

## SELECTION ON A FLORAL COLOR POLYMORPHISM IN THE COMMON MORNING GLORY (*IPOMOEA PURPUREA*): THE EFFECTS OF OVERDOMINANCE IN SEED SIZE

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Understanding the processes that maintain genetic variation in natural populations is a major focus of evolutionary biology. Theoretical investigations have shown that more than a dozen processes can explain the maintenance of genetic variation (see Hartl 1980 for a summary), whereas experiments with laboratory populations indicate that at least some of these processes can operate under some conditions (e.g., Dobzhansky 1948; Dobzhansky and Pavlovsky 1953; Ehrman 1967; Powell and Wistrand 1978; Jones and Probert 1980; Cavender and Clegg 1981). Nevertheless, detailed studies of the selective forces acting to maintain genetic variation in natural populations are rare.

The study reported here is part of an ongoing examination of the selective forces acting to maintain genetic variation at a locus influencing floral pigment intensity in the common morning glory, *Ipomoea purpurea*. This locus (designated “*W*”) is polymorphic in natural populations throughout the southeastern United States. Plants homozygous for the *w* allele have white flowers with pigmented rays (i.e., “whites”). Heterozygous plants have lightly pigmented flowers with dark rays (i.e., “lights”), and homozygotes for the *W* allele have darkly pigmented flowers with even darker rays (i.e., “darks”) (Ennos and Clegg 1983; Epperson and Clegg 1988). In natural populations, frequencies of the white allele generally range from 0 to 0.4, with a mean of about 0.1 (Epperson and Clegg 1986).

Previous work has shown that when white (*ww*) plants are in the minority, they are undervisited by bees (Brown and Clegg 1984; Epperson and Clegg 1986; Rausher et al. 1993). This undervisitation results in higher selfing rates for white plants, without an associated reduction in the contribution of whites to the outcross pollen pool (i.e., no pollen discounting, Rausher et al. 1993; Iwao 1995). Moreover, inbreeding depression is minimal or absent in North American populations that have been examined (Pear 1983; S. M. Chang, pers. comm. 1996). These observations indicate that the white allele should enjoy a transmission advantage, and thus increase in frequency, when rare (Fisher 1941) and may explain the protection of the white allele in natural populations. It is unclear, however, what forces operate to protect the dark allele. In the absence of any other selective forces acting on the *W* locus, a combination of genetic drift and active protection of the white allele is expected to result in the fixation of the white allele. The high frequency of the dark allele (0.6–1) in most natural populations therefore suggests the action of other selective forces favoring the dark allele.

Rausher and Fry (1993) examined the hypothesis that pleiotropic effects of the *W* locus on characters affecting viability or seed production contribute to selection pressure protecting the dark allele. They found no differences among *W*-locus genotypes in viability or fecundity that could contribute to selection opposing the mating advantages of the white allele. Rausher and Fry did, however, find that *W*-locus genotypes differed in the mean size of seeds produced, and in particular that heterozygotes produced larger seeds than either homozygote. Many studies on other plant species indicate that larger seeds represent higher quality offspring than smaller seeds. Larger seeds have been shown to have higher rates of germination and emergence, produce larger seedlings with greater survival, and, in some cases, result in larger adult plants with greater reproductive output (e.g., Andersson 1990; Black 1956; Cideciyan and Malloch 1982; Dolan 1984; Mazer 1987; Morse and Schmitt 1985; Roach 1987; Schaal 1980; Stanton 1984, 1985; Winn 1988; Wulff 1986a,b; see McGinley et al. 1987 for additional references). If in *I. purpurea* the larger size of seeds produced by heterozygotes translates into greater offspring fitness, then this overdominance in seed size could contribute to stabilization of the *W*-locus polymorphism.

Here, we report the results of a study designed to test the hypothesis that variation in offspring size reflects variation in offspring quality, and that the latter contributes to the maintenance of the dark allele at high frequencies, by measuring the fitness of the seed offspring produced in Rausher and Fry’s experiment. We also examine the general effects of seed size on fitness in *I. purpurea*.

### MATERIALS AND METHODS

#### *Study Organism*

The common morning glory, *Ipomoea purpurea* (L.) Roth (Convolvulaceae), is a self-compatible, annual vine that grows in disturbed habitats throughout the southeastern United States. Germination occurs between mid-May and August, depending on weather conditions and the timing of soil disturbance. Flowering begins around six weeks after germination and continues until plants are killed by the first frost. Flowers are almost exclusively pollinated by bumblebees. The fruits, which mature in approximately one month, are dehiscent capsules containing one to six seeds. The mass of viable seeds typically ranges from 8 to 40 mg (L. Mojonniere, pers. obs.).

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### Experimental Methods

Three thousand seeds were randomly chosen from the seeds produced in Rausher and Fry's (1993) experiment on the pleiotropic effects of *W*-locus genotype on fitness components. The *W*-locus genotypes of the maternal, but not the paternal, parents of these seeds were known. In mid-June 1992, each seed was scarified and weighed to the nearest 0.1 mg. Seeds were planted into randomly assigned locations in the same former agricultural field used by Rausher and Fry (1993), which was disked prior to planting. The location of each planted seed was marked with a plastic toothpick in order to distinguish the experimental plants from native morning glories. All native *I. purpurea* seedlings were removed from the field. One meter high bamboo stakes were provided for plants to twine up. Use of the stakes was intended to mimic growth in corn fields, a common habitat for *I. purpurea*, and facilitated plant identification during censuses and seed collection.

Seedling emergence and survival were recorded in three censuses: one week after planting, on July 13 and on July 21. A fourth census was conducted from August 20 to August 28, in which survival was noted and the length of each leaf was measured to the nearest millimeter. The sum of the leaf lengths of each plant served as an estimate of young plant size. During the fourth census, natural vegetation was clipped within an approximately 10 cm radius of the base of each plant, in order to facilitate the early growth of the experimental plants. This vegetation (mainly grasses) quickly grew back, and the field was otherwise not weeded during the experiment.

Flowering began in late August. The number of flowers produced by each plant was recorded daily from August 28 through October 19, when plants were killed by a frost. Because flowers last only one day, these daily censuses provided a complete count of total flower production. Flower color was also recorded in order to infer *W*-locus genotype. Mature capsules (fruits) were collected daily. The numbers of capsules and seeds produced by each plant were counted and each seed was individually weighed.

### Statistical Analyses

In addition to differing in the size of seeds that they produce, maternal *W*-locus genotypes differ in the proportions of dark, light, and white offspring that they produce. Because previous work has shown that *W*-locus genotypes may differ in fitness (Rausher and Fry 1993), analyses of the effects of maternal *W*-locus genotype on offspring fitness were designed to avoid confounding the effects offspring *W*-locus genotype on offspring fitness with the effects of maternal *W*-locus genotype.

#### Effects of Maternal *W*-locus Genotype on Offspring Survival

The appropriate test of the hypothesis that maternal *W*-locus genotypes differ in offspring survival involves comparing the survival of offspring of the same *W*-locus genotype but of different maternal *W*-locus genotype, where survival is defined as living to produce at least one flower. Because

we inferred offspring *W*-locus genotype from offspring flower color, we could not determine the *W*-locus genotype of offspring that died before flowering and thus could not directly measure percent survival for the different offspring *W*-locus genotypes. Instead, we estimated and compared the survival probabilities of genotypes using maximum-likelihood techniques.

The likelihood model used consists of two parts. The first part estimates the proportions of each offspring *W*-locus genotype expected in the seeds of each maternal *W*-locus genotype that were planted in the field. These proportions were estimated by taking an additional random sample of 1087 seeds produced in Rausher and Fry's experiment, germinating them in the greenhouse, and scoring flower color to determine *W*-locus genotype. We used counts of the number of plants of each maternal/offspring *W*-locus genotype in this greenhouse sample to estimate the corresponding proportions of the different genotypes planted into the field by using the following likelihood function:

$$L_I = K' \prod_{i=3} \prod_{j=3} p_{ij}^{m_{ij}},$$

where  $p_{ij}$  = the probability that a seed randomly drawn from a maternal parent of genotype  $i$  is genotype  $j$ ,  $m_{ij}$  = the number of offspring of genotype  $j$  produced by parents of genotype  $i$  in the greenhouse sample;  $K'$  = a combinatorial constant; and  $i, j = 1, 2, 3$  correspond to dark, light, and white genotypes, respectively. Survival to flowering within the greenhouse sample was 94%, indicating little opportunity for differential survival of the genotypes in the greenhouse to bias estimates of the  $p_{ij}$ .

A second likelihood function,  $L_{II}$ , estimates the probability of survival in the field of each offspring genotype for each maternal genotype (the  $l_{ij}$ ) using counts of the observed number of surviving offspring of each *W*-locus genotype for each maternal *W*-locus genotype (the  $s_{ij}$ ):

$$L_{II} = K'' \prod_{i=3} \left[ \sum_{j=3} p_{ij}(1 - l_{ij}) \right]^{(n_i - \sum_{j=3} s_{ij})} \prod_{j=3} (p_{ij} l_{ij})^{s_{ij}},$$

where  $l_{ij}$  = the survival probability of offspring of genotype  $j$  produced by maternal parents of genotype  $i$ ;  $n_i$  = the total number of offspring produced by parents of genotype  $i$  in the field sample;  $s_{ij}$  = the number of surviving offspring of genotype  $j$  produced by maternal parents of genotype  $i$  in the field sample, and  $K''$  = a combinatorial constant.

The following total likelihood function,  $L$ , which is the product of  $L_I$  and  $L_{II}$ , was used to jointly estimate the  $p_{ij}$  and the  $l_{ij}$ :

$$L = K \prod_{i=3} \left[ \sum_{j=3} p_{ij}(1 - l_{ij}) \right]^{(n_i - \sum_{j=3} s_{ij})} \left[ \prod_{j=3} (p_{ij})^{m_{ij}} (p_{ij} l_{ij})^{s_{ij}} \right].$$

The hypothesis that offspring survival was the same for all maternal genotypes was tested by comparing the likelihood of a model with survival probabilities constrained to be the same, within each genotype, for plants with different maternal genotypes ( $l_{11} = l_{21}$  and  $l_{12} = l_{22}$  and  $l_{23} = l_{33}$ ) to the likelihood of the unconstrained model described above. This set of constraints avoids confounding offspring genotype

with maternal genotype by comparing the survival probabilities of plants of the same genotype but of different maternal genotypes. Note that the survival probabilities  $l_{13}$  and  $l_{31}$  are undefined because dark individuals cannot produce white flowered offspring and vice versa. Likelihoods were calculated using the software package Mathematica. In accordance with standard maximum-likelihood procedures, a  $p$ -value was obtained by comparing the test statistic:

$$T = 2 \ln (\text{likelihood of the unconstrained model} / \text{likelihood of the constrained model})$$

to a  $\chi^2$  distribution with degrees of freedom equal to the difference in the number of parameters between the constrained and unconstrained models (Edwards 1972; Weir 1990).

#### *Effects of Maternal W-locus Genotype on Offspring Reproductive Success*

We used analyses of covariance (ANCOVAs) to determine the effects of maternal  $W$ -locus genotype on the number of seeds produced by surviving plants and the average size of seed produced. The analysis of seed number was restricted to plants that produced at least one flower, whereas the analysis of seed size was restricted to plants that produced at least one seed. To avoid confounding maternal  $W$ -locus genotype and offspring  $W$ -locus genotype in these analyses, the effects of both maternal and offspring genotype were included in the models. We could not perform complete two-way ANCOVAs of maternal  $W$ -locus genotype by offspring  $W$ -locus genotype because dark plants do not produce white offspring and *vice versa*. Instead, we performed two ANCOVAs for each response variable: one including plants with dark and light maternal and offspring genotypes and one including plants with light and white maternal and offspring genotypes. Response variables were square-root transformed before analysis in order to better approximate normality. The mass of the seed from which the plant germinated (initial seed mass) and plant size were included as covariates. Because there was a significant effect of maternal  $W$ -locus genotype on initial seed mass in this experiment, and because effects of maternal  $W$ -locus genotype on reproductive output may be mediated through effects on plant size, we used type-I (sequential) sums of squares with the covariates entered below the genotype effects instead of the more standard type-III sums of squares. This method statistically removes the effects of within-genotype variation in the covariates before comparing fitnesses between genotypes, and thus renders the between genotype comparison more sensitive (see the Appendix in Rausher and Fry 1993 for justification of this approach). Because the covariates are listed below the genotype effects, pleiotropic effects of  $W$ -locus genotype on the covariates were included when assessing the effects of genotype on reproductive success. By listing block before maternal and offspring genotype, the effects of block were removed from the genotype effects. Similarly, by listing offspring genotype before maternal genotype, the effects of offspring genotype were removed from, and thus not confounded with, the effects of maternal genotype.

TABLE 1. Maximum-likelihood estimates of survival probabilities, where  $l_{ij}$  = estimated survival probability of offspring of genotype  $j$  produced by plants of genotype  $i$  and  $i, j = 1, 2, 3$  correspond to dark, light, and white genotypes respectively. Survival probabilities  $l_{13}$  and  $l_{31}$  are undefined because dark individuals cannot produce white-flowered offspring and *vice versa*.

Maternal $W$ -locus genotype	Offspring $W$ -locus genotype		
	WW (dark)	Ww (light)	ww (white)
WW (dark)	$l_{11} = 0.180$	$l_{12} = 0.230$	—
Ww (light)	$l_{21} = 0.207$	$l_{22} = 0.222$	$l_{23} = 0.325$
ww (white)	—	$l_{32} = 0.216$	$l_{33} = 0.218$

#### *Overall Effects of Seed Size on Fitness*

We examined the relationship between seed size, irrespective of  $W$ -locus genotype, and four fitness components: emergence, survival from emergence to reproduction, the number of seeds produced by those plants that survived to reproduce, and the average mass of produced seeds. The effects of seed size on emergence and survival were analyzed by logistic regressions of emergence and survival on seed mass using the categorical analysis procedure CATMOD in the SAS statistical package (version 6.09). Survival was again defined as living to produce at least one flower. The effects of seed mass on number of seeds produced and the average mass of seeds produced were assessed by linear regressions of these variables on the mass of the planted seed. These analyses were performed using the general linear model (GLM) procedure of the SAS statistical package (version 6.09) and were restricted to plants that survived to produce at least one flower. Spatial block and  $W$ -locus genotype were included in the model. Response variables were square-root transformed prior to analysis.

## RESULTS

#### *Effects of Maternal W-locus Genotype on Offspring Fitness*

Estimated survival probabilities of each offspring  $W$ -locus/maternal  $W$ -locus genotype combination are presented in Table 1. We found no significant differences among of maternal  $W$ -locus genotypes in the survival of their offspring; comparison of the likelihood model with survival probabilities constrained to be identical across maternal  $W$ -locus genotypes with the unconstrained model supported the null hypothesis of no difference ( $T = 5.87$ ;  $df = 4$ ;  $0.10 < P < 0.25$ ). We also found no evidence of overdominance in offspring reproductive success: maternal  $W$ -locus genotype did not detectably affect the number or the size of seeds produced (Table 2). Thus, overdominance seed size did not appear to translate into overdominance in any measured offspring fitness component.

#### *Overall Effects of Seed Size on Fitness*

The probability that a seed emerged increased significantly with its mass ( $P < 0.0001$ , Table 3). Given that a plant emerged, the mean initial seed size of those plants that survived to produce at least one flower (mean = 20.7 mg, SE = 0.163,  $N = 811$ ) was greater than that of plants that died

TABLE 2. Effects of maternal *W*-locus genotype on offspring fitness.

A. Mean number and size (mass in milligrams) of seeds produced by plants of different maternal <i>W</i> -locus genotypes. Means and (standard errors).									
Maternal <i>W</i> -locus genotype									
Dark ( <i>WW</i> )									
Light ( <i>Ww</i> )									
White ( <i>ww</i> )									
Number of seeds	4.82 (0.490)			4.41 (0.418)			4.91 (0.538)		
Seed mass (mg)	17.28 (0.485)			17.78 (0.386)			16.90 (0.360)		

  

B. Analyses of covariance for the effects of maternal <i>W</i> -locus genotype ("maternal intensity") and offspring <i>W</i> -locus genotype ("intensity") on offspring fitness components. Sums of squares are type I. Order of effects in table reflects the order in which they were entered into the model. The covariates "mass" and "size" refer to initial seed mass and plant size, respectively. Response variables were square root transformed prior to analysis.									
Number of seeds									
Mean seed mass									
Darks vs. lights									
Lights vs. whites									
Darks vs. lights									
Lights vs. whites									
Source of variation	df	SS	<i>F</i>	SS	<i>F</i>	SS	<i>F</i>	SS	<i>F</i>
Block	5	128.68	27.48***	153.13	31.33***	3.06	1.74	3.48	2.72*
Intensity (I)	1	5.86	6.26*	0.16	0.17	0.54	1.56	3.22	12.59***
Maternal intensity (M)	1	1.91	2.05	2.74	2.80	0.65	1.84	0.21	0.82
I × M	1	0.28	0.30	0.62	0.64	1.49	4.23*	0.22	0.86
Mass	1	2.64	2.82	0.18	0.18	0.01	0.02	0.25	0.99
Size	1	307.22	328.10***	358.39	366.67***	1.26	3.57	0.39	1.54
Error	349†	326.79		374.35		81.74		58.66	
Total	359†	773.41		889.58		88.76		66.44	

\*  $P < 0.05$ ; \*\*\*  $P < 0.001$ ; † Error degrees of freedom for number of seeds: lights vs. whites = 383; for mean seed mass: darks vs. lights = 232, lights vs. whites = 229.

(mean = 20.15 mg, SE = 0.198,  $N = 590$ ), but the logistic regression of postemergence survival onto seed mass was not significant (Table 3). When we incorporated effects of seed size on both emergence probability and postemergence survival, there was a highly significant positive effect of seed size on survival to produce at least one flower ( $P < 0.0001$ , Table 3). Provided that a plant survived, seed mass had no additional effect on the number of seeds produced or on the average mass of produced seeds.

#### DISCUSSION

The overdominance in seed size found by Rausher and Fry did not translate into any detectable overdominance in offspring quality: the offspring of light plants performed no better than the offspring of white or dark plants for any of the fitness components examined. It is thus unlikely that overdominance in offspring quality could contribute strongly to the maintenance of the *W*-locus polymorphism in *I. purpurea*.

This result appears to contradict our finding that when maternal genotype is ignored, plant fitness increased with the size of the seed from which it germinated. One possible explanation for this apparent contradiction is that the seeds of heterozygotes enjoy a fitness advantage due to their larger seed size but are inferior to one or both of the homozygotes

in some other aspect of seed quality (e.g., nutrient content) that effectively cancels out the fitness advantages associated with larger seed size. Studies on agricultural plant species, for example, have revealed maternal effects on the mineral composition of seeds (Roach and Wulff 1987). Previous work on maternal effects suggests that such differences in seed quality would be most likely to influence fitness components expressed early in the life cycle, such as survival to reproductive age (Roach and Wulff 1987; Schmid and Dolt 1994). Unfortunately, analysis of our survival data cannot separate effects of maternal *W*-locus genotype that are and are not mediated through seed size, and we therefore cannot evaluate this explanation definitively. Provided that plants survived to reproduce, however, we find no evidence that maternal effects in addition to seed size influenced reproductive output: ANCOVAs with the effects of seed mass removed from the effects of maternal *W*-locus genotype show no effect of maternal *W*-locus genotype on flower or seed number.

A second, and more likely, explanation is that the larger size of the seeds of heterozygotes does reflect higher offspring quality, but the differences in seed size among *W*-locus genotypes are so small, and the effects of seed size on fitness are so weak, that these differences in offspring fitness among maternal genotypes were too small to be detected. In

TABLE 3. Maximum-likelihood ANOVAs for effects of seed mass on emergence, survival during the time interval between emergence and the production of the first flower, and overall survival (i.e., the combined effects of seed mass on emergence and postemergence survival).

Source of variation	Emergence			Postemergence survival			Overall survival		
	df	$\chi^2$	<i>P</i>	df	$\chi^2$	<i>P</i>	df	$\chi^2$	<i>P</i>
Intercept	1	61.12	<0.0001	1	0.35	>0.55	1	127.23	<0.0001
Mass	1	57.73	<0.0001	1	3.65	<0.057	1	37.38	<0.0001
Block	5	6.21	>0.28	5	5.59	>0.34	5	5.09	>0.40
Mass × block	5	5.90	>0.31	5	2.54	>0.77	5	4.56	>0.47
Likelihood ratio	1087	1404.24	<0.0001	750	970.24	<0.0001	1087	1259.24	<0.00025

TABLE 4. Equilibrium frequencies of the white ( $w$ ) allele and of the white genotype (in parentheses) predicted by the model in Equations (1–3) of the Appendix, for various combinations of parameters. The parameters  $W_d$ ,  $W_l$ , and  $W_w$  represent the relative fitnesses of offspring of the dark, light, and white genotypes, and  $s_d$ ,  $s_l$ , and  $s_w$  represent the selfing rates of the dark, light, and white genotypes. Parameter sets 1–4 (detailed in the Appendix) describe the relationship between the frequency of the white genotype ( $p_{22}$ ) and its selfing rate ( $s_w$ ).

Parameter set for the relationship between $s_w$ and $p_{22}$	$W_d = 0.938$ , $W_l = 1.0$ , $W_w = 0.976$		$W_d = 0.956$ , $W_l = 1.0$ , $W_w = 0.885$	
	$s_d = s_l = 0.0$	$s_d = s_l = 0.2$	$s_d = s_l = 0.0$	$s_d = s_l = 0.2$
(1)	0.69 (0.47)	0.68 (0.48)	0.60 (0.36)	0.56 (0.35)
(2)	0.63 (0.40)	0.62 (0.41)	0.56 (0.32)	0.53 (0.32)
(3)	0.70 (0.49)	0.67 (0.50)	0.60 (0.37)	0.56 (0.35)
(4)	0.64 (0.41)	0.63 (0.42)	0.56 (0.32)	0.52 (0.31)

Rausher and Fry's experiment, mean seed size differed by only 4% among genotypes. These small differences in seed size would have to reflect large differences in offspring quality in order for overdominance to contribute strongly to the maintenance of the dark allele. Although highly statistically significant, the relationship between seed size and plant fitness was fairly weak: the regression of relative fitness on seed mass predicts that the seed size differences found by Rausher and Fry would result in a difference in the relative offspring fitnesses of the genotypes of only 6%.

To investigate the possible contribution of overdominance in offspring quality of this magnitude, in conjunction with the Fisher effect, to stabilizing the  $W$ -locus polymorphism we analyzed a model of evolution at the  $W$ -locus that incorporates both the frequency-dependent transmission advantage of the white allele and overdominance in offspring quality. In this model (described in the Appendix), estimates of the offspring quality of the genotypes were based on (A) the observed mean seed sizes produced by each genotype, and (B) the relationship between offspring seed size and offspring fitness measured in this experiment. Equilibrium genotype frequencies were solved for using two separate sets of relative offspring fitness parameters: one set calculated using the mean seed size produced by each genotype in this experiment [mean seed sizes: dark = 17.33 mg, light = 17.86 mg, white = 16.47 mg; relative offspring fitnesses:  $W_d = 0.956$ ,  $W_l = 1.0$ ,  $W_w = 0.885$ ], and one set calculated using the mean seed sizes found in Rausher and Fry's experiment [mean seed sizes: dark = 19.70 mg, light = 20.62 mg, white = 20.21 mg; relative offspring fitnesses:  $W_d = 0.938$ ,  $W_l = 1.0$ ,  $W_w = 0.972$ ]. The frequency-dependent transmission advantage resulting from increased selfing by whites was modeled using data from previous experiments on the relationship between the frequency of the white genotype in a population and its selfing rate (see Appendix for details). For each of 16 parameter combinations that bracket the set of likely combinations (see Appendix), we solved for equilibrium genotype frequencies using the software package Mathematica. In each case, the stability of equilibria was tested by examination of the eigenvalues of the Jacobian matrix.

Analyses of the model predict that the combined effects

of biased transmission of the white allele at low frequencies, and the higher fitness of seeds produced by lights compared to seeds produced by whites, leads to a stable polymorphic equilibrium (Table 4). This result is not surprising, because the overdominance in offspring quality by itself will lead to a stable polymorphism, and adding additional frequency-dependent protection of the white allele in the form of biased transmission should not alter this basic result. More importantly, however, the model predicted in all cases examined that the equilibrium frequency of the white allele should be at least 0.5, which is much greater than typically observed frequencies (0.0–0.2). Our results, therefore, suggest that while overdominance in seed size may contribute to maintaining the  $W$ -locus polymorphism, other, as yet unknown, factors that favor the dark allele must be operating to keep the white allele at the low frequencies observed in nature.

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#### APPENDIX

##### *The Combined Effects of the Transmission Advantage of the White Genotype and Overdominance in Offspring Quality on Predicted Equilibrium Frequencies of the W-locus Genotypes*

In this appendix, we describe a model for evolution at the *W*-locus, which includes both a frequency-dependent selfing rate of the white genotype and overdominance in offspring quality. Mating among out-crossing plants is assumed to be random, and the *W*-locus genotypes are assumed not to differ in any fitness component other than offspring quality. Equations for change in genotype frequencies over one generation are

$$p'_{11} = [(p_{11}(1 - s_d)p_1 + p_1s_d)W_d + (1/2p_{12}(1 - s_l)p_1 + 1/4p_{12}s_l)W_l] \div [p_{11}W_d + p_{12}W_l + p_{22}W_w], \quad (1)$$

$$p'_{12} = [(p_{11}(1 - s_d)p_2 + (1/2p_{12}(1 - s_l)p_1 + 1/2p_{12}s_l)W_l + (p_{22}(1 - s_w)p_1)W_w] \div [p_{11}W_d + p_{12}W_l + p_{22}W_w], \quad (2)$$

$$p'_{22} = [(1/2p_{12}(1 - s_l)p_2 + 1/4p_{12}s_l)W_l + (p_{22}(1 - s_w)p_2 + p_{22}s_w)W_w] \div [p_{11}W_d + p_{12}W_l + p_{22}W_w], \quad (3)$$

where  $p_{11}$ ,  $p_{12}$ , and  $p_{22}$  equal the frequencies of the dark, light, and white genotypes, respectively,  $p_1$  equals the frequency of the dark allele,  $p_2$  equals the frequency of the white allele,  $W_d$ ,  $W_l$ , and  $W_w$  represent the relative fitnesses of offspring of the dark, light, and white genotypes, and  $s_d$ ,  $s_l$ , and  $s_w$  represent the selfing rates of the dark, light, and white genotypes.

#### *Estimating the Offspring Quality Parameters*

The relative fitnesses of offspring of dark, light and white plants,  $W_d$ ,  $W_l$ , and  $W_w$ , were considered to be a function of the mean size of the seeds produced by the parental genotype. The function relating mean offspring seed size to offspring fitness was estimated by the linear regression of the relative fitness of a plant in our field experiment onto the mass of the seed from which that plant germinated, with fitness estimated by the number of seeds produced (number of seeds =  $-0.5197 + 0.0906(\text{mass})$ ;  $df = 1, 2884$ ;  $P < 0.0001$ ).

#### *Estimating Genotype-Specific Selfing Rates*

In experimental populations of *I. purpurea*, selfing rates of dark and light plants typically range from 0 to 0.2 and do not vary with genotype frequency (Epperson and Clegg 1987; Rausher et al. 1993; Fry and Rausher 1997). Accordingly, we examined the extreme situations in which selfing rates of dark and light plants ( $s_d$  and  $s_l$ ) were considered constant at either 0 or 0.2.

The selfing rate of white plants was considered to be decreasing function of the frequency of the white genotype in the population. Previous experimental work on *I. purpurea* indicates that when the frequency of the white genotype is 0.2, the selfing rate of whites is 0.25 higher than the selfing rate of darks, and that when the frequency of the white genotype is 0.33, the selfing rate of whites is 0.11 higher than the selfing rate of darks. When the frequency of the white genotype is 0.5 or higher, there is no longer any difference in the selfing rate of whites and darks, although the exact shape of this decline is unknown (Epperson and Clegg 1987; Rausher et al. 1993; Iwao 1995; Fry and Rausher 1997). To model this relationship, we used the following equations:

$$s_w = \begin{cases} s_d & \text{if } p_{22} > \gamma, \\ s_d + ap_{22}^2 + bp_{22} + c & \text{if } p_{22} \leq \gamma \end{cases} \quad (4A)$$

$$s_w = \begin{cases} s_d & \text{if } p_{22} > \gamma, \\ s_d + ap_{22}^2 + bp_{22} + c & \text{if } p_{22} \leq \gamma \end{cases} \quad (4B)$$

where  $\gamma$  is the frequency of the white genotype above which white plants no longer have increased selfing relative to darks and lights, and  $a$ ,  $b$ , and  $c$  are parameters describing the shape of the decline in  $s_w$  with  $p_{22}$ . Because data on the shape of the decline and the value of  $\gamma$  are limited, we ran the model for four different sets of values for  $a$ ,  $b$ , and  $c$ , which correspond to values of  $\gamma$  of either 0.4 or 0.5 and either a linear or a quadratic decline in  $s_w$  with  $p_{22}$ . These values of  $\gamma$  should bracket the actual frequency at which the transmission advantage of the white allele declines to zero, since Rausher and Fry (1993) found that the white genotype still enjoyed a substantial transmission advantage when the frequency of the white genotype was 0.33, whereas Epperson and Clegg (1987) found no transmission advantage at a frequency of 0.5. Parameter values were generated by fitting either a linear or a quadratic model to the observed points ( $p_{22} = 0.2$ ,  $s_w = s_d + 0.25$ ), ( $p_{22} = 0.33$ ,  $s_w = s_d + 0.11$ ), and a third point of either ( $p_{22} = 0.5$ ,  $s_w = s_d$ ) (i.e.,  $\gamma = 0.5$ ) or ( $p_{22} = 0.4$ ,  $s_w = s_d$ ) (i.e.,  $\gamma = 0.4$ ). Parameter

set 1 ( $a = 0$ ,  $b = -0.7929$ ,  $c = 0.3843$ ) resulted from a linear fit with the third point corresponding to  $\gamma = 0.5$ . Parameter set 2 ( $a = 0$ ,  $b = -1.25$ ,  $c = 0.495$ ) resulted from a linear fit with the third point corresponding to  $\gamma = 0.4$ . Parameter set 3 ( $a = 0.1405$ ,  $b = -0.6435$ ,  $c = 0.35$ ) resulted from a quadratic fit with the third point corresponding to  $\gamma = 0.5$ , and parameter set 4 ( $a = 0.15$ ,

$b = -2.15$ ,  $c = 0.62$ ) resulted from a quadratic fit with the third point corresponding to  $\gamma = 0.4$ . Combining these four parameter sets with the two sets of offspring fitness parameters described above yielded 16 parameter combinations, which should bracket the space of all parameter combinations that are likely to be found in nature.

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## THE EFFECT OF INBREEDING ON POPULATION-LEVEL GENETIC CORRELATIONS IN THE RED FLOUR BEETLE *TRIBOLIUM CASTANEUM*

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There have been very few empirical studies on the effect of inbreeding on genetic correlations (Bryant and Meffert 1988; Goodnight 1989). Additive theory predicts that genetic correlations should not change following a bottleneck (Lande 1980; but see Avery and Hill 1977). However, this prediction is based on the assumption that the additive genetic variance-covariance matrix  $\mathbf{G}$  remains constant (Lande 1980), and theoretical and empirical evidence on the changing nature of  $\mathbf{G}$  is beginning to accumulate (e.g., Turelli 1988; Shaw et al. 1995). Although nonadditive theory makes no predictions about how  $\mathbf{G}$  should change following a bottleneck (Goodnight and Schwartz, in press), empirical evidence suggests that the nature of  $\mathbf{G}$  for nonadditive traits is unstable and inconstant (Bryant and Meffert 1988, 1990; Goodnight and Schwartz, in press). The changing nature of  $\mathbf{G}$  suggests that genetic correlations have a changing nature too (even for additive traits; see Avery and Hill 1977).

The empirical data available on the effect of inbreeding on genetic correlations are not sufficient to reveal a general trend. Bryant and Meffert (1988) studied the effect of three different sizes of bottlenecks (one-pair, four-pair, and 16-pair) on correlations among morphometric traits in the housefly *Musca domestica*. After several generations of inbreeding, the four-pair and 16-pair treatments showed significantly greater positive genetic correlations than the outbred control, but the one-pair treatment showed “significantly disrupted trait interrelationships” (Bryant and Meffert 1988). Goodnight (1989) examined population-level correlations in two species of slightly inbred *Tribolium* beetles and concluded that “correlations may occur between traits at the group level,” but he observed significant results for only one species.

Most of the research being done on the variable nature of  $\mathbf{G}$  (see Shaw et al. 1995, and references therein) and the specific effect of inbreeding on  $\mathbf{G}$  and genetic correlations (Bryant and Meffert 1988) focuses on individual traits, Goodnight’s (1989) study being a notable exception. This is not surprising, given

that Lande’s selection theory that originally sparked much of the interest in the constancy of  $\mathbf{G}$  is individual selection theory (Lande 1979), and evolutionary biology has been dominated by Darwin’s individual selection paradigm. The increasing evidence in the genetic school of multilevel selection theory (e.g., Wade 1977; Craig 1982; Goodnight 1985; Wade and Goodnight 1991; Stevens et al. 1995; Muir 1996; see also Goodnight and Stevens, in press) requires that we gain more familiarity with population-level analyses and an understanding of how evolutionary processes such as inbreeding affect population-level traits and phenomena.

A population-level trait is defined as a group-level phenotype (Goodnight and Stevens, in press). It can be the population mean of an individual trait or a trait that can only be expressed at the population level, e.g., population density (Goodnight and Stevens, in press). An analysis of population-level traits is analogous to an analysis of individual-level traits. That is, an additive genetic variance-covariance matrix  $\mathbf{G}_g$  is generated (Goodnight 1989), and parameters such as group-level genetic correlations and group heritabilities can be estimated.  $\mathbf{G}_g$  is the group-level analog of  $\mathbf{G}$ ; it is the matrix of group-level additive genetic variances and covariances:

$$\begin{bmatrix} G_{ii-g} & G_{ij-g} \\ G_{ij-g} & G_{jj-g} \end{bmatrix}, \quad (1)$$

where  $G_{ii-g}$  and  $G_{jj-g}$  are the group-level additive genetic variances, or more simply “the variance among lineages,” for two different group-level traits  $i$  and  $j$ ; and  $G_{ij-g}$  is the additive genetic covariance between the two traits  $i$  and  $j$  (from Goodnight 1989). A population-level correlation can be estimated as  $G_{ij-g}/(G_{ii-g}G_{jj-g})^{1/2}$  (from Goodnight 1989); it is the correlation between population-level traits.

There are at least two reasons why an investigation of population-level genetic correlations is a necessary means to examine certain evolutionary phenomena, and there are sev-