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## DO FLORAL PIGMENTATION GENES ALSO INFLUENCE RESISTANCE TO ENEMIES? THE *W* LOCUS IN *IPOMOEA PURPUREA*

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**Abstract.** Biochemical pathways that produce floral pigments often also produce secondary compounds that are believed to protect plants from natural enemies. Mutations that affect floral pigment patterns are thus also expected to influence the production of compounds conferring resistance to natural enemies, suggesting that the evolution of floral pigment patterns may be guided not just by interactions with pollinators, but also by indirect selection exerted by enemies on resistance. In particular, mutations that block production of floral pigments may be expected either to block production of defensive compounds or, alternatively, to lead to increased production of defenses, depending on the position of the blockage in the pathway. This expectation was examined in the morning glory *Ipomoea purpurea* by assessing whether white-flowered genotypes are more or less susceptible to attack by natural enemies than pigmented genotypes. No such difference in susceptibility was detected, although resistance to capsule-feeding insects exhibited a pigment genotype  $\times$  background genotype interaction. While this negative result indicates that natural enemies probably do not influence the evolution of floral pigment pattern in *I. purpurea*, this remains a possibility in other systems.

**Key words:** anthocyanins; floral pigments; herbivory; *Ipomoea*; resistance.

### INTRODUCTION

The evolution of floral displays is thought to be influenced largely by selection imposed by pollinators (Baker 1961, Grant and Grant 1965, Baker and Hurd 1968, Feinsinger 1983). Floral color, for example, is thought to evolve to attract specific types of pollinators (Faegri and van der Pijl 1979, Miller 1981, Waser and Price 1981, Feinsinger 1983), while some floral pigment patterns (e.g., nectar guides) are believed to have arisen because they increase the efficiency at which pollinators exploit floral resources and hence transport pollen (Knoll 1926, Manning 1956). Seldom considered, however, is the possibility that variation in genes affecting floral pigment hue, intensity, or pattern, which has been reported for a number of plant species (e.g., Epling and Dobzhansky 1942, Harding and Mankinen 1967, Ennos and Clegg 1983, Stanton et al. 1986), may have pleiotropic effects on other characters affecting fitness, and that therefore the evolution of floral displays may be constrained, or even driven, by indirect selection imposed on these other characters (Lande 1979, Charlesworth 1990).

A consideration of the biochemical pathways that produce floral pigments, however, suggests that this

possibility should be considered seriously. In particular, many floral pigments are the products of biochemical pathways that also produce plant secondary compounds that are thought to function primarily as defenses against herbivores and pathogens (Shaver and Lukefahr 1969, Harborne 1976, 1979, Stotz et al. 1985). It thus seems plausible that mutations that affect these pathways may in many cases influence not only floral pigmentation, but also susceptibility to natural enemies. In other words, the evolution of floral displays may often be guided by selection imposed by both pollinators and herbivores.

An illustration of this idea is provided by the flavonoid pathway (Fig. 1). Flavonoids are ubiquitous in angiosperms and are common in gymnosperms and some ferns (Swain 1975). Moreover, the basic branching structure of the flavonoid pathway is conserved across these taxa. Anthocyanins, which are widely distributed floral pigments, are primary products of one branch of the pathway (the "main," vertical branch in Fig. 1). Other branches produce classes of compounds such as dihydro-chalcones, flavones, flavonols, and tannins, all of which include compounds that are biologically active against natural enemies (Harborne and Ingham 1978, Elliger et al. 1980a, b, Dreyer and Jones 1981, Lane and Schuster 1981, see also Harborne et al. 1975 for a general survey). Polyphenols and tannins, for example, are often considered archetypal defensive compounds that protect plants from pathogens and herbivores (Feeny 1976, Rhoades and Cates 1976). Sim-

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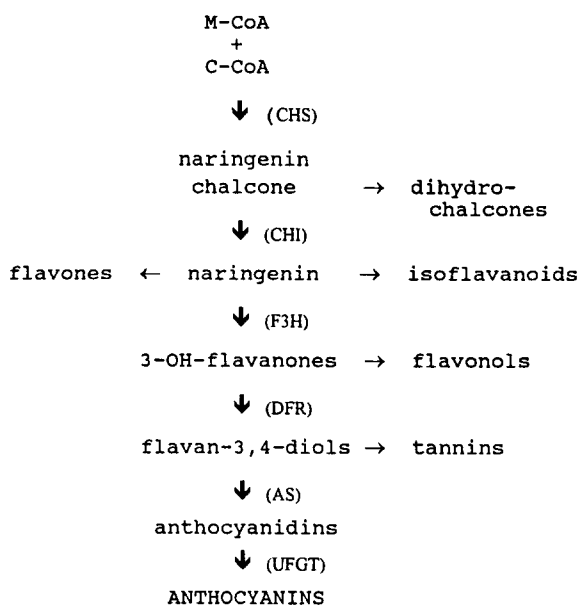


FIG. 1. Simplified diagram of the flavonoid pathway. The main branch in the figure (heavy arrows) portrays intermediate products leading to production of anthocyanin pigments. Side branches (light arrows) indicate end products of these branches. Abbreviations in parentheses indicate the enzyme that catalyzes the corresponding reaction: chalcone synthase (CHS); chalcone isomerase (CHI); flavanone 3-hydroxylase (F3H); dihydroflavonol reductase (DFR); anthocyanidin synthase (AS); UDPglucose flavonoid 3-oxy-glucosyltransferase (UFGT).

ilarly, some flavones, flavonols, and isoflavones (including rotenones) have been shown to be toxic to, or disrupt growth or reproduction of, a range of animals (McClure 1975, Harborne 1979). Moreover, genetic analyses have shown that mutants affecting anthocyanin production also often affect the production of other flavonoids (Stotz et al. 1985, Mo et al. 1992, Saito et al. 1994). For example, in *Petunia* a mutant that blocks anthocyanin production also inhibits the production of the flavonol quercetin (Stotz et al. 1985). It is thus clear that in many cases, both floral pigments and defensive compounds may be competing for common biochemical precursors.

The implications of such competition for the evolution of floral pigments are potentially profound. Consider, for example, a mutation that inactivates an enzyme that catalyzes one step in the pathway branch leading to anthocyanins. Such a mutation would result in the absence of anthocyanins, and hence white rather than pigmented flowers, as is commonly seen in molecularly characterized mutants of Snapdragons, *Petunias*, and *Arabidopsis* (e.g., Coen et al. 1986, Linn et al. 1990, Martin et al. 1991). Depending upon the location of the gene/enzyme affected relative to branch points in the pathway, such a mutation could affect production of potentially defensive compounds in two different ways. On the one hand, if the mutation affects

a step before a branch, then that mutation will block the production of potentially defensive compounds as well as anthocyanins. For example, a mutation that inhibits chalcone isomerase (CHI), which catalyzes the transformation of naringenin chalcone to naringenin, would not only produce white flowers but would also block the production of tannins, flavonols, and flavones and isoflavonoids (Fig. 1). A mutant of this type would presumably be less well defended against natural enemies, leading to selection exerted by those enemies against the mutant allele, even if white flowers were favored by pollinators. On the other hand, if the white-flowered mutation affects a step in the anthocyanin pathway after another pathway branches off (Fig. 1), intermediates may accumulate and be channeled into the production of more defensive compounds than would normally occur (see Gould 1988 and Berenbaum and Zangerl 1988 for discussion and examples of such substrate competition in the production of alternative defensive compounds). For example, the same mutant affecting chalcone isomerase discussed above could cause naringenin chalcone to accumulate and, in plants possessing the appropriate enzymes, be channeled toward the production of excess dihydrochalcones. To the extent that dihydrochalcones are defensive, the white mutant in this case would be better defended than the wild type and hence favored by selection imposed by natural enemies.

In many plant species, some (but not all) enzymes in the anthocyanin pathway are represented by multi-gene families (Koes et al. 1987, Beld et al. 1989, Harker et al. 1990, O'Neill et al. 1990). The different genes in these families often allow for either tissue-specific or environmental-specific expression of those genes (Dooner and Robbins 1991, van der Meer et al. 1993). Tissue specificity, in particular, may allow an evolutionary uncoupling of the effects of anthocyanin mutants on floral pigments and defensive compounds. Thus, to the extent that the effects of, for example, a chalcone synthase mutant can be localized to just floral tissue, the production of tannins, flavonols, etc. in roots, leaves, and stems may be unaffected. However, examples of mutations that affect floral pigmentation also being expressed in vegetative tissues (Schoen et al. 1984, Simms and Bucher, *in press*) indicate that such evolutionary uncoupling is not universal. Whether these situations involve primarily single-copy genes is not known. Nevertheless, they do indicate that foliage-eating herbivores may have the potential to impose selection on variation affecting floral pigmentation.

These considerations suggest that *because the production of defensive compounds and floral pigments are dynamically connected by a common biosynthetic network, mutations in that network may often be subject to selection by both pollinators and natural enemies.* Consequently, the evolution of floral displays may often be influenced by herbivores, while the evolution of

chemical defenses may often be influenced by indirect selection imposed by pollinators. This reciprocal influence of pollinators and herbivores, if it occurs, should not be surprising, as it is simply a concrete manifestation of Sewell Wright's principle of universal pleiotropy.

Several evolutionary patterns are expected if this type of reciprocal influence is common in nature:

1. *The evolution of pigmentation should tend to involve genes coding for enzymes involved in later steps of the anthocyanin pathway.* This tendency should be especially true of "loss of function" mutations, i.e., mutations that completely inactivate either expression or functioning of one of the structural genes in the anthocyanin pathway. When such a mutation affects an early step in the pathway (e.g., chalcone synthase or chalcone isomerase; see Fig. 1), it will block the production not only of anthocyanins, but also of end products of many of the other branches in the pathway. Since all of these compounds are potentially defensive—and most certainly perform other useful functions for a plant—the detrimental consequences of such mutations likely will counterbalance any beneficial effects conferred by alteration of the floral display. By contrast, a mutation of this type acting at a late stage (e.g., affecting anthocyanidin synthase or UFGT) will block production only of anthocyanins. While biochemical imbalances may result from precursor accumulation, we would expect any detrimental effects of these imbalances to be much less severe than those due to failure to produce needed compounds.

2. *Certain types of evolutionary change in pigmentation may be more constrained than others.* For example, we would expect loss of pigmentation (e.g., evolution of white flowers) to be more constrained than alteration of pigment hue, since many loss of pigmentation mutations are likely to have highly deleterious effects. By contrast, most mutations that affect floral hue do so by altering the hydroxylation state of anthocyanin precursors, i.e., by adding or removing one or two hydroxy groups from particular positions on the B ring of the precursors (Forkmann 1991, Holton et al. 1993). This type of mutation has similar effects on other end products of the flavonoid pathway (i.e., potentially defensive compounds; Forkmann 1991, Stich et al. 1992). While in some cases minor alteration of the of the hydroxylation state of compounds can greatly alter the toxicity of a compound (McClure 1975), in many (most?) cases there is little effect on toxicity (e.g., Elliger et al. 1980b). In general, then, mutations affecting floral hue can be expected to have only minor effects on susceptibility to natural enemies.

3. *The evolution of pigmentation should tend to preferentially involve steps in the anthocyanin pathway whose enzyme is coded by multigene families.* This pattern is expected because multigene families are more likely to involve tissue-specific regulation, thus in-

creasing the likelihood that the pleiotropic effects of a floral-pigment mutation will be restricted to floral tissues. There will thus be less of a likelihood that fixation of such mutations will be opposed by indirect selection imposed by natural enemies or other environmental factors.

4. *Floral pigment evolution should be more evolutionarily labile in taxa that are defended by compounds other than flavonoids than in taxa that are defended primarily by flavonoids.* This pattern is expected because in plants that rely on non-flavonoid defenses, the defensive compounds are biochemically isolated from the flavonoid pathway. Consequently, mutations affecting production of anthocyanins are not likely to influence the production of defensive compounds, and hence are not likely to be subject to indirect selection by natural enemies. A special case of this prediction is that the evolution of floral pigmentation should be more constrained in taxa that are defended primarily by tannins and other flavonoid phenolics. In the context of plant-apparency theory (Feeny 1976, Rhoades and Cates 1976), this suggests that floral pigment evolution may be more constrained in plants that rely on "quantitative" defenses than in plants that rely on "qualitative" defenses.

#### *An empirical test of the pleiotropy hypothesis*

Although these predictions could be examined directly by using joint phylogenetic analyses of floral color evolution and evolution of the underlying flavonoid pathway, such an analysis will not be achievable in the near future because the flavonoid pathway and its associated genes have not yet been characterized in plant taxa that exhibit extensive evolution of floral pigmentation. Consequently, direct tests of these hypotheses are unlikely in the near future. However, another way of evaluating whether floral pigment evolution is constrained or influenced by selection imposed by natural enemies is to determine whether pigment variants are differentially susceptible to attack by natural enemies, since as long as natural enemies reduce plant fitness, differential susceptibility will lead to selection on pigment loci. Here we describe experiments designed to determine whether such differential susceptibility exists for a floral pigment polymorphism in the annual morning glory *Ipomoea purpurea*.

*Ipomoea purpurea* is an annual vine that grows in disturbed habitats throughout southeastern North America. Throughout this range, the pattern of anthocyanin pigmentation in flowers is quite variable. In particular, populations of *Ipomoea purpurea* exhibit variation at four unlinked loci affecting floral pigment hue and intensity (Ennos and Clegg 1983, Epperson and Clegg 1988). In this study, we concentrated on the *W* locus, which affects pigment intensity. *WW* homozygotes are darkly pigmented, *Ww* heterozygotes are lightly pigmented, and *ww* homozygotes are white with

pigmented rays (nectar guides). In one study of seventeen natural populations of *I. purpurea*, the frequency of the *w* allele ranged from 0.0 to  $\approx 0.4$ , with a mean of  $\approx 0.1$  (Epperson and Clegg 1986).

The most striking effect of variation at the *W* locus is on floral pigmentation. Moreover, pollinators differentiate between the floral morphs produced by that variation, leading to higher selfing rates for white-flowered than for pigmented genotypes (Brown and Clegg 1984, Epperson and Clegg 1987, Rausher et al. 1993). Coupled with absence of pollen discounting (Rausher et al. 1993) and minimal inbreeding depression (Pear 1983, S-M. Chang and M. D. Rausher, unpublished data), these observations imply that pollinators impose selection that contributes to the protection of the white allele in natural populations. The *W* locus has thus been characterized as being primarily a mating-system modifier, with selection on this locus being determined primarily by pollinators (Schoen and Clegg 1985, Epperson and Clegg 1987). Nevertheless, two lines of evidence suggest that variation at the *W* locus may have pleiotropic effects influencing susceptibility to herbivores. First, Rausher and Fry (1993) showed that *W*-locus variation influences plant size and flower and seed production. In particular, dark-flowered plants are smaller at the initiation of flowering and produce  $\approx 4\%$  fewer flowers and seeds. These observations are consistent with the possibility that the dark-flowered genotype is more susceptible to natural enemies. Second, Simms and Bucher (*in press*) showed that survival of larvae of the tortoise beetle *Charidotella bicolor* feeding on foliage of *I. purpurea* decreases with increasing doses of the white allele. There are thus measurable pleiotropic effects of *W*-locus variation on the quality of foliage for herbivores, which might conceivably cause differential susceptibility to herbivory. Moreover, the pattern of survival in Simms and Bucher's experiments are consistent with the idea that reductions in size and fitness reported by Fry and Rausher in pigmented plants is due to greater susceptibility to herbivores: survival is highest on darks, which is the genotype that produces the smallest plants and the fewest flowers and seeds. It thus seems plausible that *W*-locus genotypes vary in susceptibility to herbivory.

## METHODS

### *Study organisms*

*Ipomoea purpurea* is fed on by a variety of insect herbivores, which can be grouped into four categories based on the type of damage they cause. Three types of insects consume foliage: (1) flea beetles, *Chaetocnema confinis* (Chrysomelidae), (2) tortoise beetles (Chrysomelidae), *Deloyala guttata* and *Charidotella bicolor*, and (3) generalist herbivores, including several species of orthopterans and lepidopteran larvae. In addition, an unidentified species of fleahopper (Hemiptera: Miridae) causes a speckled bleaching pattern on

the leaves. Developing seed capsules are fed upon by several species of lepidopterans, but primarily by the corn earworm, *Heliothis zea* Boddie (Noctuidae), which also feed on the flowers. Finally, the rust fungus *Coleosporium ipomoea* (Uredinales: Coleosporaceae) produces orange lesions on the foliage (for further details on these organisms, see Rausher and Simms, 1989; Simms and Rausher, 1987, 1989, 1993; Simms, 1993).

### *Experiment 1: damage to foliage*

The purpose of this experiment was to determine whether *W*-locus genotypes differ in susceptibility to the four types of folivorous insects or to rust. Experimental seed of known genotype at the *W* locus and with the remainder of the genetic background (i.e., genes unlinked to the *W* locus) randomized were generated by three generations of crosses. In the first generation of crosses, plants collected as seedlings from an agricultural field in Orange County, North Carolina, were crossed in pairs ("great-grand parental pairs"), each pair consisting of one dark and one white plant. One heterozygote offspring ("grandparent") from each pair was then allowed to produce a large number of seeds by selfing. Heterozygous offspring from this selfing were discarded. The homozygous dark and white offspring ("parents") were then crossed among themselves to produce experimental seed known to be either white, light, or dark. All experimental seed descended from a common great-grand-parental pair is termed a "unit." There were six units. Within each unit, several (between 4 and 10) pairs of parents were crossed to produce seed of each genotype, and each plant of a pair was used both as seed parent and as pollen parent to allow detection of possible maternal effects. Each maternal parent contributed three seeds to the final experimental design, producing a total of 738 experimental plants.

Experimental seeds were germinated in Rootainers in a greenhouse. Approximately 1 wk later, seedlings were transferred to a disked field in the Duke Forest, Orange County, North Carolina.

The seedlings were planted in a randomized block design, with one seedling from each maternal parent in each of three spatial blocks. Spacing between plants was 1 m. Plants were allowed to twine up 1.3 m bamboo poles to mimic growth in corn fields. Naturally occurring plants were allowed to grow in the field, except for *I. purpurea*, which was removed by weeding.

Four weeks after transplantation, the plants were censused for leaf area, herbivore damage, and rust lesions. Leaf length and width was measured for every leaf, and leaf area was estimated from a regression of area on length and width ( $R^2 = 0.995$ ). Damage was estimated on the second, third, fifth and sixth leaves of the main stem, using transparent grids to estimate the amount of area lost to various types of herbivores (see Simms and Rausher 1987, 1989 for details of dam-

TABLE 1. Damage experienced by different *W*-locus genotypes in field experiments. (A) Mean percentage damage to foliage by different types of herbivores and a pathogen in experiment 1. (B) Means of percent damage to flowers and capsules in Experiment 2. Numbers in parentheses are standard errors.

Damage type	Genotype		
	<i>WW</i>	<i>Ww</i>	<i>ww</i>
A) Experiment 1			
Generalist herbivores	2.54 (0.24)	2.14 (0.20)	2.46 (0.22)
Tortoise beetles	0.10 (0.02)	0.08 (0.01)	0.10 (0.10)
Flea beetles	28.07 (0.96)	28.25 (0.97)	27.35 (0.91)
Flea hoppers	53.79 (2.10)	55.11 (2.13)	54.90 (2.21)
Rust	6.04 (0.93)	5.53 (0.91)	8.90 (1.17)
B) Experiment 2			
Flowers	4.19 (0.47)	3.70 (0.41)	4.09 (0.45)
Capsules	20.28 (0.94)	21.46 (0.94)	22.99 (0.99)

age measurement). Rust infection was assessed by recording the proportion of censused leaves that exhibited lesions.

#### *Experiment 2: damage to flowers and capsules*

The purpose of this experiment was to determine whether *W*-locus genotypes differ in susceptibility to damage caused by insect herbivores that feed on flowers and developing seed capsules. Plants used in this experiment were the same as those used by Rausher and Fry (1993). The crossing design used to generate the experimental seeds for this experiment was similar to that described above, except that in the final generation of crosses, all parent plants descended from one set of great grandparents were mated to parental plants descended from a second set of great grandparents to prevent possible inbreeding depression. The experimental seed thus descended from two sets of great grandparents is termed a "unit" in this experiment. There were seven units in the experiment.

A total of 2,688 experimental seeds were planted in eight spatial blocks in a randomized block design in a disked field in Durham County, North Carolina and were allowed to twine up 1.3 m bamboo poles (further details of the experimental design and field site are described in Rausher and Fry 1993). Weeds were allowed to grow naturally, except for *I. purpurea* plants that were not part of the experiment.

On five days within a 2-wk period beginning  $\approx 8$  wk after planting, we scored the proportion of flowers exhibiting damage to the corolla or stigma. Approximately 1 mo after the flower censuses, the proportion of developing capsules that were damaged (i.e., exhibited a perforated capsule wall) was scored for each plant. Normally, capsules with perforated walls produce no seeds, since larvae consume most of the developing capsule.

#### *Statistical analyses*

The proportion of flowers and capsules damaged was either arcsine square-root transformed or log trans-

formed to render distributions more normal before subsequent analysis. The contribution of various factors to determining amount of damage was assessed in both experiments using a mixed-model nested analysis of variance on proportion damaged. When necessary, appropriate denominator mean squares for approximate (Satterthwaite) *F*-tests were constructed based on the Type III expected mean square as listed by the output of the SAS analysis (SAS Institute 1988, Neter et al. 1990). When compound denominators were necessary or when an interaction or nested effect mean square was the appropriate denominator, nonsignificant mean square components with *F*-values  $< 1.5$  were dropped to simplify the *F*-test (Neter et al. 1990; D. Burdick, *personal communication*).

## RESULTS

#### *Experiment 1: damage to foliage*

Although levels of herbivore and rust damage were comparable to that seen in previous experiments, we obtained little evidence indicating that *W*-locus genotypes differ in susceptibility of foliage to herbivores or rust. For none of the four herbivores did the amount of damage differ significantly among genotypes (Tables 1, 2, Intensity effect). Differences among genotypes in the proportion of leaves exhibiting rust lesions were barely significant (Table 1), with dark (*WW*) and light (*Ww*) plants exhibiting less damage than white (*ww*) plants. However, when the significance level is corrected for conducting multiple comparisons (because five types of damage were examined), this effect becomes no longer significant. In addition, of the ten two-way interactions involving pigment intensity genotype, only one is significant (Table 2, Unit  $\times$  Intensity and Block  $\times$  Intensity effects), and this significance also disappears when corrections for multiple comparisons are made. We thus obtained no indication that the effect of genotype on damage depends either on the genetic background (Unit effect) or on environmental quality, as reflected by spatial blocks, even though damage dif-

TABLE 2. Mixed-model analysis of covariance for leaf damage. Proportion damaged was arcsine square-root transformed before analysis. All effects were tested over the error mean square except: for generalist herbivores, flea hoppers and rust fungus, Block and Unit effects were tested over the Block  $\times$  Unit mean square; for tortoise beetles, Unit and Intensity effects were tested over the Unit  $\times$  Intensity mean square; for flea beetles, the Block, Unit, Intensity, Block  $\times$  Unit, Block  $\times$  Intensity, Unit  $\times$  Intensity, Pair(Unit  $\times$  Intensity) and Block  $\times$  Unit  $\times$  Intensity effects were tested over the Block  $\times$  Pair(Unit  $\times$  Intensity) mean square.

Source	df	Generalist herbivore		Tortoise beetle		Flea beetle		Flea hopper		Rust fungus	
		ss	F	ss	F	ss	F	ss	F	ss	F
Date [D]	1	0.0944	9.60**	0.0023	4.17*	0.1985	9.59**	0.7218	3.25	0.1715	2.75
Block [B]	2	0.4448	14.7**	0.0508	45.2***	6.2975	118*	18.2441	23.5***	2.5098	11.6**
Unit [U]	5	0.1202	1.59	0.0022	0.40	0.2475	1.86	2.1880	1.13	1.9316	3.58*
Intensity [I]	2	0.0053	0.27	0.0008	0.36	0.0366	0.69	0.1917	0.43	0.3882	3.11*
B $\times$ U	10	0.1508	1.53	0.0058	1.04	0.2714	1.02	3.8796	1.75	1.0785	1.73
B $\times$ I	4	0.0144	0.37	0.0003	0.13	0.1055	0.99	0.8015	0.90	0.0810	0.32
U $\times$ I	10	0.0756	0.77	0.0110	1.96*	0.3621	1.36	1.8964	0.85	0.4786	0.85
Pair [P(U $\times$ I)]	105	1.2054	1.17	0.7027	1.19	2.4865	0.89	20.9123	0.90	7.0364	0.77
B $\times$ P(U $\times$ I)	210	1.6264	0.79	0.1419	1.20	5.5965	1.29*	38.7074	0.83	13.3864	1.08
Dam(P(U $\times$ I))	123	1.0789	0.89	0.0842	1.22	3.1038	1.22	24.3706	0.89	7.5894	1.02
B $\times$ U $\times$ I	20	0.1571	0.80	0.0147	1.30	0.3364	0.63	3.0159	0.68	1.1221	0.90
Error	236	2.3214		0.1327		4.8832		52.3961		14.7074	

Note: Correction for multiple comparisons has not been applied when calculating indicated significance levels; \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

ferred significantly among blocks for all herbivores and for rust.

#### Experiment 2: damage to flowers and capsules

The results of this experiment provide little indication that *W*-locus genotypes differ in susceptibility to flower or capsule damage. The Intensity main effect showed no evidence of even approaching significance for either type of damage (Tables 1, 3). Among main effect interactions involving Intensity, only the Unit  $\times$  Intensity interaction for capsule damage was significant (Table 3). However, this effect remained significant (at  $P < 0.05$  level) even after applying a Bonferroni correction for multiple comparisons. Graphical examination of capsule damage for the three intensity genotypes (Fig. 2) reveals a "crossing" type interaction: in some

units, darks exhibited more damage than whites, while in other units, darks exhibited less damage than whites.

#### DISCUSSION

The results of this study provide little indication that genotypes at the *W* locus vary in susceptibility to natural enemies, and thus give little support to the notion that floral color variation is subject to indirect selection imposed by natural enemies. Similarly, although resistance to a variety of natural enemies is generally genetically variable in *I. purpurea* (Simms and Rausher 1987, 1989, Fineblum and Rausher 1995), our results indicate it is unlikely that indirect selection exerted by pollinators on the *W* locus will influence the evolution of resistance in this species. Nevertheless, we do not believe that these results should discourage further at-

TABLE 3. Analysis of variance for flower and capsule damage (proportion of flowers or capsules damaged). All effects were tested over the error mean square except: for capsule damage, the B effect was tested over B  $\times$  I mean square, the U effect was tested over the U  $\times$  I mean square, and the I effect was tested over a synthesized mean square. Correction for multiple comparisons has not been applied when calculating the indicated significance levels. Type III Sums of Squares (ss) were used.

Source	df	Flower damage		Capsule damage	
		ss	F	ss	F
Block [B]	7	3.569	11.68**	22.214	13.00***
Unit [U]	6	0.141	0.54	3.631	1.99
Intensity [I]	2	0.016	0.19	0.326	0.42
B $\times$ U	42	1.792	0.98	7.256	1.11
B $\times$ I	14	0.443	0.72	3.414	1.56†
U $\times$ I	12	0.476	0.91	3.651	1.95*
Pair [P(U $\times$ I)]	59	2.385	0.93	11.266	1.22
B $\times$ P(U $\times$ I)	413	17.397	0.96	68.274	1.06
B $\times$ U $\times$ I	84	3.832	1.04	10.832	0.83
Error	1910	83.385		298.210	

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; †  $P < 0.10$ .

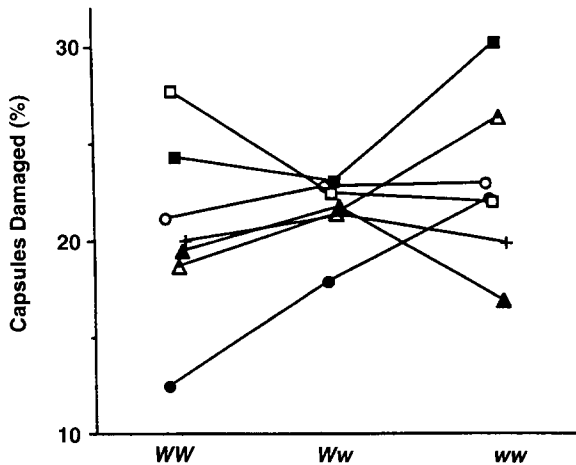


FIG. 2. Mean percentage of capsules damaged as a function of *W*-locus genotype for the seven units in Experiment 2. Each unit is represented by a different symbol.

tempts to detect such constraints, for a variety of reasons. First, although we detected no differences among *W*-locus genotypes in susceptibility to natural enemies, there are demonstrable effects of the *W* locus on foliage characteristics that influence herbivore survival (Simms and Bucher, *in press*). The type of pleiotropic effects that are necessary for natural enemies to generate indirect selection on pigment variation thus occur in this system. The failure of this type of variation in *I. purpurea* to generate differences in damage under field conditions may simply reflect the idiosyncracies of herbivore population regulation in this system (e.g., density-dependent larval survival could lead to mortality compensation, resulting in no difference among genotypes in damage even though density-independent survival, as measured in Simms and Bucher's experiment, varies among *W*-locus genotypes). Under other regulatory conditions, it is possible that the pleiotropic effects detected by Simms and Bucher could cause *W*-locus genotypes to experience different amounts of damage.

Second, although we detected little evidence of pleiotropic effects of the *W* locus on resistance, we can not rule out the possibility that this locus has pleiotropic effects on tolerance to natural enemies. Recent investigations suggest that many natural plant populations, including those of *I. purpurea*, are genetically variable for tolerance (Simms and Triplett 1994, Fineblum and Rausher 1995). Although little is known about the underlying mechanisms responsible for tolerance, because the *W* locus affects plant growth (Rausher and Fry 1993), it may also affect the ability of a plant to compensate for damage. Under these circumstances, natural enemies could select for pigment-intensity genotypes having the greatest tolerance, even though all genotypes are equally resistant. A direct test of this possibility would require quantification of the selective impact of natural enemies on the *W* locus.

Third, while we detected no main effect of *W*-locus genotype on susceptibility to any natural enemy, we did observe a strong genotype  $\times$  unit interaction for capsule damage. One explanation for this effect is that it is caused by a locus linked to the *W* locus that affects susceptibility. This explanation is not of great relevance to the general thesis of this study. However, two other explanations are of relevance: either pleiotropic effects of the *W* locus on damage depend upon the genetic background inherited by individuals of a unit from a particular set of great grandparents; or there is intra-allelic variation that affects damage to capsules (e.g., different copies of the *w* allele inherited by different units from different great grandparents do not have the same pleiotropic effects on damage). In either case, the interaction suggests that while in our experiments *W*-locus genotype did not *on average* influence resistance to herbivores or pathogens, such an influence might arise in populations with a different mix of background genotypes or a different mix of intra-allelic variants. Since previous work has shown that differential resistance to capsule-feeding insects imposes strong selection on *I. purpurea* (Rausher and Simms 1989), it is conceivable that in some *I. purpurea* populations such insects might indirectly impose selection on the *W* locus.

Finally, as described in the introduction, different types of mutations affecting the flavonoid pathway are likely to constrain or influence pigment evolution to a greater or lesser extent. Even if the *W* locus proves never to be subject to indirect selection by natural enemies, this may be more a reflection of the particular nature of the *W*-locus gene and its (currently unknown) position in the pathway than of the validity of the general hypothesis that natural enemies often help determine the course of evolution of floral displays. Similar experiments on different genes in the anthocyanin pathway (e.g., the *A* locus in *I. purpurea*, which affects both flower and stem color) could easily yield results very different from those reported here. For this reason, we believe that further examination of this possibility is warranted.

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#### LITERATURE CITED

- Baker, H. G. 1961. The adaptations of flowering plants to nocturnal and crepuscular pollinators. *Quarterly Review of Biology* 36:64-73.
- Baker, H. G., and P. D. Hurd. 1968. Intrafloral ecology. *Annual Review of Entomology* 13:385-415.
- Beld, M., C. Martin, H. Huits, A. R. Stuitje, and A. G. M. Gerats. 1989. Flavonoid synthesis in *Petunia hybrida*: partial characterization of dihydroflavonol-4-reductase genes. *Plant Molecular Biology* 13:491-502.
- Berenbaum, M. R., and A. R. Zangerl. 1988. Stalemates in

- the coevolutionary arms race: syntheses, synergisms, and sundry other sins. Pages 113–132 in K. C. Spencer, editor. Chemical mediation of coevolution. Academic Press, New York, New York, USA.
- Brown, B. A., and M. T. Clegg. 1984. The influence of flower color polymorphisms on genetic transmission in a natural population of the common morning glory, *Ipomoea purpurea*. *Evolution* **38**:796–803.
- Charlesworth, B. 1990. Optimization models, quantitative genetics, and mutation. *Evolution* **44**:520–538.
- Coen, E. S., R. Carpenter, and C. Martin. 1986. Transposable elements generate novel spatial patterns of gene expression in *Antirrhinum majus*. *Cell* **47**:285–296.
- Dooner, H. K., and T. P. Robbins. 1991. Genetic and developmental control of anthocyanin biosynthesis. *Annual Review of Genetics* **25**:173–199.
- Dryer, D. L., and K. C. Jones. 1981. Feeding deterrence of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae*: aphid feeding deterrents in wheat. *Phytochemistry* **20**:2489–2493.
- Elliger, C. A., B. G. Chan, A. C. Waiss, R. E. Lundin, and W. F. Haddon. 1980a. C-Glycosylflavones from *Zea mays* that inhibit insect development. *Phytochemistry* **19**:293–297.
- Elliger, C. A., B. C. Chan, and A. C. Waiss. 1980b. Flavonoids as larval growth inhibitors. *Naturwissenschaften* **67**:358–360.
- Ennos, R. A., and M. T. Clegg. 1983. Flower color variation in the morning glory, *Ipomoea purpurea*. *Journal of Heredity* **74**:247–250.
- Epling, C., and T. Dobzhansky. 1942. Genetics of natural populations: VI. Microgeographical races in *Linanthus parryae*. *Genetics* **27**:317–332.
- Epperson, B. K., and M. T. Clegg. 1986. Spatial autocorrelation analysis of flower color polymorphisms within substructured populations of morning glory (*Ipomoea purpurea*). *American Naturalist* **128**:840–858.
- Epperson, B. K., and M. T. Clegg. 1987. Frequency-dependent variation in outcrossing rate among flower color morphs of *Ipomoea purpurea*. *Evolution* **41**:1302–1311.
- Epperson, B. K., and M. T. Clegg. 1988. Genetics of flower color polymorphism in the common morning glory (*Ipomoea purpurea*). *Journal of Heredity* **79**:64–68.
- Faegri, K., and L. van der Pijl. 1979. The principles of pollination ecology, Third edition. Pergamon Press, New York, New York, USA.
- Feeny, P. P. 1976. Plant apparency and chemical defense. *Recent Advances in Phytochemistry* **10**:1–40.
- Feinsinger, P. 1983. Coevolution and pollination. Pages 282–310 in D. J. Futuyma and M. Slatkin, editors. *Coevolution*. Sinauer, Sunderland, Massachusetts, USA.
- Fineblum, W. L. and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. *Nature* **377**:517–520.
- Forkmann, G. 1991. Flavonoids as flower pigments: the formation of the natural spectrum and its extension by genetic engineering. *Plant Breeding* **106**:1–26.
- Gould, F. 1988. Genetics of pairwise and multispecies plant-herbivore coevolution. Pages 13–55 in K. C. Spencer, editor. *Chemical mediation of coevolution*. Academic Press, New York, New York, USA.
- Grant, V., and K. A. Grant. 1965. Flower pollination in the phlox family. Columbia University Press, New York, New York, USA.
- Harborne, J. B. 1976. Functions of flavonoids in plants. Pages 736–779 in T. W. Goodwin, editor. *Chemistry and biochemistry of plant pigments*. Academic Press, New York, New York, USA.
- . 1979. Flavonoid pigments. Pages 619–655 in G. A. Rosenthal and D. H. Janzen, editors. *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York, New York, USA.
- Harborne, J. B., and J. L. Ingham. 1978. Biochemical aspects of the coevolution of higher plants with their fungal parasites. Pages 343–405 in J. B. Harborne, editor. *Biochemical aspects of plant and animal coevolution*. Academic Press, New York, New York, USA.
- Harborne, J. B., T. J. Mabry, and H. Mabry, editors. 1975. *The flavonoids*. Academic Press, New York, New York, USA.
- Harding, J., and C. B. Mankinen. 1967. Genetics of *Lupinus*. I. Variations in flower color from natural populations of *Lupinus nanus*. *Canadian Journal of Botany* **45**:1831–1836.
- Harker, C. L., T. H. Noel Ellis, and E. S. Coen. 1990. Identification and genetic regulation of the chalcone synthase multigene family in pea. *The Plant Cell* **2**:185–194.
- Holton, T. A., F. Brugilera, D. R. Lester, Y. Tanaka, C. D. Hyland, J. G. T. Menting, C.-Y. Lu, E. Farcy, T. W. Stevenson, and E. C. Cornish. 1993. Cloning and expression of cytochrome P450 genes controlling flower color. *Nature* **366**:276–279.
- Knoll, F. 1926. *Insecten und Blumen. Abhandlungen der Zoologisch-botanischen Gesellschaft, Wien* **12**:1–646.
- Koes, R. E., C. E. Spelt, J. N. M. Mol, and A. G. M. Gerats. 1987. The chalcone synthase multigene family of *Petunia hybrida* (V30): sequence homology, chromosomal localization and evolutionary aspects. *Plant Molecular Biology* **10**:159–169.
- Lande, R. 1979. Quantitative genetic analyses of multivariate evolution, applied to brain:body size allometry. *Evolution* **37**:1210–1226.
- Lane, H. C., and M. F. Schuster. 1981. Condensed tannins of cotton leaves. *Phytochemistry* **20**:425–427.
- Linn, F. I. Heidmann, H. Saedler, and P. Meyer. 1990. Epigenetic changes in the expression of the maize A1 gene in *Petunia hybrida*: Role of numbers of integrated gene copies and state of methylation. *Molecular and General Genetics* **222**:329–336.
- Manning, A. 1956. The effects of honey-guides. *Behaviour* **9**:114–139.
- Martin, C., A. Prescott, S. Mackay, J. Bartlett, and E. Vrijlandt. 1991. Control of anthocyanin biosynthesis in flowers of *Antirrhinum majus*. *Plant Journal* **1**:37–49.
- McClure, J. W. 1975. Physiology and functions of flavonoids. Pages 970–1055 in J. B. Harborne, T. J. Mabry, and H. Mabry, editors. *The flavonoids*. Academic Press, New York, New York, USA.
- Miller, R. B. 1981. Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution* **35**:763–774.
- Mo, Y., C. Nagel, and L. P. Taylor. 1992. Biochemical complementation of chalcone synthase mutants defines a role for flavonols in functional pollen. *Proceedings of the National Academy of Science (USA)* **89**:7213–7217.
- Neter, J., W. Wasserman, and M. H. Kutner. 1990. *Applied linear statistical models*. Irwin, Boston, Massachusetts, USA.
- O'Neill, S. D., Y. Tong, B. Sporlein, G. Forkmann, and J. I. Yoder. 1990. Molecular genetic analysis of chalcone synthase in *Lycopersicon esculentum* and an anthocyanin-deficient mutant. *Molecular and General Genetics* **224**:279–288.
- Pear, J. R. 1983. Viability and fecundity consequences of breeding system in *Ipomoea purpurea* (L.) Roth, the common morning glory. Thesis. University of Georgia, Athens, Georgia, USA.
- Rausher, M. D., D. Augustine, and A. VanderKooi. 1993. Absence of pollen discounting in a genotype of *Ipomoea purpurea* exhibiting increased selfing. *Evolution* **47**:1688–1695.

- Rausher, M. D., and J. D. Fry. 1993. Effects of a locus affecting floral pigmentation in *Ipomoea purpurea* on female fitness components. *Genetics* **134**:1237–1247.
- Rausher, M. D., and E. L. Simms. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. *Evolution* **43**:563–572.
- Rhoades, D. F., and R. G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. *Recent Advances in Phytochemistry* **10**:168–213.
- Saito, N., J. Cheng, M. Ichimura, M. Yokoi, Y. Abe, and T. Honda. 1994. Flavonoids in the acyanic flowers of *Pharbitis nil*. *Phytochemistry* **35**:687–691.
- SAS Institute. 1988. SAS/STAT user's guide, release 6.03. SAS Institute, Cary, North Carolina, USA.
- Schoen, D. J., and M. T. Clegg. 1985. The influence of flower color on outcrossing rate and male reproductive success in *Ipomoea purpurea*. *Evolution* **39**:1242–1249.
- Schoen, D. J., D. E. Giannasi, R. A. Ennos, and M. T. Clegg. 1984. Stem color pleiotropy of genes determining flower color in the common morning glory. *Journal of Heredity* **75**:113–116.
- Shaver, T. N., and Lukefahr, M. J. 1969. Effects of flavonoid pigments and gossypol on growth and development of the bollworm, tobacco budworm, and pink bollworm. *Journal of Economic Entomology* **62**:643–646.
- Simms, E. L. 1993. Genetic variation for pathogen resistance in tall morningglory. *Plant Disease* **77**:901–904.
- Simms, E. L., and M. A. Bucher. Pleiotropic effects of flower color intensity on herbivore performance in *Ipomoea purpurea*. *Evolution*, in press.
- Simms, E. L., and M. D. Rausher. 1987. Costs and benefits of plant resistance to herbivory. *American Naturalist* **130**:570–581.
- Simms, E. L., and M. D. Rausher. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. *Evolution* **43**:573–585.
- Simms, E. L., and M. D. Rausher. 1993. Patterns of selection on pathogen resistance in *Ipomoea purpurea*. *Evolution* **47**:970–976.
- Simms, E. L., and J. Triplett. 1994. Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* **48**:1973–1985.
- Stanton, M. L., A. A. Snow, and S. N. Handel. 1986. Floral evolution: Attractiveness to pollinators influences male fitness. *Science* **232**:1625–1627.
- Stich, K., T. Eidenberger, F. Wurst, and G. Forkmann. 1992. Enzymatic conversion of dihydroflavonols to flavan-3,4-diols using flower extracts of *Dianthus caryophyllus* L. (carnation). *Planta* **187**:103–108.
- Stotz, G., P. de Vlaming, H. Wiering, A. W. Schram, and G. Forkmann. 1985. Genetic and biochemical studies on flavonoid 3'-hydroxylation in flowers of *Petunia hybrida*. *Theoretical and Applied Genetics* **70**:300–305.
- Swain, T. 1975. Evolution of flavonoid compounds. Pages 1096–1129 in J. B. Harborne, T. J. Mabry, and H. Mabry, editors. *The flavonoids*. Academic Press, New York, New York, USA.
- van der Meer, I. M., A. R. Stuitje, and J. N. M. Mol. 1993. Regulation of general phenylpropanoid and flavonoid gene expression. Pages 125–155 in D. P. S. Verma, editor. *Control of plant gene expression*. CRC Press, Boca Raton, Florida, USA.
- Waser, N. M., and M. V. Price. 1981. Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution* **35**:376–390.