

Floral-Color Polymorphism in *Ipomoea purpurea*: Biased Inheritance of the Dark Allele is not a General Explanation for its Maintenance

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A previous investigation reported the existence in a single population of the morning glory (*Ipomoea purpurea*) of non-Mendelian inheritance at the *W* locus influencing flower color. In addition, it was shown that the magnitude of biased inheritance in that population was sufficient to maintain a floral-color polymorphism at that locus at frequencies approximating those observed in natural populations. The current investigation was undertaken to determine whether this biased inheritance was characteristic of other *I. purpurea* populations, and thus whether it provides a general explanation for maintenance of the polymorphism. The current study found no evidence for biased inheritance in two additional polymorphic populations examined. Non-Mendelian inheritance thus seems unlikely to constitute a general explanation for the maintenance of this floral-color polymorphism in *I. purpurea*.

Understanding the maintenance of genetic variation remains one of the challenges of evolutionary biology. Although theoretical investigations have identified several mechanisms that can maintain a polymorphism at a single locus [for a summary see Hartl (1980)], there are few unequivocal demonstrations that such mechanisms operate in nature (Endler 1986; Hartl 1980), nor is it clear what mechanisms most commonly operate to maintain variation in nature.

Flower color variation displayed by natural populations of the tall morning glory (*Ipomoea purpurea*) has been intensively studied to determine the processes responsible for its maintenance. Both the intensity and the hue of pigmentation are highly variable and appear to be under the control of four major, unlinked loci (Ennos 1981; Ennos and Clegg 1983; Epperson and Clegg 1988) that exhibit variation in natural populations. The *W* locus, which determines whether the corolla is white (*ww*), lightly pigmented (*Ww*), or darkly pigmented (*WW*), has been the major focus of investigations attempting to elucidate mechanisms responsible for maintaining this variation. Typically, the white allele occurs at low frequencies, ranging from zero to approximately 0.4, with an average of 0.1 (Epperson and Clegg 1986).

The *W* locus polymorphism appears to be protected, as judged by perturbation experiments. Subramaniam and Rausher (2000) established populations with either low or atypically high frequencies of the *w* allele. After one generation gene frequencies converged toward the more typical, intermediate frequencies found in natural populations. Moreover, a series of investigations has revealed the operation of a mechanism that can account for protection of the white allele. In particular, white-flowered plants, when rare, appear to enjoy a transmission advantage that arises because of increased selfing. When white flowers are in the minority, bumblebees fail to visit them as frequently as they do lightly or darkly pigmented flowers (Brown and Clegg 1984; Epperson and Clegg 1987; Fry and Rausher 1997; Rausher et al. 1993). This undervisitation appears to cause increased selfing without any detectable pollen discounting (Rausher et al. 1993). In addition, inbreeding depression is minimal in this species (Chang and Rausher 2000). Because the additional transmission pathway through self-pollination is not compensated for by inbreeding depression or pollen discounting, white-flowered plants are expected to en-

joy a greater overall success in passing copies of their genes to offspring compared to the pigmented genotypes. The frequency-dependence of this transmission advantage—visitation and selfing rates are equal for the three genotypes when whites are common (Epperson and Clegg 1987; Rausher et al. 1993)—implies that any additional selective advantage of appropriate magnitude associated with the dark allele can balance this transmission advantage and yield a stable polymorphism (Fry and Rausher 1997).

The nature of such a dark-allele advantage has been more difficult to identify. For example, in an experimental population in which the frequency of the dark allele was lower than normal (0.5), Rausher and Fry (1993) failed to find an advantage for the dark allele in either viability or seed production. Although there was some evidence for overdominance in seed size, subsequent experiments failed to reveal offspring fitness effects substantial enough to account for protection of the dark allele (Mojonnier and Rausher 1997). Analysis of reproductive success in the same experimental population also failed to find any increased success of light or dark plants over whites as pollen donors (Fry and Rausher 1997).

One potential mechanism for protecting the dark allele has been identified. Fry and Rausher (1997) found evidence for non-Mendelian inheritance favoring the dark allele in pollen produced by heterozygous plants in their experimental population. In addition, controlled crosses indicated variation among pollen parents in the proportions of pollen carrying the light and dark alleles. Finally, using a simple genetic model with parameters estimated from experimental populations, Fry and Rausher showed that jointly the two factors—biased inheritance for the dark allele from heterozygous pollen parents and frequency-dependent transmission bias favoring whites when rare—can produce a stable polymorphism at the *W* locus. When 60% of the pollen produced by heterozygous plants carries the *W* allele, the model produced equilibrium frequencies approximating those seen in natural populations.

The objective of the investigation reported here was to determine whether this explanation holds generally for most populations of *I. purpurea*, or is specific to just some, such as the one examined by Fry and Rausher (1997). Specifically we used controlled crosses involving a large number of replicated genotypes from two different populations to determine wheth-

er non-Mendelian inheritance of paternal alleles at the *W* locus is characteristic of other natural populations of this species, and thus provides a general explanation for why the dark allele is maintained in polymorphic populations.

Materials and Methods

Biased inheritance acting through the pollen parent can be detected readily as a deviation from expected Mendelian ratios in the offspring produced by crossing a heterozygous pollen parent to a homozygous seed parent. We adopted this approach by performing experimental crosses using plants of each genotype collected in the field as newly germinated seedlings. Each heterozygous plant chosen for testing as a pollen parent was crossed to three seed-parent plants from the same population, one each of the *WW*, *Ww*, and *ww* genotypes. Different seed parents were used for each pollen parent. The offspring from the *Ww* × *ww* crosses, which are either white or light in color, were scored first in both populations sampled because determination of their offspring genotype is unambiguous. The crosses to *Ww* and *WW* seed parents, whose offspring are more difficult to score, were included for one of the two populations sampled to determine whether any biased inheritance that existed depended on the maternal genotype at this locus.

We collected newly germinated seedlings from two populations in Durham County, North Carolina. Approximately 1000 plants were sampled from the Duke University Field Station ("Field Station" population), and another 1000 from an agricultural field in northern Durham County (the "Riverlea" population). Seedlings were raised to first flowering to identify their genotypes. The *ww* genotype was rare, and its number limited the number of crosses performed. Thus, in total, 27 heterozygotes were tested as pollen parents from the Field Station population and 30 from the Riverlea population.

We performed all crosses in the greenhouse. The flowers on plants serving as seed parents were emasculated as buds in the afternoon prior to their opening the next morning. We then hand-pollinated each seed parent by rubbing the stigma with a dehiscent anther collected from its paired heterozygous pollen parent. We strove to perform enough pollinations to produce 120 seeds from each cross; however, a number of plants did not survive long enough or bloom sufficiently to attain

this number. Each seed produced was scarified to promote germination and the seedlings were raised in flats in the greenhouse to minimize differential survival as a source of bias. We scored flower color when the plants first bloomed. While the progeny from white seed parents were easily distinguished as either white or light, the light and dark offspring produced from the crosses to light and dark seed parents can be difficult to distinguish. Consequently we assigned light and dark phenotypes to progeny only after we had scored each plant's flower at least twice on separate days, and the scorings agreed. In addition, the identity of the capsule of each seed was recorded so that if bias was observed it could be linked to differences in embryo survival among capsules.

To test for biased inheritance we applied an exact binomial test (Sokal and Rohlf 1969) to the progeny of $Ww \times ww$ and $Ww \times WW$ crosses. For the cross to a heterozygous seed parent we tested the progeny using a Chi-square goodness-of-fit test (Sokal and Rohlf 1969) to the Mendelian expectations. In addition, a G test (Sokal and Rohlf 1969) was applied to each population to test for heterogeneity among pollen parents in the probability of transmitting the dark allele.

Results

We did not detect biased inheritance in either population sampled. For the Field Station population, the progeny of 27 heterozygous pollen parents crossed only to white seed parents were scored. Offspring germination and survival to first flowering was high, 99.34% (3019 of 3039 seeds planted germinated), eliminating the possibility that differential survival obscured the true inheritance rates of each allele. The proportion of offspring inheriting the dark allele exhibits a unimodal distribution centered on 0.5 (Figure 1), as would be expected if there were no average deviation from Mendelian inheritance. Individually, only two sets of progeny differed significantly from Mendelian expectation using the exact binomial test. One cross produced a ratio of 49 whites:71 lights ($P = .055$) while the other produced a 43:70 ratio ($P = .014$). Given the number of tests performed, two significant outcomes would be expected by chance with reasonable probability. Because excess, unscored seeds were available for these two pollen parents, we repeated the test for biased inheritance. For the first pollen par-

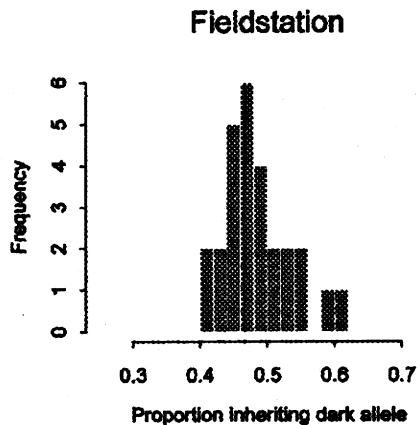


Figure 1. Frequency distribution of proportion of offspring from a particular cross that inherited the dark allele from the Ww pollen parent. All plants crossed were collected from the Field Station population. A total of 27 crosses between Ww pollen parent and ww seed parent were conducted. The number of offspring tested per cross ranged from 89 to 123, with a median of 113.

ent, extra seeds from the cross to the white seed parent were planted and scored. These seeds produced a ratio of 50 whites:48 lights, suggesting that the earlier "significant" result was spurious. For the second pollen parent with apparent significant inheritance bias, there were no additional seeds from the cross with the white seed parent to score. Instead we planted the seeds produced from this pollen parent crossed to the Ww seed parent. A ratio of 37 ww :69 Ww :27 WW was produced, which does not differ significantly from Mendelian expectations ($P = 0.43$). It thus appears unlikely that any of the 27 crosses truly exhibited biased inheritance. This pattern is confirmed by the absence of detectable heterogeneity among pollen parents in the proportion of transmitted alleles carrying the dark allele (G test: $P = .209$), and the absence of a significant deviation from Mendelian expectation for the entire data set, pooled over pollen parents (1555 ww :1464 Ww , $P = .101$, two-tailed test).

Overall offspring survival for the Riverlea population was 98.02% (3576 of 3648 seeds planted), again virtually precluding the possibility of differential survival among genotypes influencing apparent inheritance rates. For this population we tested 30 Ww plants as pollen parents crossed to white-flowered seed parents. The number of offspring tested per cross ranged from 10 to 120, with a median of 50. Once again, the overall distribution of inheritance frequencies for crosses to ww seed parents exhibited little evidence of

deviation from equal inheritance of dark and white alleles (Figure 2). Moreover, none of the 30 crosses to white seed parents individually exhibited a significant deviation from the Mendelian expectations ($P > .05$ in all cases). There was no detectable heterogeneity in the transmission ratio among crosses (G test: $P = .57$), and pooling over the crosses did not reveal any significant deviation from the expected 1:1 ratio of whites:lights (811:783; $P = .499$).

For 13 of the Riverlea pollen parents tested above we also scored the progeny from their crosses to heterozygous light (Ww) and homozygous dark (WW) seed parents, and again failed to detect biased inheritance. The number of offspring tested per cross ranged from 18 to 127, with a median of 71 for the crosses to light seed parents and ranged from 40 to 122 with a median of 75 for the crosses to the dark seed parent. Among all these crosses, only one produced progeny that differed significantly from Mendelian expectation: a cross to a heterozygous seed parent produced progeny in a 21 ww :66 Ww :40 WW ratio ($P = .05$). As before, one anomalous significant result would be expected by chance. The same pollen parent, when crossed to a white seed parent, produced a ratio of 54 ww :43 Ww , and when crossed with a dark seed parent produced a ratio of 50 Ww :32 WW , neither result being significant. (The latter result produced $P = .06$, but the bias is in the direction opposite that anticipated.) It thus seems that the nominally significant ratio when crossed with a heterozygous seed parent is probably an artifact of chance. This inference is confirmed by the absence of heterogeneity in inheritance ratios among crosses ($P = .65$ and $P = .13$ for light and dark seed parents, respectively), and the failure of the pooled samples to deviate significantly from Mendelian expectations (225 ww :481 Ww :246 WW , $P = .597$ and 523 Ww :507 WW , $P = .640$).

Discussion

The principle implication of our results is that non-Mendelian inheritance of paternal alleles at the W locus in seeds sired by heterozygote pollen parents is not a general explanation for the widespread maintenance of the W -locus polymorphism in *I. purpurea*. While this explanation may apply to the population studied by Fry and Rausher (1997), it clearly does not apply to the two populations examined in this study. Moreover, our failure to detect bias

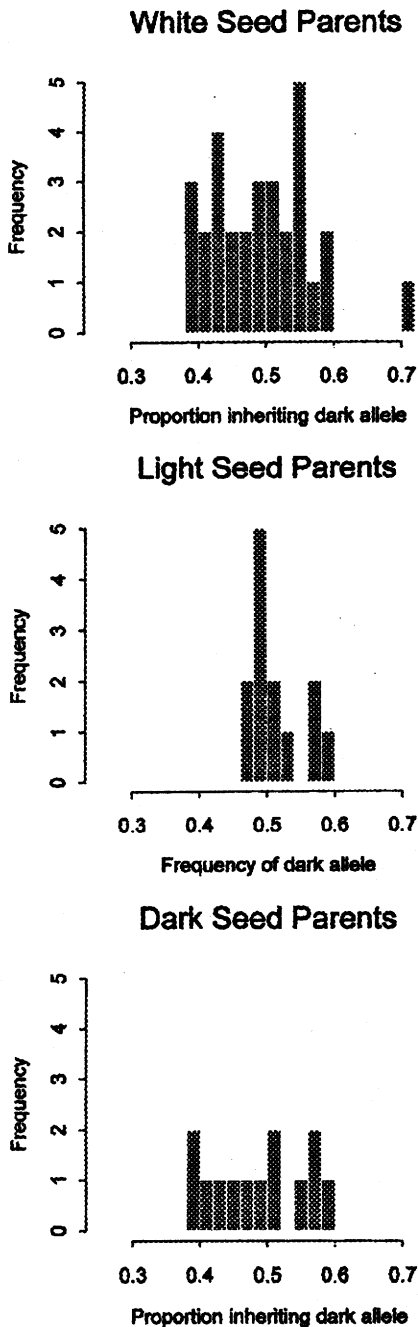


Figure 2. Frequency distributions, by seed parent genotype, of proportion of offspring that inherited the dark allele in crosses from *Ww* pollen parents. All plants crossed were collected from the Riverlea population. See text for variation in number of offspring tested per cross. (A) A total of 30 *Ww* pollen parents each crossed to a different *ww* seed parent. (B) A total of 13 of the *Ww* pollen parents used in (A) were also each crossed to 13 *Ww* seed parents. The frequency of the dark allele in the offspring is reported rather than the proportion of offspring inheriting the dark allele from the pollen parent. (C) The same 13 *Ww* pollen parents used in (B) were each crossed to a different *WW* seed parent.

is not due to lack of statistical power: if anything, the trend in both populations was for the white allele to be inherited more frequently than the dark allele.

The difference between our results and those of Fry and Rausher (1997) may be explained in two reasonable ways: (1) populations differ in whether they contain alleles modifying inheritance bias, either at "restorer" loci or at the *W* locus itself (the "modifier-variation" hypothesis); and (2) the biased inheritance detected by Rausher and Fry was artifactual ("artifact" hypothesis). Although the modifier-variation hypothesis is made plausible by the apparent existence in other plant species of variation among populations in the presence or absence of restorer genes for characters such as male sterility and sex ratio (Boutin-Stadler et al. 1990; Koelewijn and Van Damme 1995; Taylor 1994), we at this point have no direct evidence that it is applicable to the *W* locus in *I. purpurea*, or even that restorer or modifier alleles exist.

It seems to us unlikely that Fry and Rausher's results were completely artifactual because replicate crosses from their population exhibited significant heterogeneity in the proportion of dark alleles inherited from the heterozygous pollen parent and because two of six crosses exhibited significant deviations from the expected 1:1 ratio of whites to lights. There thus appear to be real deviations from Mendelian inheritance in that population. However, the evidence that there is a net bias favoring the dark allele in that population is less compelling. The crossing experiments reported suggest no net bias: of the two crosses exhibiting significant bias, one favored the dark allele while the other favored the white allele. This pattern suggests that there may be segregating in this population a locus linked to the *W* locus that exhibits biased inheritance. If true, the small number of replicate crosses used in the field experiment (the 2155 experimental plants were derived from just 28 wild-collected progenitor plants) means that it is quite possible that the dark allele was accidentally associated with the biasing allele at the linked locus in the experimental plants, producing artifactual evidence of non-Mendelian inheritance at the *W* locus.

While these considerations suggest to us that the existence of inheritance biases are unlikely, we cannot completely rule out the possibility that they occur in some populations and contribute to active maintenance of the *W* locus polymorphism, as

envisioned by Fry and Rausher (1997). This possibility raises the question of whether the dark allele can be maintained in populations without bias by gene flow from populations with bias. Such a phenomenon is conceivable, since at intermediate to high frequencies there is no difference in selfing rates among the *W* locus genotypes, and thus no selection favoring the white allele (Brown and Clegg 1984; Rausher et al. 1993). If *W* locus variation were truly neutral at these frequencies, very little gene flow would be required to maintain similar gene frequencies in populations with and without biased inheritance as (Hartl and Clark 1989).

It seems unlikely, however, that in the face of even small amounts of gene flow some populations would be biased while others remained bias free. Thus the results of this study indicate that either (1) all populations, including that studied by Fry and Rausher (1997), truly lack biased inheritance, or (2) some populations have biased inheritance and others lack it, implying that gene flow between populations is minimal. An additional possibility is that we may have inadvertently biased our study against finding very strong biased inheritance: in order to test heterozygotes collected from natural populations for biased inheritance, we sampled populations known to be polymorphic and disregarded populations which appeared to be fixed for the dark allele. Populations of the latter type may be expected to harbor any alleles with the strongest inheritance bias, alleles for which the bias is strong enough to overcome the transmission advantage enjoyed by the white allele because of increased selfing. However, alleles with very strong bias are not relevant to understanding the maintenance of variation in polymorphic populations, the goal of our investigation, because by definition they lead to fixation of the dark allele.

In conclusion, our results appear to rule out biased inheritance of alleles from heterozygous pollen parents as a universal mechanism for protecting the dark allele in polymorphic populations of *I. purpurea*. Similarly, other investigations have failed to detect a dark-allele advantage in post-germination viability or in either the male or female components of fitness (Fry and Rausher 1997; Mojonnier and Rausher 1997; Rausher and Fry 1993). The one stage of the life cycle in which selection on the *W* locus has not yet been examined is the seed stage, in which seeds lay buried in the soil for at least 7 months. It is conceivable that pleiotropic effects of the

W locus acting during this period may generate a selective advantage that protects the dark allele. This possibility deserves examination.

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Genetic Variability in the Iberian Imperial Eagle (*Aquila adalberti*) Demonstrated by RAPD Analysis

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RAPD analysis was used to estimate the genetic diversity in an Iberian imperial eagle (*Aquila adalberti*) population, one of the most threatened bird species in the world. Forty-five of 60 arbitrarily designed primers amplified 614 loci in 25 individual eagles, 59.7% of which were polymorphic. In contrast to the traditional allozyme analysis performed in a previous study, the RAPD method has revealed a high level of heterozygosity in this species ($H = 0.267 \pm 0.008$). The genetic distances estimated between 25 eagles can serve to establish more adequate mating in order to preserve genetic variability. Conservation efforts being carried out in Spain in this species might be successful based on the results obtained in the present work.

The Iberian imperial eagle (*Aquila adalberti*) is a diurnal species of prey, living almost exclusively in the southwest of the Iberian peninsula (González and González 1991). Despite being one of the most threatened bird species in the world (Collar and Andrew 1988) with a decreasing census of less than 126 pairs, until recently genetic studies had not been carried out on this species. Recently, molecular and cytogenetic studies have shown a clear separation between *A. adalberti* and eastern imperial eagle (*A. heliaca*) (Padilla et al. 1999; Seibold 1994; Seibold et al. 1996). A possible consequence of the small number of breeding animals is the loss in the amount of genetic variation present in this species. This loss of variability can result in a significant decrease in fitness (inbreeding depression) and consequently a high risk of extinction (Bijlsma et al. 1994; Frankel and Soule 1981; Hedrick and Miller 1992; Schonewald-Cox et al. 1983; Soule 1987). Knowledge of genetic variation is considered to