Clegg 1987a; Rausher et al. 1993), apparently due to reduced visitation by bumblebees, which are virtually the sole pollinator (Ennos 1981; Brown and Clegg 1984; Stucky 1984; Epperson and Clegg 1987a; Rausher et al. 1993). In addition, current evidence indicates that when in low frequency, white plants do not experience pollen discounting (a reduction in transmission of pollen to other plants by genotypes that exhibit increased selfing; Rausher et al. 1993), nor is the magnitude of inbreeding depression more than about 10% (Pear 1983; S.-M. Chang, pers. comm. 1995). As originally shown by Fisher (1941; see also Wells 1979; Lloyd 1979; Holsinger et al. 1984), under these conditions, the white allele, which is essentially an allele that increases the rate of selfing, should tend to increase in frequency. This increase, which we term the “Fisher effect,” arises because white-flowered plants transmit more copies of the white allele to the next generation through self pollen than dark plants transmit copies of the dark allele, assuming all three genotypes are equally successful at transmitting alleles at the *W* locus through ovules and outcross pollen.

An important result of previous studies (Epperson and Clegg 1987a; Rausher et al. 1993) is that this advantage of the white allele is frequency dependent. When the frequency of white-flowered plants in a population reaches 0.5, bees no longer

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discriminate between color morphs and selfing rates of the *W*-locus genotypes are equal. Thus, at low frequencies of the white allele, the Fisher effect tends to cause the frequency of that allele to increase, whereas at high frequencies of the white allele, this effect disappears.

The Fisher effect has been observed to operate in an experimental population in which 20% of the plants were white-flowered (Rausher et al. 1993). If the Fisher effect were the only selective process affecting variation at the *W* locus, frequencies of white plants in natural populations should be higher than 20%. Because most populations have fewer than 10% whites (Epperson and Clegg 1986), however, it seems almost certain that some other selective process(es) operate that favor the dark allele. This inference is consistent with an observed increase in the frequency of the dark allele in experimental populations when that allele is at low initial frequency (Subramaniam 1994).

The frequency dependence of the Fisher effect suggests that any factor that provides an advantage to the dark allele, *whether that advantage is frequency dependent or not*, may act in concert with the Fisher effect to stabilize the *W*-locus polymorphism. This stabilization can occur because at high frequencies of the white allele, only the dark-allele advantage would operate, thus tending to protect the dark allele. At low frequencies, by contrast, the Fisher effect could protect the white allele, as long as the Fisher effect is stronger than the dark-allele advantage when the white allele is rare.

Previous investigations of selection acting at the *W* locus have found no evidence of any mechanism that might provide such a dark-allele advantage. In particular, Rausher and Fry (1993) found no evidence of a dark-allele advantage either for viability or for seed production, though there was some indication that there might be overdominance in seed size. Here, we consider three other possibilities that have been insufficiently investigated.

Superiority of Darks to Whites in Success as Pollen Parents.—If, when white plants are in relatively high frequency, darks have higher net success as pollen parents than whites, this would tend to reduce the frequency of the white allele, potentially stabilizing the polymorphism in conjunction with operation of the Fisher effect when whites are in low frequency. Although two previous studies have compared the success of darks and whites as pollen parents, neither found evidence for superiority of darks (Schoen and Clegg 1985; Rausher et al. 1993). Both studies, however, were based on small arrays of potted plants that were grown in the greenhouse, trimmed to have equal numbers of flowers, and exposed to pollinators for a single morning. Such artificial conditions may not allow full expression of differences between the genotypes that could affect their success as pollen parents under more natural conditions, such as differences in frequency of flowering.

Superiority of Lights to Whites in Success as Pollen Parents.—If, when white plants are in high frequency, lights have greater success as pollen parents than whites, this could also protect the dark allele. No study has examined the success of lights as pollen parents, although Brown and Clegg (1984) showed that lights and darks apparently do not differ in selfing rate. It is also possible that lights are superior to darks as

pollen parents; like the Fisher effect, such a difference would tend to protect the white allele.

A Non-Mendelian Transmission Bias Favoring the Dark Allele in Pollen Produced by Heterozygotes, and/or a Similar Non-Mendelian Bias in Ovules of Heterozygotes.—Clegg and Epperson (1988) mention unpublished observations of departures from Mendelian ratios at the *W* locus. As we will show below, a transmission bias favoring the *W* allele, in conjunction with the Fisher effect, could stabilize the polymorphism.

To determine whether any of these processes are operating, we analyzed the breeding system of an experimental population of *I. purpurea*. In this population, the frequency of whites (= 0.33) was by design higher than that observed in the vast majority of natural populations. We therefore anticipated that the Fisher effect would be weak or absent and that the only important processes acting on the *W* locus would be those affording a dark-allele advantage. We also conducted observations on pollinator visitation, which potentially provide insight into any differences in breeding-system parameters we may observe between the *W* locus genotypes. These observations also permit a simplification of the estimation of breeding system parameters, as described below.

MATERIALS AND METHODS

Experimental System

The tall morning glory, *I. purpurea* (L.) Roth is a weedy annual that is common in agricultural fields and other disturbed sites throughout southeastern North America. Plants typically germinate between late May and August, depending upon timing of soil disturbance and rainfall, and are killed by the first frost, which ranges from late September through early December in the piedmont of North Carolina. The showy flowers open at dawn and close and wither by late morning of the same day.

Experimental Plants

The experimental population used in this study was the same as that described in Rausher and Fry (1993; see this reference for details of crossing and planting designs). In this experiment, equal numbers of dark (*WW*), light (*Ww*), and white (*ww*) plants (over 2600 in all) were planted at the seedling stage in a randomized-block array in early July 1990. To facilitate progeny analysis, the experimental plants were bred so that all dark and white plants were homozygous for the fast allele of an electrophoretically detectable esterase polymorphism (Ennos and Clegg 1983), and all light experimental plants were homozygous for the slow esterase allele. This arrangement allows unambiguous identification of the paternal genotype of progeny of the experimental plants in most cases from their esterase and *W*-locus genotypes (Fig. 1; the one ambiguous case is genotype 6, the double heterozygote produced on light seed parents). The crossing scheme (Rausher and Fry 1993) should have effectively randomized the genetic background of the experimental plants with respect to genes not linked to either the *W* locus or the esterase locus. All of the plants in the experiment had the blue (*P*-) and nonintense (*I*-) phenotypes described by Ennos and Clegg (1983). During

		Pollen Parent		
		D	L	W
		<i>FF</i>	<i>SS</i>	<i>FF</i>
Seed Parent	D	<i>FF</i>	<i>WWFF</i> (1) <i>WWFS</i> (2) <i>WwFS</i> (3)	<i>WwFF</i> (4)
	L	<i>SS</i>	<i>WWFS</i> (5) <i>WwFS</i> (6)	<i>WWSS</i> (7) <i>WwSS</i> (8) <i>wwSS</i> (9)
	W	<i>FF</i>	<i>WwFF</i> (11) <i>WwFS</i> (12) <i>wwFS</i> (13)	<i>wwFF</i> (14)

FIG. 1. The 14 combinations of seed-parent genotype and offspring genotype produced in the experimental population. *FF* and *SS* = fast and slow esterase homozygotes, respectively. *D*, *L*, and *W* = dark, light, and white *W*-locus genotypes.

the experiment, all nonexperimental *I. purpurea* that germinated in the vicinity of the experimental plot were removed.

Bee Observations

Pollinator activity in the experimental plot was observed biweekly from August 29 to October 16, 1990, on a total of 15 mornings. Workers and drones of the bumblebee *Bombus pennsylvanicus* accounted for > 99% of all observed insect visits to *I. purpurea* flowers during this period. On the same mornings that pollinators were observed, the numbers of flowering plants of each genotype were recorded. The total number of plants flowering ranged from 38 to 982, with the proportion of whites among the flowering plants ranging from 0.28 to 0.37.

Bees were followed as they visited plants in the plot and the *W*-locus genotype of each plant visited was recorded. Bees were usually observed until they left the plot, except no bee was observed for more than one-half hour; also, in a few cases, bees remained in the plot but ceased foraging, in which case the observer switched to another bee after five minutes. Because bees were not individually marked, the same individuals may have been observed more than once. However, most observations on most mornings were probably of different bees, because observations usually ended when bees flew a considerable distance away from the plot, many bees were usually simultaneously present in the plot, and the bees observed showed marked individual variation in size (as well as consisting of both sexes on most mornings: 30% of the observations were of drones).

Bees visited more than one flower on a plant only about 15% of the time. For simplicity, we ignore visits to multiple flowers on the same plant and consider only visits to plants. A total of 4816 such visits in 192 foraging bouts were observed; the number of visits observed per foraging bout ranged from 1 to 218.

On a few warm mornings, the pigments of dark-flowered plants began to fade late in the morning, and it became difficult

for the observer to distinguish between light- and dark-flowered plants. Data collected under these circumstances were used for analyses in which lights and darks were pooled, but not for analyses in which lights and darks were distinguished.

Data for Breeding-System Analysis

As the capsules matured in fall, a total of 47,462 seeds were collected from the 2155 plants that produced seeds; seeds from each capsule were stored separately. For the progeny analysis, 1158 seeds sampled randomly from this collection were scarified and planted in seedling flats. After approximately two weeks, a small sample of leaf tissue was taken for determination of esterase genotype (see Ennos and Clegg 1983, for methods), and flower color was scored a few weeks later. Six percent of the seeds failed to germinate, so that the final progeny analysis sample consisted of 1087 plants, with 365 from dark seed parents, 354 from lights, and 368 from whites.

The breeding system was analyzed using two different models, which are described in Appendix 1.

Controlled Crosses

As an assessment independent of the field experiment of whether biased transmission occurs in either pollen or ovules of lights (*Ww*), controlled crosses were conducted in the greenhouse. In one set of crosses, six light pollen parents were crossed to six white seed parents, yielding six full-sib families. In a second set, seven white pollen parents were crossed to seven light seed parents, yielding seven full-sib families. In each set of crosses, the *W*-locus alleles of different families were descended from different ancestral plants, which were derived from the same population as the plants used for the field experiment. Between 54 and 71 seeds from each family, depending upon availability, were grown in flats in a greenhouse and scored for *W*-locus genotype.

TABLE 1. Comparison of observed numbers of bumblebee visits to light- versus dark-flowered plants with expected numbers based on relative frequencies of the two types among plants flowering each morning (ignoring visits to white-flowered plants). The last two columns give the results of *G*-tests for heterogeneity among bees in visit proportions to lights versus darks on each morning, using only bees with at least 10 visits to lights and darks to avoid low expected cell numbers, and ignoring visits to whites (thus, results of the heterogeneity tests are given only for mornings on which at least two bees made at least 10 visits to lights and darks).

Date (M/D)	Visits to lights		Visits to darks		Heterogeneity		
	Observed	Expected	Observed	Expected	<i>G</i> (1 df)	<i>G</i>	df
8/29	52	54.2	53	50.8	0.179	1.20	2
8/31	158	148.1	141	150.9	1.319	3.56	7
9/4	146	149.8	171	167.2	0.187	4.77	7
9/7	189	182.0	190	197.0	0.523	7.43	5
9/11	14	17.4	18	14.6	1.470	—	—
9/14	106	106.4	92	91.6	0.003	7.49	4
9/18	112	112.6	102	101.4	0.006	5.40	7
9/21	31	29.5	31	32.5	0.141	—	—
9/25	167	175.5	106	97.5	1.139	3.63	5
9/27	106	105.1	82	82.9	0.017	7.29	8
10/2	156	157.4	149	147.6	0.025	6.21	8
10/4	128	113.5	66	80.5	4.565*	5.08	4
10/9	91	81.3	47	56.7	2.865 ^a	2.93	4
10/15	17	15.1	10	11.9	0.539	—	—

^a 0.05 < *P* < 0.10; * *P* < 0.05 (neither significant after adjusting for multiple comparisons; Rice 1989).

RESULTS

Bee Observations

In this section, we address two questions: (1) Did pollinators discriminate between dark and light genotypes? (2) Did pollinators discriminate between white and pigmented genotypes? In each case, we examine not only whether bees undervisited or overvisited particular genotypes when data from all bees are considered together, but also whether there was heterogeneity among bees (strictly speaking, among foraging bouts) in visit proportions to the different genotypes. Net under- or overvisitation of certain genotypes could cause variation in selfing rates of the genotypes and/or variation in success of the genotypes as outcross pollen parents. Heterogeneity among bees in visit proportions could result in assortative outcrossing (e.g., an excess of matings between whites and other whites compared to the random expectation), which in turn could effect interpretation of some of the estimates of breeding system parameters reported below.

Lack of Discrimination between Lights and Darks

While it is clear that bumblebees often undervisit white-flowered *I. purpurea* (Brown and Clegg 1984; Epperson and Clegg 1987a; Rausher et al. 1993), it is less clear from previous work whether bees discriminate between light- and dark-flowered plants. We observed 2731 visits to lights and darks (Table 1), and used *G*-tests of goodness-of-fit (Sokal and Rohlf 1981) to compare the observed total numbers of visits to lights and darks on each morning with the expected numbers based on the hypothesis that bees visit lights and darks in proportion to their abundance; for this analysis, visits to whites were ignored. For only one day of 14 was there nominally significant (0.01 < *P* < 0.05) evidence for preferential visitation; if a

TABLE 2. Comparison of observed numbers of bumblebee visits to white- versus pigmented-flowered (light or dark) plants with expected numbers based on relative frequencies of the two types among plants flowering each morning. The last two columns give the results of *G*-tests for heterogeneity among bees in visit proportions to white versus pigmented plants on each morning, using only bees with at least 15 total visits (thus, results of the heterogeneity tests are given only for mornings on which at least two bees made at least 15 visits).

Date (M/D)	Visits to lights and darks				<i>G</i> (1 df)	Heterogeneity	
	Visits to whites		Visits to darks			<i>G</i>	df
	Observed	Expected	Observed	Expected			
8/29	97	79.2	131	148.9	5.98*	0.33	3
8/31	373	315.1	501	558.9	16.28*** ^b	29.96*** ^b	12
9/4 ^c	276	264.1	482	493.9	0.82	52.65*** ^b	10
9/7	218	189.3	379	407.7	6.23*	24.1*** ^b	5
9/11 ^c	5	12.7	32	24.3	8.38*** ^b	—	—
9/14 ^c	57	83.9	198	171.1	13.79*** ^b	7.42 ^a	3
9/18 ^c	76	90.6	214	199.4	3.55 ^a	6.04	7
9/21	18	22.7	62	57.3	1.44	—	—
9/25 ^c	104	106.3	273	270.7	0.07	11.00 ^a	5
9/27	85	79.2	188	193.8	0.59	1.93	7
10/2	151	143.1	305	312.9	0.63	22.10*** ^b	8
10/4	113	110.8	194	196.2	0.07	1.70	4
10/9	85	79.5	138	143.5	0.59	2.90	4
10/15 ^c	19	15.7	27	30.3	1.00	—	—
10/16	6	5.6	9	9.4	0.05	—	—

^a 0.05 < *P* < 0.10; * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

^b Significant at tablewide 0.05 level by sequential Bonferroni test (Rice 1989).

^c Mornings on which the earliest bee visits to the plot were observed.

sequential Bonferroni test (Rice 1989) is used to control for multiple comparisons, however, none of the *G*-tests are significant. Furthermore, the observed number of visits to lights was greater than expected on half of the mornings and less than expected on the other half. In addition, *G*-tests for heterogeneity among bees in their relative numbers of visits to lights versus darks (Table 1), for each morning for which sufficient data were available, gave no evidence for heterogeneity in visit proportions (*P* > 0.10 in each case). We conclude that for all practical purposes, bumblebees did not discriminate between light- and dark-flowered plants in our experimental plot.

Discrimination between Whites and Nonwhites

The conclusion that bees did not discriminate between lights and darks means that visits to these two forms can be pooled into a single category. Comparing the observed numbers of visits to whites versus nonwhites with the expected numbers based on the hypothesis that bees visit the two categories in proportion to their abundance indicates that the bees that were observed on some mornings showed significant overvisitation of whites, while the bees observed on other mornings showed significant undervisitation (Table 2). It cannot be concluded that the (statistical) population of bees as a whole showed net over- or undervisitation of whites on the mornings for which these *G*-tests are significant, however, because there was highly significant heterogeneity among bees in visit proportions to whites on four of 11 mornings for which tests could be conducted (Table 2). Furthermore, for two of the other mornings, the heterogeneity tests approached significance, and the morn-

ings for which the heterogeneity tests were not significant tended to be those on which fewer total visits and/or smaller numbers of bees were observed. Thus bees were heterogeneous in their visit proportions to whites versus nonwhites, and the significant over- or undervisitation of whites on some mornings may have resulted from the observer happening to watch one or a few bees showing strong over- or undervisitation. Nonetheless, the *G*-tests comparing observed and expected numbers of visits make it clear that at least some bees over-visited whites, while others undervisited whites.

Further insight into whether the (statistical) population of bees as a whole showed net undervisitation or overvisitation of whites requires different analytic methods. One factor that needs to be taken into account is time of morning. Rausher et al. (1993) found evidence that bees tended to undervisit whites before 9:00 A.M. on mornings in July, but not later in the same mornings. Time of morning is of particular interest because the first pollen deposited on a stigma is likely to result in the majority of fertilizations (Epperson and Clegg 1987b), and most pollen is removed from the anthers by the first few visits (Fry and Rausher, unpubl. obs.). Thus the earliest visits are probably responsible for the majority of pollination events.

Because in our study, the timing of first bee activity varied considerably (from ca. 7:00 A.M. to ca. 10:00 A.M.), a simple partition of the data into visits observed before and after a certain time would be inappropriate. Furthermore, the number of observations on any one day was too small to allow realistic detection of anything but very strong time effects. To provide an analysis that uses data from all days, but nonetheless takes into account the *relative* timing of visits within days, we fitted the following linear model to the data:

$$v_{ij} - f_i = \mu_i + \beta (t_{ij} - t_{i1}) + \text{error}. \quad (1)$$

Here, v_{ij} is the observed proportion of visits to whites made by the j th bee on the i th day, f_i is the observed proportion of whites among plants flowering on the i th day, t_{ij} is the midpoint of the time interval during which the j th bee was observed (thus, t_{i1} is the time of observation of the first bee on the i th morning), β is the change in mean proportion of whites visited per hour, and μ_i is the average (population) deviation of the actual proportion of visits to whites from the proportion of whites among flowering plants at the time of observation of the first bee on the i th day. Thus if bees tend to undervisit whites early in the morning but increase the proportion of visits to whites as the morning wears on, the estimates of μ_i should be negative (at least for the mornings in which the earliest visits to the experimental plot were observed; see below) and the estimate of β should be positive. The statistical properties and limitations of this model are discussed in Appendix 2.

Applying this model showed that the proportion of visits to whites tended to increase over time within mornings. The estimate of $\beta \pm \text{SE}$ was 0.0224 ± 0.0098 per hour in the analysis in which observations were weighted by the number of visits a bee made (see Appendix 2 for rationale), and 0.0291 ± 0.0169 per hour in the unweighted analysis. The weighted estimate was significantly different from zero ($F_{1,176} = 5.30$, $P = 0.023$; these and subsequent *F*-statistics were calculated using the general linear model methodology described by Neter et al. 1990), but the unweighted estimate was not quite

TABLE 3. Estimated difference between the mean proportion of visits that bees made to whites and the proportion of whites among flowering plants, at the time that the first bee was observed on each morning ($\hat{\mu}_i$; see eq. 1 in text). The $\hat{\mu}_i$ are compared to zero by *t*-tests (Neter et al. 1990).

Date (M/D)	$\hat{\mu}_i$ (SE), weighted analysis	$\hat{\mu}_i$ (SE), unweighted analysis
8/29	0.074 (0.036)*	0.066 (0.094)
8/31	0.024 (0.026)	0.038 (0.050)
9/4 ^c	-0.056 (0.037)	-0.082 (0.057)
9/7	0.021 (0.025)	-0.098 (0.056) ^a
9/11 ^c	-0.240 (0.090)**	-0.275 (0.065)***.b
9/14 ^c	-0.137 (0.036)***.b	-0.151 (0.060)*
9/18 ^c	-0.086 (0.035)*	-0.137 (0.050)**
9/21	-0.074 (0.061)	-0.090 (0.057)
9/25 ^c	-0.049 (0.033)	-0.106 (0.056) ^a
9/27	-0.001 (0.034)	-0.009 (0.043)
10/2	-0.007 (0.027)	-0.065 (0.055)
10/4	-0.010 (0.032)	0.017 (0.055)
10/9	0.004 (0.037)	-0.001 (0.051)
10/15 ^c	0.057 (0.080)	0.142 (0.077) ^a
10/16	0.025 (0.139)	-0.145 (0.094)

^a Difference from zero: $0.05 < P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^b Significant at tablewide 0.05 level by sequential Bonferroni test (Rice 1989).

^c Mornings on which the earliest bee visits to the plot were observed.

significant ($F_{1,176} = 2.95$, $P = 0.088$). Nonetheless, the two estimates are in good agreement, and both are in the direction previously observed (Rausher et al. 1993). The estimates mean that on average, the proportion of visits that bees made to whites increased by about 0.02–0.03 per hour. While significant, the time effect accounts for only 2.4% and 1.3% of the total variance in visit proportions in the weighted and unweighted analyses, respectively, and does not explain all of the heterogeneity among bees in visit proportions (JDF, unpubl. calc.).

Estimates of μ_i for each of the 15 mornings are shown in Table 3. On six of the 15 mornings we were confident that we observed the earliest visits of bees to the plot, as judged by the fact that no bees were present when the observer arrived and pollen on anthers was undisturbed. Because the earliest visits are likely to result in the majority of pollinations, the estimates from these six mornings are of particular interest. In both the weighted and unweighted analyses, the μ_i estimates for three of these six mornings are significantly negative, indicating undervisitation of whites early in the morning, while none of the estimates are significantly positive. In both the weighted and unweighted analysis, one of the μ_i estimates remains significantly negative after a sequential Bonferroni test to take into account the 15 tests being performed (if instead the Bonferroni test is based on only the six mornings when the earliest visits were observed, two remain significant in each case). Considering all mornings, in only one case was the μ_i estimate significantly positive (8/29, weighted analysis); it may be notable that this was a day when observations began relatively late (9:20 A.M.) compared to when bee activity probably started (because this was a warm morning in August, bee activity probably began by 7:00 A.M.).

Transition Matrix of Plant-to-Plant Flights

A likely consequence of the observed heterogeneity among bees in visit proportions to whites is assortative pollen move-

TABLE 4. Matrix of observed plant-to-plant transitions by bumblebees in the experimental plot, summing data from all days.

		Flights originating at:		
		Darks	Lights	Whites
Flights arriving at:	Darks	411	437	412
	Lights	466	552	462
	Whites	376	482	518

ment, the magnitude of which may be roughly assessed by examining the matrix of observed plant-to-plant flights by pollinators, pooling data from all days (Table 4). Overall, there is a highly significant dependence of genotype visited on the genotype previously visited ($G = 17.83$, $df = 4$, $P = 0.001$). Considering only flights between lights and darks, however, there is no such dependence ($G = 1.34$, $df = 1$, $P = 0.25$). In contrast, if lights and darks are pooled to examine transitions between whites and nonwhites, there is still highly significant dependence ($G = 13.41$, $df = 1$, $P = 0.0003$), with an excess of like-to-like transitions. These results are exactly what one would expect from the observations that pollinators did not discriminate between lights and darks, but varied in the proportion of visits to whites.

In summary, the results of the bee observations indicate that (1) pollinators did not discriminate between dark and light genotypes; (2) pollinators did discriminate between white and nonwhite genotypes; (3) pollinators on average undervisited whites early in the morning, when most pollination takes place; and (4) pollinators exhibited an excess of transitions between whites and whites, and between nonwhites and nonwhites, compared to the random expectation.

Breeding System Analysis

In this section, we report the results of analyses of the breeding system. The purpose of these analyses was to estimate and compare breeding-system parameters for each of the *W*-locus genotypes. These comparisons allow us to determine whether lights and/or darks are more successful as pollen parents than whites, and whether dark alleles are transmitted with greater frequency than white alleles in the successful pollen and/or ovules of light parents. Any of these results would provide evidence for a dark-allele advantage.

Estimation of Proportion of Seeds Sired by Each Genotype (Model 1)

Using this model (see Appendix 1), we estimated the proportions of seeds sired by each genotype in the experimental population as a whole, without distinguishing between selfed and outcrossed offspring (Table 5). Because plants of the three *W*-locus genotypes were initially equally abundant and survival did not differ among the genotypes (Rausher and Fry 1993), the expectation, if all genotypes are equally successful as male parents, is that each genotype should sire one-third of the seeds. The estimate for darks is somewhat lower than one-third, that for lights is somewhat higher than one-third, and that for whites is almost exactly one-third. Testing the hypothesis of equal proportions of seeds sired by each genotype gives $-2\ln \Lambda$ (twice the difference in log-likelihoods

TABLE 5. Estimates of the proportion of seeds sired by each *W*-locus genotype in the experimental population as a whole (θ_D , etc.; see Appendix 2).

Genotype	Estimate	SE
Darks	0.306	0.015
Lights	0.360	0.014
Whites	0.334	0.015

between constrained and unconstrained models) = 4.86, $df = 2$, $P = 0.088$. Even though this result approaches significance, the near significance arises mostly from the difference between darks and lights. (Constraining the proportion of seeds sired by darks and lights to be equal, without constraining the proportion of seeds sired by whites, gives $-2\ln \Lambda = 4.82$, $df = 1$, $P = 0.028$; in contrast, constraining both the proportions sired by whites and lights, or by whites and darks, to be equal gives $P > 0.25$). Thus, there is no compelling evidence that either darks or lights were more successful as pollen parents than whites.

Estimation of Transmission Biases, Selfing Rates, and Contributions to Outcross Pollinations (Model 2)

As described in Appendix 1, model 2 predicts frequencies of the 14 maternal genotype-offspring genotype combinations in terms of nine free parameters (Table 6). The model allows determination of whether there were departures from Mendelian transmission in the pollen and ovules produced by light heterozygotes. The model also allows estimation of genotype specific selfing rates and contributions to the outcross pollen pool, though there are some biases in these estimates.

Departures from Mendelian Ratios.—One of the main goals of this study was to determine whether biased transmission of alleles from heterozygous parents occurs, since a bias favoring the dark allele could provide a dark-allele advantage. Model 2 allows testing for three types of departures from Mendelian ratios among the progeny of light (*Ww*) individuals: (1) departures from a 1:2:1 ratio among the selfed progeny of lights; (2) departures from equal transmission of the two alleles when lights act as pollen parents in outcross events; and (3) departures from equal transmission when lights act as seed parents in outcross events.

Estimates of the proportions of *WW*, *Ww*, and *ww* genotypes among the selfed progeny of lights ($q_1 - q_3$, Table 7) suggest an excess of heterozygotes. The departure from a 1:2:1 ratio is not quite statistically significant, however ($-2\ln \Lambda = 4.76$, $df = 2$, $P = 0.092$). In contrast, in outcross events, significantly more *W* alleles than *w* alleles were transmitted by *Ww* pollen parents (a , Table 3; $-2\ln \Lambda = 8.39$, $df = 1$, $P = 0.004$). This bias can be observed directly in the offspring of dark and white maternal plants known to have been sired by light pollen parents (Table 6): of these, 116 inherited the dark allele from the pollen parent, while only 76 inherited the white allele. Note that the estimate of a , 0.604, equals $116/(76 + 116)$; also, a standard *G*-test for departure of 116 and 76 from a 1:1 ratio (Sokal and Rohlf 1981) gives the same test statistic as above, 8.39. The estimated proportion of *W* alleles transmitted by *Ww* seed parents in outcross events (b , Table 7) is almost exactly one-half ($-2\ln \Lambda = 0.006$, $df = 1$, $P = 0.94$).

TABLE 6. Probabilities of observing each of the genotypic combinations listed in Figure 1 as a function of the parameters of the general model of genetic transmission in the experimental population, and of the simplified model (model 2) that makes use of the fact that pollinators did not discriminate between light and dark flowers. See Appendix 2 for full model description; s_i = probability ovule of genotype i is selfed (= selfing rate); s_w^* = "effective" selfing rate of whites in simplified model; m_{ij} = probability outcrossed ovule of genotype i is fertilized by pollen from genotype j (simplified model assumes $m_{ij} = m_{dj}$ for $j = d, l, \text{ or } w$); q_i = probability selfed seed produced by light plant is genotype i ($i = 1, 2, 3$ corresponds to dark, light, and white, respectively); a = probability outcrossed pollen of heterozygote transmits dark allele; b = probability outcrossed ovule of heterozygote transmits dark allele. Note that the probabilities are defined conditional on the seed parent. Observed numbers of each genotype in the progeny analysis sample are also given.

Offspring genotype	Probability under general model	Probability under simplified model (model 2)	Observed number
1	$s_d + (1 - s_d)m_{dd}$	$s_d + (1 - s_d)m_{dd}$	174
2	$(1 - s_d)am_{dl}$	$(1 - s_d)am_{dl}$	66
3	$(1 - s_d)(1 - a)m_{dl}$	$(1 - s_d)(1 - a)m_{dl}$	39
4	$(1 - s_d)m_{dw}$	$(1 - s_d)m_{dw}$	86
5	$(1 - s_l)bm_{ld}$	$(1 - s_l)bm_{ld}$	39
6	$(1 - s_l)[bm_{lw} + (1 - b)m_{ld}]$	$(1 - s_l)[bm_{dw} + (1 - b)m_{dd}]$	83
7	$s_lq_1 + (1 - s_l)abm_{ll}$	$s_lq_1 + (1 - s_l)abm_{dl}$	43
8	$s_lq_2 + (1 - s_l)m_{ll}[a(1 - b) + (1 - a)b]$	$s_lq_2 + (1 - s_l)m_{dl}[a(1 - b) + (1 - a)b]$	114
9	$s_lq_3 + (1 - s_l)m_{ll}(1 - a)(1 - b)$	$s_lq_3 + (1 - s_l)m_{dl}(1 - a)(1 - b)$	40
10	$(1 - s_l)(1 - b)m_{dl}$	$(1 - s_l)(1 - b)m_{dw}$	35
11	$(1 - s_w)m_{wd}$	$(1 - s_w^*)m_{dd}$	76
12	$(1 - s_w)am_{wl}$	$(1 - s_w^*)am_{dl}$	50
13	$(1 - s_w)(1 - a)m_{wl}$	$(1 - s_w^*)(1 - a)m_{dl}$	37
14	$s_w + (1 - s_w)m_{ww}$	$s_w^* + (1 - s_w^*)m_{dw}$	205

Because there was no evidence that b differed from one-half, this parameter was constrained to 0.5 for the subsequent analyses. Setting $b = 0.5$ rather than estimating it from the data had the effect of reducing the standard errors of the remaining parameters considerably, while having negligible effects on the estimates themselves. Possible consequences of departures of b from 0.5 are discussed in Appendix 3. In contrast, because there was evidence that q_1 - q_3 and especially a differed from their Mendelian expectations, these parameters were left unconstrained in the subsequent analyses.

Selfing Rates.—The results of model 1 showed that white-flowered plants were neither more nor less successful as pollen parents than pigmented-flowered plants, when both selfed and outcrossed offspring are considered. This result could have arisen in two different ways. Whites may have self-fertilized more than lights and darks, as previous studies have found (Brown and Clegg 1984; Epperson and Clegg 1987a; Rausher et al. 1993), while being less successful as pollen donors to other plants than lights and darks. Alternatively, whites and nonwhites may have been equal in both selfing rates and success as outcross pollen parents. Model 2 gives some, albeit limited, information to allow discrimination between these possibilities.

TABLE 7. Estimates of genotypic ratios produced in matings in the experimental population involving light-flowered (Ww) plants; q_1 , q_2 , and q_3 are the proportions of darks, lights, and whites, respectively, produced upon self-fertilization of lights; a is the proportion of W alleles transmitted by light pollen parents in outcross events; b is the proportion of W alleles transmitted by light seed parents in outcross events.

Parameter	Estimate	SE
q_1	0.140	0.075
q_2	0.650	0.071
q_3	0.210	0.063
a	0.604	0.035
b	0.508	0.078

Because bees apparently did not discriminate between darks and lights, these genotypes are expected a priori to have similar selfing rates (see also Brown and Clegg 1984). In contrast, previous investigations, as well as our own pollinator observations, lead to the a priori expectation that whites self more than nonwhites. The hypothesis of most interest is thus that the selfing rate of whites is greater than the average selfing rate of nonwhites. Point estimates of selfing rates of darks and lights, and of the "effective" selfing rate of whites, which may overestimate the true selfing rate because of assortative pollinator movement (see Appendix 1), are consistent with higher selfing by whites (Table 8). A one-tailed test (Gaines and Rice 1990) of the hypothesis that the effective selfing rate of whites is greater than the mean of the selfing rates of lights and darks can be provided by estimating $c = s_w^* - (s_d + s_l)/2$ (c is analogous to a "contrast" in analysis of variance). From Table 8, this gives $\hat{c} = 0.108$. The approximate standard error of \hat{c} , estimated from the large-sample variances and covariances of the selfing rate estimates, is 0.061. A test of the null hypothesis $c = 0$ against the one-sided alternative $c > 0$ therefore gives $z = 0.108/0.061 = 1.77$, $P = 0.039$. Thus, this result gives evidence that either whites self-fertilized at a higher rate than

TABLE 8. Estimates of mating system parameters in the experimental population; s_d and s_l are the selfing rates of darks and lights, respectively, while s_w^* is the "effective" selfing rate of whites (see Appendix 2); m_{dd} , m_{dl} , and m_{dw} are the proportions of outcross seeds on dark or light seed parents that were sired by dark, light and white pollen parents, respectively. Estimates were obtained from model 2 with the constraint $b = 0.5$ added.

Parameter	Estimate	SE
s_d	0.222	0.051
s_l	0.295	0.050
s_w^*	0.366	0.044
m_{dd}	0.328	0.029
m_{dl}	0.371	0.025
m_{dw}	0.301	0.026

TABLE 9. Numbers of light (*Ww*) and white (*ww*) progeny produced in controlled crosses (*Ww* × *ww*). Results of *G*-tests (Sokal and Rohlf 1981) for departure from a 1:1 ratio are given.

Light seed parents				Light pollen parents			
Family	<i>Ww</i>	<i>ww</i>	<i>G</i>	Family	<i>Ww</i>	<i>ww</i>	<i>G</i>
1	31	40	1.14	1	41	31	1.39
2	28	34	0.58	2	39	25	3.09 ^a
3	30	36	0.55	3	36	33	0.13
4	35	30	0.39	4	20	39	6.23*
5	25	29	0.30	5	34	37	0.13
6	27	30	0.16	6	43	22	6.91**
7	29	42	2.39				
Total	205	241	2.91 ^a	Total	213	187	—

^a 0.05 < *P* < 0.10; * *P* < 0.05; ** *P* < 0.01.

lights and darks, or that assortative pollination mimicked the effects of increased selfing by whites. In contrast, comparing the selfing rates of lights and darks in similar fashion gives no evidence that these two genotypes differed in selfing rates ($z = 0.93$, two-tailed $P = 0.35$).

Contributions of the Genotypes to Outcross Pollinations.—Model 2 also allows estimation of the proportion of outcross seeds on light and dark plants that were sired by whites, lights, and darks (m_{dw} , m_{dl} , and m_{dd} , respectively). If whites were less successful as outcross pollen parents in the population as a whole than lights and darks (i.e., when white as well and light and dark seed parents are considered), then we would expect m_{dw} to be less than m_{dl} and m_{dd} . (However, this result could also be due to the effects of assortative pollinator movement, which should cause whites to be underrepresented as outcross pollen parents on light and dark seed parents). The estimate of m_{dw} is somewhat less than one-third, that of m_{dl} is somewhat greater than one-third, and that of m_{dd} is close to one-third (Table 8). Although the point estimates suggest that whites may have been responsible for fewer outcross pollinations than lights and darks, comparing log-likelihoods of the unconstrained model to that of a model with the constraint $m_{dw} = m_{dl} = m_{dd}$ does not allow the null hypothesis to be rejected ($-2\ln \Lambda = 2.84$, $df = 2$, $P = 0.24$). In addition, if a one-tailed test similar to that above for selfing rates is used, \hat{m}_{dw} is not significantly lower than the mean of \hat{m}_{dl} and \hat{m}_{dd} ($z = -1.21$, $P = 0.11$).

Controlled Crosses

In crosses of light (*Ww*) seed parents to white (*ww*) pollen parents, families exhibited no significant heterogeneity (*G*-test for heterogeneity; Sokal and Rohlf 1981) in the proportion of progeny that were light (Table 9; $G = 2.60$, $df = 6$, $P = 0.86$). When families were pooled, the white allele was transmitted to seeds slightly more frequently than expected under Mendelian transmission: 54% of the progeny were light. While this trend suggests there may be a slight transmission bias favoring the white allele, the trend was not significant at the 0.05 level ($P = 0.09$). This result is consistent with the absence of a dark-allele transmission bias through ovules of heterozygotes in the field experiment.

In crosses of light (*Ww*) pollen parents to white (*ww*) seed parents, there was significant heterogeneity among families in the proportion of progeny that were light (Table 9; $G = 16.18$,

$df = 5$, $P = 0.006$). It is thus not legitimate to pool families to determine whether there is an overall bias. Instead, the deviation of each family from the expected 1:1 ratio was assessed individually. Family 6 exhibited a significant bias favoring the dark allele, while family 4 exhibited a significant bias favoring the white allele. Other families exhibited no significant biases, although family 2 exhibited a bias favoring the dark allele that approached significance ($P = 0.08$).

DISCUSSION

Stabilization of the W-Locus Polymorphism

The primary objective of this study was to determine what factor or factors provide a dark-allele advantage that, in conjunction with the Fisher effect, could stabilize the *W*-locus polymorphism in *I. purpurea*. We found no evidence that dark-flowered plants had greater net success as pollen parents, considering both selfed and outcrossed offspring, than white-flowered plants; the estimated proportion of seeds sired by darks was in fact slightly lower than that for whites (Table 5). We also found little evidence that light-flowered plants had greater success as pollen parents than white-flowered plants; the estimated proportion of seeds sired by lights was slightly higher than that for whites, but the difference was not statistically significant. Thus, differential success of the genotypes as pollen parents did not appear to provide a dark-allele advantage in our experimental population. Furthermore, we found no evidence that the dark allele was transmitted in greater than expected frequency through the ovules of light heterozygotes.

In contrast, analysis by model 2 revealed that, of seeds sired by lights, approximately 60% inherited the dark allele from the paternal parent, significantly more than the 50% expected under strict Mendelian inheritance. In conjunction with the Fisher effect, this type of transmission bias has the potential to stabilize the polymorphism. Because the Fisher effect is not expected to operate when the dark allele is at low to intermediate frequencies, the transmission bias will operate to increase the frequency of the dark allele, thus protecting it when rare. When the white allele is rare, the situation is more complicated: both the Fisher effect and the transmission bias presumably operate. Whether the white allele will increase when rare will thus depend on the relative magnitudes of these two forces. At this point, because frequency dependence of the Fisher effect has been characterized only qualitatively, we can not definitively say that the Fisher effect will dominate and thus stabilize the polymorphism. However, a simple model incorporating just these two effects and using realistic estimates of parameter values suggests that this may be the case.

This model makes the following assumptions: First, we assume that heterozygous *Ww* individuals produce pollen in which the frequency of the *W* allele is α . Second, because pollinators behave identically toward dark and light plants and there is no evidence that the selfing rate of these genotypes differs or shows frequency dependence, we assume both genotypes have the same, constant outcross rate (probability that an ovule is fertilized by pollen from another plant) of T_j . However, because selfing rate of whites differs from that of darks and lights when the frequency of whites is low, we assume that the (not necessarily constant) outcross rate of whites is T_3 .

The recursion equations incorporating these assumptions are:

$$G_1' = (1 - T_1)(G_1 + 0.5G_2\alpha) + T_1(G_1 + \alpha G_2) \cdot (G_1 + 0.5G_2) \quad (2a)$$

$$G_2' = 0.5G_2(1 - T_1) + (G_1 + \alpha G_2)(0.5G_2T_1 + G_3T_3) + (G_3 + [1 - \alpha]G_2)(0.5G_2T_1 + G_1T_1) \quad (2b)$$

$$G_3' = G_3(1 - T_3) + 0.5G_2(1 - T_1)(1 - \alpha) + (G_3 + [1 - \alpha]G_2)(0.5G_2T_1 + G_3T_3), \quad (2c)$$

where G_1 , G_2 , and G_3 are the genotype frequencies of darks, lights, and whites, respectively. Sufficient information is available to estimate all of the parameters of this model, though admittedly the estimates are crude. In this study, we estimated the value for α to be 0.6. Estimates of T_1 from four different studies (Brown and Clegg 1984; Epperson and Clegg 1987a; Rausher et al. 1993; this study) range between 0.70 and 0.85, with a mean of 0.78. Estimates of T_3 are more problematic. Although previous results indicate that the outcross rate of whites declines from being equal to that of darks and lights when whites constitute more than approximately 50% of the individuals (e.g., Epperson and Clegg 1987a; Rausher et al. 1993), the exact form of this decline is not known. The simplest assumption is that this decline is linear with slope ϕ , which may be represented by

$$T_3 = \begin{cases} T_1 & \text{if } G_3 > \gamma \\ T_1 - (\gamma - G_3)\phi & \text{if } G_3 \leq \gamma, \end{cases} \quad (3a)$$

$$(3b)$$

where γ is the white-genotype frequency below which T_3 starts to decline and ϕ is the slope of the decline. Although we do not have estimates of either γ or ϕ , we do have estimates of two of the points that equation (3b) passes through (anchor points). In two previous experiments (Epperson and Clegg 1987a; Rausher et al. 1993), in which whites constituted 20% of the population, the average deviation between T_1 and T_3 (i.e., $T_1 - T_3$) is 0.25; using the best estimate of T_1 as 0.78, this gives the point ($G_3 = 0.2$, $T_3 = 0.53$). In the experiment reported here, the frequency of the white genotype was 0.33. The deviation between the mean outcross rate of lights and darks and the outcross rate of whites is 0.11, giving the point ($G_3 = 0.33$, $T_3 = 0.67$). Fitting equation (3b) through these two anchor points gives $\gamma = 0.432$ and $\phi = 1.077$.

Substituting these values for the parameters into the recursion equations and solving numerically yields a stable polymorphism, at which the frequency of the dark allele is 0.696 (Table 10, line 1). This result suggests that the Fisher effect and observed transmission bias acting together are sufficient to stabilize the W -locus polymorphism. Moreover, the equilibrium gene frequency is within the range observed in natural populations (0.6–1.0), though it is lower than the mean (= 0.9) of 17 populations reported by Epperson and Clegg (1986). It should be realized, however, that not only are estimates of the parameters γ and ϕ imprecise, but also that the form of the relationship between T_3 and T_1 may not be linear. By altering the parameters slightly and allowing for a nonlinear relationship using the model

TABLE 10. Equilibrium frequencies of the dark (W) allele, \hat{p} , and of white-flowered plants, \hat{G}_3 , for various parameter combinations as predicted by the model in Equations (2–4). T_1 , γ , ϕ , ψ and α are parameters described in the text T_3' and T_3'' are the values of T_3 corresponding to the anchor point frequencies of whites, $G_3 = 0.2$ and $G_3 = 0.33$, respectively. The first two rows give estimates of equilibrium frequencies, T_3' and T_3'' for linear model ($\psi = 0$) using best estimates of parameters ϕ and γ from available data.

T_1	γ	ϕ	ψ	α	\hat{p}	\hat{G}_3	T_3'	T_3''
0.78	0.432	1.077	0	0.6	0.696	0.142	0.530	0.670
0.78	0.432	1.077	0	0.605	0.765	0.100	0.530	0.670
0.78	0.420	0.400	2.0	0.6	0.797	0.081	0.595	0.728
0.78	0.450	0.400	1.63	0.6	0.782	0.090	0.578	0.709
0.78	0.450	0.400	1.63	0.605	0.845	0.057	0.578	0.709
0.78	0.450	0.500	1.55	0.617	0.857	0.054	0.558	0.698

$$T_3 = \begin{cases} T_1 & \text{if } G_3 > \gamma \\ T_1 - (\gamma - G_3)\phi - (\gamma - G_3)^2\varphi & \text{if } G_3 \leq \gamma, \end{cases} \quad (4a)$$

where φ becomes another parameter of the model, one can obtain slightly different relationships between T_3 and T_1 that pass near the anchor points, but that give equilibria nearer to $\hat{p} = 0.9$ (Table 10). Thus, if we recognize that there is some uncertainty in our estimates of the model's parameters and form, a model incorporating a balance between the Fisher effect and a transmission bias in pollen produced by heterozygotes can account for most aspects of the W -locus polymorphism.

Possible Mechanisms Causing Biased Transmission

The biased transmission favoring the dark allele in the pollen of light heterozygotes could have been produced by any of three mechanisms: segregation distortion in the pollen itself, differential pollen-tube growth rates of pollen bearing the two alleles, and differential abortion of seeds sired by the two pollen types (Epperson and Clegg 1987b). Differential abortion does not appear to be consistent with the data, for the following reasons. Differential abortion determined by zygotic genotype would lead to the prediction that a bias favoring the W allele should occur when lights act as seed parents, as well as when they act as pollen parents, but no such bias was observed. Differential abortion determined by an interaction between zygotic genotype and maternal genotype would lead to the prediction that the bias in progeny of light pollen parents should differ depending on maternal genotype, but a similar bias occurred whether the seed parent was dark or white (Table 6).

The possibility that differential pollen-tube growth may contribute to biased transmission by heterozygotes is suggested by the findings that in maize (Coe et al. 1981) and petunia (Mo et al. 1992; Taylor and Jorgensen 1992; Pollak et al. 1993), unpigmented anthers of flowers are partially or wholly male-sterile. In these plants, disruption of chalcone synthase function results in failure to produce flavonoids. Because certain flavonoids (e.g., kaempferol) are required for proper pollen-tube growth, genotypes that lack flavonoids, and that thus have white flowers, also have reduced male fertility. It thus seems plausible that reduced synthesis of flavonoids by white (w) gametophytes in *Ipomoea* could inhibit pollen-tube growth,

thus rendering *w*-bearing pollen tubes less competitive than *W*-bearing tubes.

One potential problem with differential pollen-tube growth as an explanation for the transmission bias is that if pollen carrying the dark allele tends to outcompete pollen carrying the white allele, we might expect darks to be much more successful as outcross pollen parents than whites. Yet we found no evidence for such a difference (Table 8). Epperson and Clegg (1987b), however, demonstrated strong first-pollen primacy in *I. purpurea*: differences of less than a minute in time of pollen deposition onto the stigma result in much greater success of the pollen deposited earlier. In this situation, competition among dark (*W*) and light (*w*) pollen is likely to lead to biased transmission only when the pollen arrives simultaneously, which would be the case if the pollen comes from a light heterozygote. By contrast, since *W* and *w* pollen from dark and white pollen parents are likely to arrive at the same stigma at different times, the first-pollen primacy effect is likely to swamp any effects of differential competitive ability of *W* and *w* pollen. We would thus not necessarily expect differential competitive ability of these two types of pollen to cause darks to be more successful outcross pollen parents than lights. We therefore believe that current evidence does not allow us to distinguish between segregation distortion and biased pollen competition as causes of the observed transmission bias in seeds sired by heterozygote pollen parents.

Caveats

An alternative explanation for the apparent bias in the allelic composition of successful pollen produced by lights is gene flow from outside the experiment. In particular, simple calculations indicate that in the worst-case scenario in which all "foreign" pollen (pollen from outside the experimental population) carried the dark allele at the *W* locus and the slow allele at the esterase locus, an apparent bias of the magnitude of that observed could be produced if foreign pollen was responsible for approximately 7% of all outcross events. To the extent that the foreign pollen carried the white and fast alleles, it would have to be responsible for a greater fraction of all outcross events to produce the apparent bias.

We believe that this alternative explanation is unlikely for several reasons. First, we eradicated other *I. purpurea* plants from the vicinity of our experimental population. Very few, if any, native *I. purpurea* plants occurred within 1 km of our study site. Second, in our observations of foraging patterns by bumblebees, we commonly observed bees entering our experimental population of morning glories after foraging on other flower species nearby. It is thus likely that even if a bee had visited a native morning glory plant prior to entering our experimental population, it would have first visited other flower species, where much of the morning glory pollen may have been removed. Given these two considerations, we find it difficult to imagine that foreign pollen could have accounted for as much as 7% of all outcross pollen in our experiment. Finally, the controlled greenhouse crosses provide convincing evidence that biased transmission occurs in pollen produced by lights. These results suggest that the bias observed in the field experiment was real and not the result of gene flow.

An issue that is not resolved, however, is whether the ob-

served bias favoring the *W* allele in pollen in the field experiment is typical of what occurs in most populations. Both the field experiment and the controlled crosses involved a small number of independently derived white and dark alleles (14 of each in the field experiment; Rausher and Fry 1993). Given indications from the controlled crosses that pollen parents with different copies of white and dark alleles can show bias in different directions, the net bias favoring the dark allele in the field experiment could be due to a chance failure to include in the experiment white alleles that are strongly competitive, either during meiosis or pollen-tube growth. It is thus conceivable that a complete replication of our field experiment, using independently derived dark and white alleles, could yield a bias in the opposite direction. The crucial question raised by our results is thus whether there is a net bias in natural populations. Resolving this issue will require a set of controlled crosses involving a large number of heterozygote pollen parents with independently derived copies of the alleles at the *W* locus.

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APPENDIX 1

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In this appendix, we describe the statistical analysis used to estimate breeding system parameters. Data were analyzed according to two models. Model 1 estimates the proportion of seeds sired by each genotype in the experimental population, without regard to whether the seeds were the product of self-fertilization or outcrossing. This model requires relatively few assumptions (see below). Model 2 was formulated to estimate transmission biases in pollen and ovules of heterozygotes, as well as the selfing rate of each genotype and the proportional contribution of each genotype to the outcross pollen pool. For reasons that will be described below, however, the estimated selfing rate of whites from this model (but not those of lights and darks) is likely to be an overestimate of the true selfing rate, and the estimates of the proportional outcross contributions of all three genotypes are likely to be biased to some extent.

For both models, likelihood methods (Edwards 1972; Weir 1990) were used to obtain parameter estimates and their standard errors and to conduct hypothesis tests. The use of likelihood methods requires the assumption that the genotype of each sampled progeny is independent of that of every other sampled progeny. This assumption is violated when different progeny are the result of fertilizations resulting from the same pollinator visit sequence. While this type of nonindependence may have an important effect on the estimates obtained from some designs (Schoen and Clegg 1984, 1986), it is unlikely to have had a major effect in this study, for two reasons. First, *I. purpurea* flowers remain open for only one morning, and the seeds used for the progeny analysis were derived from plants that flowered over a two-month period; therefore most seeds came from flowers that were open on different days. Second, the large number of plants in the experimental population means that even seeds derived from flowers open on the same day were unlikely to have been fertilized in the same visit sequence. Of the 1087 progeny analyzed, only 37, or 3%, were from the same capsule as another progeny analyzed.

The observed numbers of the 14 possible maternal genotype-offspring genotype combinations in the progeny sample are given in Table 6.

Estimation of Proportion of Seeds Sired by Each Genotype (Model 1).—We will denote the (true) proportion of seeds on genotype *i* that were sired by genotype *j* by θ_{ij} ; for example, the proportion of seeds on dark seed parents that were sired by light pollen parents is θ_{DL} . For dark and white seed parents, the paternal genotype of each seed can be determined unambiguously (Fig. 1); thus, maximum-likelihood estimates of the θ_{ij} s are given directly by the sample proportions (Weir 1990). (For example, the estimate of θ_{DL} , $\hat{\theta}_{DL}$, is $[N_2 + N_3]/N_{D,tot}$, where N_2 and N_3 are the observed numbers of progeny of genotypes 2 and 3, and $N_{D,tot}$ is the total number of analyzed progeny of dark seed parents; the estimated variance of $\hat{\theta}_{DL}$ is $\hat{\theta}_{DL} [1 - \hat{\theta}_{DL}]/N_{D,tot}$.) θ_{LL} can similarly be estimated directly. The situation for θ_{LD} and θ_{LW} is a bit more complicated, however, because genotype 6 could have been sired by either darks or whites (Fig. 1). If light seed parents are assumed to transmit *W* and *w* alleles in equal proportions, it can be shown (by differentiating the likelihood expression for light seed parents with respect to two of the θ_{Lj} s and setting the resulting expressions equal to zero) that the maximum-likelihood estimate of θ_{LD} is

$$\hat{\theta}_{LD} = \frac{N_5(N_5 + N_6 + N_{10})}{(N_5 + N_{10})N_{L,tot}} \quad (A1)$$

The estimated variance of $\hat{\theta}_{LD}$, obtained from inverting the expected information matrix (Weir 1990), is

$$\frac{\hat{\theta}_{LD}[2 + \hat{\theta}_{LD}\hat{\theta}_{LL} - 2(\hat{\theta}_{LD} + \hat{\theta}_{LL})]}{(1 - \hat{\theta}_{LL})N_{L,tot}} \quad (A2)$$

The formulae for θ_{LW} are analogous. Although we have found suggestive evidence from greenhouse crosses that light seed parents may transmit a slight excess of *w* over *W* alleles (see text for details), allowing for this excess does not change the conclusions from model 1. In addition, a model in which the proportion of *W* versus *w* alleles transmitted by light seed parents in the field experiment was estimated jointly with the θ_{Lj} s gave only a slightly higher log-likelihood than the model assuming equal proportions (twice the difference in log-likelihoods $\sim \chi^2 = 0.52$, $P = 0.47$).

Because the three *W*-locus genotypes produced approximately equal numbers of seeds (Rausher and Fry 1993), the estimated proportion of seeds sired by a given genotype in the population as a whole, $\hat{\theta}_{.j}$, is simply the average of the $\hat{\theta}_{ij}$ s for that paternal genotype over the three

maternal genotypes. The variance of this estimator is one-ninth the sum of the variances of the three component θ_{ij} s, because the latter are statistically independent. Of particular interest is whether the three genotypes sired equal proportions of seeds in the population as a whole. The hypothesis $\theta_D = \theta_L = \theta_W = 1/3$ can be tested by comparing the log-likelihood of a model with this constraint introduced to that of the model without the constraint (Edwards 1972; Weir 1990). If the hypothesis is true, the test statistic $-2 \ln \Lambda$, which is equal to twice the difference in log-likelihoods between the unconstrained and constrained models, should have an approximately chi-square distribution with two degrees of freedom (because in this case two parameters are being constrained). The likelihood of the entire dataset can be written as the product of the likelihoods for each seed parent genotype: $L = L_D L_L L_W$, where

$$L_D = C_1 \theta_{DD}^{N_1} \theta_{DL}^{(N_2+N_3)} (1 - \theta_{DD} - \theta_{DL})^{N_4}, \quad (A3a)$$

$$L_L = C_2 \theta_{LD}^{N_5} (1 - \theta_{LL})^{N_6} \theta_{LL}^{(N_7+N_8+N_9)} (1 - \theta_{LD} - \theta_{LL})^{N_{10}} \quad (A3b)$$

and

$$L_W = C_3 \theta_{WD}^{N_{11}} \theta_{WL}^{(N_{12}+N_{13})} (1 - \theta_{WD} - \theta_{WL})^{N_{14}},$$

here, the C s are constants that do not depend on the parameters. $\theta_j = 1/3$ implies that $\theta_{Wj} = 1 - \theta_{Dj} - \theta_{Lj}$ (because $\theta_j = [\theta_{Dj} + \theta_{Lj} + \theta_{Wj}]/3$); thus, the constraints $\theta_D = \theta_L = \theta_W = 1/3$ can be introduced into the likelihood expressions by making the substitutions $\theta_{WD} = 1 - \theta_{DD} - \theta_{LD}$ and $\theta_{WL} = 1 - \theta_{DL} - \theta_{LL}$ (because the three θ_{ij} s for a given maternal genotype must sum to one, the constraint $\theta_{WW} = 1 - \theta_{DW} - \theta_{LW}$ is implied by the above two constraints). Maximizing $\ln L$ with the constraints introduced requires numerical methods; we used the "Find-Minimum" routine in Mathematica (Wolfram 1991). For this case and the cases described below, several widely different starting points within the parameter space were used, and these always resulted in the same estimates.

Estimation of Transmission Biases, Selfing Rates, and Proportional Contributions to Outcross Pollinations (Model 2).—To introduce model 2, we first consider a general model of patterns of genetic transmission in the experimental population. The model is based on the following parameter definitions: (1) seeds on darks, lights, and whites are the products of self-fertilization with probabilities s_d , s_l , and s_w , respectively; (2) outcross seeds produced by the i th genotype ($i = d, l, \text{ or } w$) are sired by the j th genotype with probability m_{ij} (thus $\sum_j m_{ij} = 1$ for each i); (3) when lights self-fertilize, they produce dark, light, and white offspring with probabilities q_1 , q_2 , and $q_3 (= 1 - q_1 - q_2)$, respectively; (4) when lights act as pollen parents in outcross events, they transmit W and w alleles with probabilities a and $1 - a$, respectively; (5) when lights act as seed parents in outcross events, they transmit W and w alleles with probabilities b and $1 - b$, respectively.

Given these definitions, the probabilities of observing each of the genotypic combinations shown in Fig. 1, conditional on the seed parent, can be written as in Table 6 (second column). Under this general model, there are more parameters than can be estimated from the data: the model has 13 free parameters, versus only 11 degrees of freedom in the dataset (viewing the total number of seeds sampled from each maternal genotype as given). Thus some simplifying assumptions need to be made before the model can be useful. We have used a set of simplifying assumptions about pollen transmission based on our observations of pollinators foraging on morning glories in the experimental population. These observations indicate that the following two constraints may reasonably be placed on the m_{ij} :

Constraint 1: $m_{dj} = m_{lj}$ for $j = d, l, \text{ or } w$, that is, pollen arriving at dark seed parents and light seed parents should have the same set of frequencies of the three pollen parent genotypes. This follows from the observation that bees do not discriminate between darks and lights. As a consequence of this constraint, we can substitute m_{dd} , m_{dl} , and m_{dw} for m_{ld} , m_{ll} , and m_{lw} into the expressions for the probabilities of observing genotypes 5–10 in Table 6.

Constraint 2: $m_w/m_{wd} = m_{dl}/m_{dd}$. In words, the ratio of pollen arriving from lights to pollen arriving from darks should be the same regardless of seed parent. This follows because bees did not vary in their relative probabilities of visiting lights versus darks, even though they did differ in their probabilities of visiting whites versus lights or darks (see text). Therefore, we can substitute $m_{dl}m_{wd}/m_{dd}$ for m_{wl} into the expression for genotypes 12 and 13 in Table 6. Furthermore, note that $m_{ww} = 1 - m_{wd}$

– m_{wd} . From constraint 2, we have $m_{ww} = 1 - m_{dl}m_{wd}/m_{dd} - m_{wd} = 1 - m_{wd}(1 + m_{dl}/m_{dd})$. This may be substituted into the expression for genotype 14.

The above constraints would be violated by spatial clumping of lights and darks in the experimental plot, even if pollinators fail to discriminate between them; however, in our study, the genotypes were planted at random locations in the plot.

The above substitutions reduce the number of parameters from 13 to 10. Although there are 11 degrees of freedom in the dataset, it is still not possible to estimate two of the remaining parameters, s_w and m_{wd} . Rearrangement of the expressions for the probabilities of observing genotypes 11–14 show that these two parameters are confounded, always occurring in the combination $(1 - s_w)m_{wd}$. As an alternative to estimating s_w and m_{wd} , we define what we will call the effective selfing rate of whites, s_w^* :

$$s_w^* = 1 - (m_{wd}/m_{dd})(1 - s_w). \quad (A4)$$

Rearranging, the actual selfing rate of whites can be expressed in terms of the effective rate:

$$s_w = 1 - (m_{dd}/m_{wd})(1 - s_w^*). \quad (A5)$$

This can be substituted into the expressions for genotypes 11–14 in Table 6.

Making all of the substitutions and simplifying results in the expressions in the third column of Table 6. Note that the only m_{ij} s that now appear are m_{dw} , m_{dl} , and m_{dd} , the proportions of the donor genotypes in the pollen arriving at dark or light seed parents. The proportions arriving at white seed parents have disappeared; these are encompassed in the composite parameter, s_w^* , which depends on both s_w , the true selfing rate of whites, and the ratio m_{wd}/m_{dd} . The expressions in the third column of Table 6 also reveal why the term "effective selfing rate" is appropriate for s_w^* : these expressions are identical to those that would be derived under a model in which s_w^* was the true selfing rate of whites, but in which outcrossing took place randomly with respect to flower color (with whites, lights, and darks making proportional contributions to the outcross pollen pool of m_{dw} , m_{dl} , and m_{dd} , respectively).

The important feature of the simplified model (model 2) is that all of the parameters in it are estimable by standard likelihood methods. Estimates of s_l and s_d are estimates of the true selfing rates of lights and darks, respectively. In contrast, an estimate of s_w^* is likely to be an overestimate of the true selfing rate of whites, for the following reason. Our observations of pollinator behavior (see text) indicate that when whites and nonwhites are contrasted, there was net assortative movement of pollinators, so that a bee on a white was more likely to make its next visit to a white than a bee on a nonwhite. Such assortative movement implies $m_{wd}/m_{dd} < 1$; that is, the frequency of dark pollen arriving at whites is lower than that arriving at darks or lights. From (A4), therefore, $s_w^* > s_w$. In intuitive terms, assortative pollinator movement mimics the effects of increased selfing by whites.

The parameters m_{dw} , m_{dl} , and m_{dd} estimated under model 2 are the proportions of outcross seeds on light and dark seed parents sired by each of the three genotypes. Model 2 does not allow estimation of the proportions of outcross seeds on white seed parents sired by each genotype, nor of the proportions in the population as a whole sired by each genotype; the latter proportions are the weighted average of the proportions of outcross seeds sired on all three seed parent genotypes, weighted by the outcrossing rate of the genotype. If the proportions of outcross seeds sired by each genotype in the population as a whole are denoted by m_{wv} , m_{lv} , and m_{dv} , respectively, the observed pattern of assortative outcrossing implies that $m_{wv} > m_{dw}$, $m_{lv} < m_{dl}$, and $m_{dv} < m_{dd}$. In words, m_{dw} underestimates the overall outcross contribution of white pollen parents, and m_{dl} and m_{dd} overestimate the overall outcross contributions of light and dark pollen parents.

If the probabilities in the third column of Table 6 are denoted by P_i , where i is the genotype number, then the likelihood of the entire dataset is given by

$$L = C \prod_i^{14} P_i^{N_i}; \quad (A6)$$

here, C is a constant that does not depend on the parameters being estimated, and the N_i are the observed numbers of each genotype in the progeny sample. Parameter estimates that maximized $\ln L$ for the un-

constrained and various constrained models (see text) were found numerically. In addition, standard errors of the parameter estimates, as well as of linear combinations of the estimates, can be calculated from the large-sample variance-covariance matrix of the estimates, which is found by inverting the expected information matrix evaluated at the maximum-likelihood estimates (Weir 1990). With large samples, the estimates should have an approximately multivariate normal distribution, a fact that allows testing hypotheses that cannot be tested by comparing log-likelihoods of constrained and unconstrained models. For example, to test the hypothesis $c = s^*_w - (s_d + s_l)/2 = 0$ against the one-sided alternative $c > 0$, one can use the fact that $\hat{c}/SE(\hat{c})$ should be approximately distributed as a unit normal random variable if the hypothesis is correct.

The unconstrained version of model 2 has nine free parameters, compared to 11 degrees of freedom in the dataset. This means that two degrees of freedom are available for testing the fit of the model, which can be done by comparing $\ln L$ from model 2 to $\ln L$ from a "saturated" model in which all observed and expected frequencies are equal. One degree of freedom of this test can be viewed as being used to test the assumption that the parameter a is homogeneous among dark and white seed parents; that is, that $P_2P_3 = P_{12}P_{13}$ (see Fig. 1). The hypothesis tested by the other degree of freedom is less simple to express in either mathematical or biological terms; however, certain forms of nonrandom outcrossing could lead to lack of fit with regard to this last degree of freedom (but not that caused by assortative pollinator movement of the type observed). Comparing the log-likelihood of model 2 to that of the saturated model gives no evidence for lack of fit ($-2\ln \Lambda = 1.10$, $df = 2$, $P = 0.58$). Thus, insofar as can be determined by this test, the data appear to conform to the assumptions of model 2.

The results in Table 4 also permit direct tests of constraints assumed in model 2. According to constraint 1, pollen arriving at dark seed parents and light seed parents should have the same set of frequencies of the three pollen parent genotypes. This can be tested by determining whether there is significant dependence of genotype visited on the genotype previously visited within the 2×3 matrix obtained by deleting the last row of Table 4. Such a test gives $G = 2.03$, $df = 2$, $P = 0.36$. According to constraint 2, the ratio of pollen arriving from lights to pollen arriving from darks should be the same on all three seed parents. This can be tested by determining whether there is significant dependence within the 3×2 matrix obtained by deleting the last column of Table 4. Such a test gives $G = 3.74$, $df = 2$, $P = 0.15$. Therefore there is no evidence to reject either constraint.

APPENDIX 2

In this appendix, we describe the statistical properties, limitations, and assumptions of the statistical analysis based on equation (1). There are two statistical difficulties in applying this model. First, the observed visit proportions are in many cases based on small numbers of visits; thus, errors cannot be normally distributed. Simulations of data with the same structure as the actual data, however, showed that non-normality of errors should not have greatly affected Type I error rates (JDF, unpubl. data). More importantly, the large range in the number of visits per bee means that the data are severely heteroscedastic. One solution to this problem is to weight the observations by the numbers of visits each bee made, on the logic that the variance of a sample proportion is inversely proportional to the sample size. This solution is not entirely satisfactory, however, because if bees show true interindividual variability in visit proportions that is not entirely described by the linear effect in equation (1), the error variance will be the sum of two components: the variance of a binomial proportion, which is inversely proportional to the number of visits, and the true interindividual variance, which is independent of the number of visits. The ideal set of weights, however, must lie somewhere in between weighting the observations by the number of visits and giving all observations equal weight, because the former corresponds to the case where the error variance is entirely due to the binomial component, and the latter to the case where the error variance is entirely due to the interindividual component. For this reason, we performed both the weighted and unweighted analysis and compared the results; agreement between the two analyses should allow confidence that the conclusions are robust.

Another assumption of the above analysis is that there was a simple linear relationship between time and the average proportion of visits bees made to whites, with the same slope on different days. This assumption

would be violated if the slope of the relationship was different on different days, or if the relationship was nonlinear. To check the first possibility, we examined a model containing a day by time interaction. The F -test for this interaction was not significant in the weighted analysis ($F_{14,162} = 0.75$, $P = 0.72$) but approached significance in the unweighted analysis ($F_{14,162} = 1.63$, $P = 0.075$). However, the near significance of the interaction in the unweighted analysis was apparently caused by the undue influence of points based on small numbers of visits: when the unweighted analysis was repeated after deleting points based on four or fewer visits, the interaction was nonsignificant ($F_{13,115} = 0.64$, $P = 0.81$), while the other conclusions were hardly effected. To check the linearity assumption, we added a square term involving time to the model described by equation (1); the square term was nonsignificant in both the weighted ($F_{1,175} = 0.11$, $P = 0.74$) and unweighted analyses ($F_{1,175} = 0.004$, $P = 0.95$). Thus the simple linear model described by (1) appears to adequately describe the dependence of the mean proportion of visits to whites on time.

A more serious caveat regarding the above analysis is that because bees were not individually marked, we may have observed the same individuals more than once. To the extent that individuals show consistent differences in visit proportions to whites, observing the same individuals more than once would violate the assumption of independent errors and lead to liberal significance tests. It is difficult to evaluate the effects that such nonindependence may have had on our conclusions. Nonetheless, as pointed out in the text, it is likely that most observations on most mornings were of different bees. Furthermore, the conclusions of the analysis are consistent with the results of previous work showing net undervisitation of whites early in the morning, when whites are in minority (Brown and Clegg 1984; Epperson and Clegg 1987a; Rausher et al. 1993), and an increase in the proportion of visits to whites over time within mornings (Rausher et al. 1993).

APPENDIX 3

In this appendix, we describe the effects of setting $b = 0.5$ on the estimates of the other parameters in model 2. The parameter b (the proportion of W vs. w alleles transmitted by light seed parents) plays an important role in the analyses of selfing rates and outcross pollinations by model 2. This role arises because b influences the probability that a seed in the ambiguous class (genotype 6, Fig. 1) was sired by a dark versus a white parent. Higher values of b make it relatively more likely that such a seed was sired by a white individual, while lower values have the opposite effect. The estimate of b was close to its Mendelian expectation of 0.5, so for the analysis of selfing rates and proportional contributions to the outcross pollen pool reported above, b was set to 0.5. The standard error of the estimate of b was fairly large (Table 7), however, so it is important to examine the influence that departures of b from 0.5 could have.

Two lines of evidence suggest that if b departs from 0.5, it is likely to be in the direction of a bias in favor of the w allele (i.e., $b < 0.5$). First, in greenhouse crosses between light seed parents and white pollen parents, there was a slight, though nonsignificant, bias in favor of the white allele (see text). The second line of evidence comes from using a slightly different model for the progeny analysis, in which the proportions of the three genotypes produced when light individuals self-fertilize are assumed to equal those produced when two different light individuals cross. In terms of the parameters of model 2, this assumption means setting $q_1 = ab$ and $q_2 = a(1 - b) + b(1 - a)$. The estimate of b from this model is 0.42, much lower than before (although still not significantly different from 0.5: $-2\ln \Lambda = 2.56$, $df = 1$, $P = 0.11$). (Because the above constraints on the q s may not be biologically realistic, we have preferred to report estimates of the other parameters based on a model in which the constraints were not present.)

Because lower values of b make it relatively more likely that genotype 6 progeny were sired by dark rather than white individuals, repeating the model 2 analyses after setting b to a value less than 0.5 (e.g., $b = 0.45$) increases the estimate of m_{dd} and decreases that of m_{dw} , while having little effect on the standard errors (results not shown). In parallel fashion, the estimate of s_d decreases, while that of s^*_w increases. Thus, given the evidence that $b \leq 0.5$, the tests reported above (i.e., using $b = 0.5$) are conservative, because using an overestimate of b should make it less likely to detect higher selfing and lower contribution to outcross pollinations by whites compared to the other genotypes.