Individual and Population Shifts in Flower Color by Scarlet Gilia: A Mechanism for Pollinator Tracking

Abstract. *Individual plants and populations of scarlet gilia* (Ipomopsis aggregata) *shift from darker to lighter corolla colors during the flowering season. Shifts to lighter color coincide with emigration of hummingbirds from the system. In the absence of hummingbirds, lighter colors attract the remaining pollinator, a hawkmoth. Comparison of plants that shift to lighter colors with those that fail to shift shows that shifting is adaptive in that it enhances reproductive success because of the preference of hawkmoths for lighter colored flowers. Color shifting therefore provides a mechanism for plants to track changing pollinator abundances.*

Although shifts in the foraging behavior of pollinators in response to changing rewards have been widely demonstrated (*1–3*), the adaptive significance of shifts in the floral characteristics of plants has been much less explored. We studied the extensive temporal variation in corolla colors that occurs between flowers of the same inflorescence and between early and late-flowering individuals of scarlet gilia (*Ipomopsis aggregata*). We report that the shift from darker to lighter corolla colors is adaptive because it permits plants to track different floral color preferences of the changing pollinator species. Such flexibility in flowering permits these plants to take advantage of the remaining pollinator when another pollinator is lost from the system.

Studies of color shifts in *I. aggregata* were conducted during the summers of 1981 through 1983 on Fern Mountain (elevation, 2500 m), near Flagstaff, Arizona. Although scarlet gilia is noted for the bright red coloration of its flowers, on Fern Mountain and other high-elevation sites an array of flower colors from red through various shades of pink to white are observed. Flowering begins in early to mid-July and lasts until early September; up to 250 flowers are produced per inflorescence per plant. Since *Ipomopsis* is essentially self-incompatible (*4–6*), seed set is primarily a result of pollen transfer between plants. Pollinators include the resident broad-tailed hummingbird (*Selasphorus platycercus*), the migrant rufous hummingbird (*Selasphorus rufus*), and a single species of hawkmoth, the white-lined sphinx (*Hyles lineata*). Pollinator densities were censused during standardized walks at approximately 3-day intervals. Individual plants were censused for flower color with a standardized corolla color scale (*7*). Censuses of over 4200 individually tagged plants were taken every 2 weeks along designated transects. Plants were added to the census as they initiated flowering.

As plants initiated flowering at different times during the 6- to 8-week flowering season, the population shifted from darker to lighter flower color. Red flowers were most commonly produced by plants that initiated flowering on or near 14 July, whereas plants that initiated flowering 1½ months later produced predominantly lighter colored flowers, and no red flowers were found [χ^2 (4) = 74.30, $P < 0.001$] (Fig. 1A).

In addition to color shifts in which lighter colored plants initiate flowering later in the season, similar shifts occur within the same inflorescence. Repeated censusing of the same inflorescences showed that flowers produced later in the season were lighter in color than earlier flowers (Fig. 1B). Over a 2-week span individuals typically shifted from one color shade to the next lighter shade (for example, from red to dark pink flowers); however, occasionally an individual shifted two shades lighter (for example, from red to pink). Late flowering plants were also more likely to shift colors than plants that flowered early. For example, over a 2-week period at the beginning of the flowering season, only 2 percent of all plants shifted colors, while among plants that initiated flowering 1½ months later, 26 percent shifted during a 2-week period [$\chi^2(1) = 107.94$, $P < 0.001$]. Consequently, color shifts in the same inflorescence and population color shifts occurred at the same time.

Shifts to lighter flower color by *I. aggregata* coincided with hummingbird emigration (Fig. 1, A to C). Hummingbird populations remained stable through July, then steadily declined through August until none remained by early September. When hummingbirds had emigrated hawkmoths became the sole pollinator, and their abundances remained stable to the end of the flowering season (Fig. 1C). Each year, on average, 77 percent of all within-plant color shifts and 80 percent of all population shifts took place during hummingbird emigration (August). Although hummingbird emigration from high elevations is associated with the change in flower color by *Ipomopsis*, the change in color is not the cause of emigration; other red-flowered hummingbird-pollinated plant species are abundant at high elevations, and yet hummingbirds emigrate from these populations (*8*).

Shifts to lighter corolla colors were reflected in the nocturnal foraging preferences of hawkmoths for lighter colored flowers. At sunset hawkmoths selectively visited red flowers of *Ipomopsis* (Fig. 2). As light intensity decreased there was a progressive shift to the use of lighter

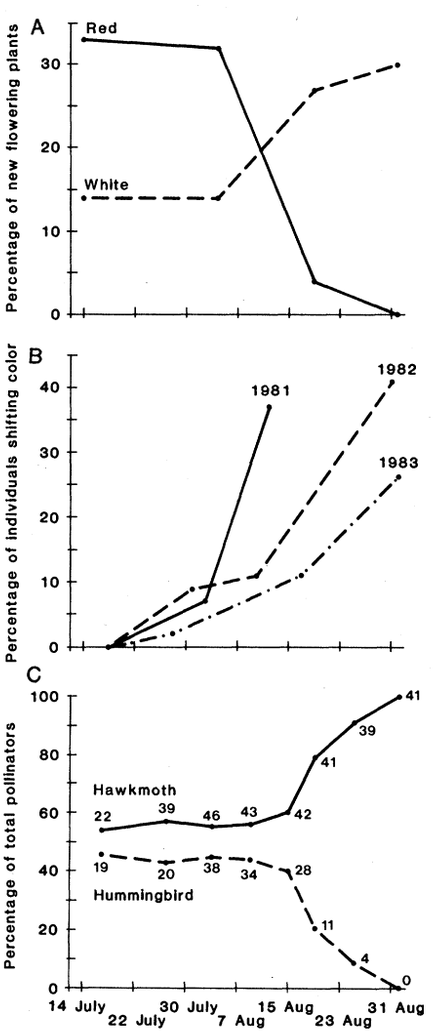

Fig. 1. Shifts in corolla color by populations (A) and individuals (B) of scarlet gilia in relation to pollinator abundance (C). (A) Plants initiating flowering early in the season were darker in color, whereas plants initiating flowering later produced lighter corollas. Pink individuals (not shown) displayed flower patterns intermediate to those of red and white individuals. (B) Individual plants shifted color during the flowering season. Three years of data are shown. Sample sizes for 1981, 1982, and 1983 are 482, 1282, and 2463 plants, respectively. (C) The two primary pollinators of *I. aggregata* changed in abundance during the flowering season. Numbers adjacent to points represent the number of pollinators observed on a particular date. Data shown are for 1983. Pollinator abundance data collected in 1982 also correspond with floral shifts.

and lighter colored flowers. By dark, the hawkmoths were visiting white flowers almost exclusively (Fig. 2). They continued to forage for at least 2 hours after dark, thus giving lighter colored plants (light pinks and whites) a higher probability of being pollinated (*9*).

The different foraging patterns of hawkmoths and hummingbirds led us to hypothesize that the shift to lighter flower color is an adaptation that takes advantage of the remaining pollinator when hummingbirds emigrate. The importance of hawkmoth and hummingbird pollination on each of the color variants of *Ipomopsis* was determined by placing nylon tricot–covered wire cages over the plants to exclude one of the two pollinators. Thirty plants (six per color variant) were covered only at night to exclude hawkmoths, while an additional 30 were covered only during the day to exclude hummingbirds (*10*). As a control, ten plants (two per color variant) were exposed to both hawkmoth and hummingbird pollination. At the end of the flowering season, comparisons were made in terms of fruit-set and seed-set. Since fruit-set reflects seed-set ($r = 0.71$, $n = 110$, $P < 0.02$), discussion will be restricted to fruit-set.

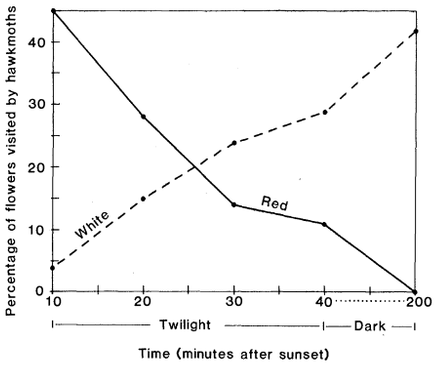

Fig. 2. Change in the preference of hawkmoths for corolla color during a single evening. During twilight, preference shifted progressively to lighter colored corollas. Preference for pink corollas (not shown) was intermediate. In total, far more lighter colored plants were visited than darker plants. The data represent 718 observed movements between plants.

Table 1. Percentage of scarlet gilia color variants setting fruit. In the absence of hummingbirds, lighter colored flowers set the most fruit because of the color preference of hawkmoths. The data show fruit-set percentages for six plants per color variant for each pollinator species and two plants per color variant for the control. Values in parentheses are total numbers of flowers examined.

Color variant	Hawkmoth-pollinated	Hummingbird-pollinated	Hawkmoth- and hummingbird-pollinated (controls)
Red	23 (604)	22 (666)	52 (130)
Dark pink	27 (438)	11 (613)	45 (163)
Pink	28 (945)	17 (863)	55 (66)
Light pink	33 (713)	17 (523)	42 (162)
White	40 (1203)	11 (862)	57 (104)

Statistical evaluation

Comparison	Test	Degrees of freedom	Value	*P*
Red versus white, hawkmoth-pollinated	χ^2	1	51.38	<0.001
	t	10	2.42	<0.04
Red versus white, hummingbird-pollinated	χ^2	1	34.43	<0.001
	t	10	2.86	<0.02
Controls; between color variants	χ^2	4	8.19	>0.08
	F	4, 5	0.36	>0.10
Hawkmoth-pollinated + hummingbird-pollinated versus controls	χ^2	4	2.12	>0.25
	t	8	1.19	>0.25

Table 2. Fruit-set for individuals of scarlet gilia that shifted color and those that remained colorfast. Results are based on two plants per color variant for each category. Values in parentheses are total numbers of flowers examined. Fruit-set differed significantly between flowers that shifted color and those that remained colorfast [$\chi^2(1) = 7.07$, $P < 0.001$ and paired $t(7) = 3.24$, $P < 0.01$]. No comparison possible for whites, all remain colorfast.

Color variant	Fruit-set (percent)	Color variant	Fruit-set (percent)
Colorfast plants		*Plants shifting color*	
Red	29 (180)	Red to dark pink	37 (181)
Dark pink	23 (60)	Dark pink to pink	42 (44)
Pink	29 (102)	Pink to light pink	30 (142)
Light pink	31 (74)	Light pink to white	53 (74)

When both pollinators were present no significant differences in fruit-set between color variants were observed (Table 1). Furthermore, when fruit-set in plants exposed only to hawkmoths is added to fruit-set in plants exposed only to hummingbirds and compared to the controls, no significant differences are observed. Thus the effects of the two pollinators on fruit-set were additive.

When the plants were exposed to only one species of pollinator, however, different color variants had a selective advantage. With only hummingbirds present, red flowers were twice as likely to set fruit as white flowers (Table 1). Just the opposite occurred when only hawkmoths were present; lighter colored flowers were nearly twice as likely to set fruit as red flowers. Fruit-set comparisons were also made on a per plant basis (Table 1).

Since the hawkmoth population remains stable while the hummingbird population declines, plants that naturally shift from darker to lighter colors should set more fruit than plants that remain colorfast. Although plants that remained colorfast and those that changed produced the same number of flowers ($P > 0.80$, two-tailed Mann-Whitney U test), plants that remained colorfast had a lower percentage of fruit-set than plants that shifted to the next lighter shade (Table 2). A shift of only one shade increased fruit-set by as much as 22 percent (mean, 13 percent). No plants shifted in the opposite direction (from lighter to darker), which should be least favored.

In another test of the effects of flower color on plant reproduction, we found that red flowers painted white achieved a higher percentage of fruit-set than controls. In late August, 105 flowers from ten red plants were painted white and, as a control, 115 flowers from ten red plants were painted red (a water-based latex paint was used to avoid damaging the flowers). Since all hummingbirds had emigrated by this time, only hawkmoths remained to pollinate these plants. As predicted, red flowers painted white had a significantly higher percentage of fruit-set than the control flowers [50 versus 31 percent, respectively; $\chi^2(1) = 7.59$, $P < 0.01$]. These results accurately reflect natural conditions. Red flowers painted white set 38 percent more fruit than red flowers painted red, while natural whites set 42 percent more fruit than natural reds.

In light of these results, flower color or colors within populations may be an evolutionary consequence of local pollinator abundance and foraging behavior. Popu-

lations of scarlet gilia on the San Francisco Peaks produce red and white morphs that have different flowering phenologies as well as individual plants that shift color within an inflorescence, whereas populations growing only a few kilometers away but 500 m lower in elevation do not shift color and produce only red flowers. Notably, scarlet gilia populations that do not shift color do not lose their hummingbird pollinators. In comparison, high-elevation populations that shift color do lose their hummingbird pollinators halfway through the flowering season (*8*). Since evolutionary changes in the characteristics of populations over short distances have been shown to be related to differences in microhabitat (such as competition, disturbance, and heavy metals) (*11–13*), it should also be expected that plants can become locally adapted to patterns of pollinator abundance and behavior (*14*). Color shifts within and between individual plants of *I. aggregata* permit these plants to track local changes in pollinator abundance in ecological time. Whether such local variation in flower color represents a stable polymorphism or is dynamic, favoring an even greater proportion of lighter colored flowers, remains to be determined.

KEN N. PAIGE
Department of Biological Sciences, Northern Arizona University, Flagstaff 86011
THOMAS G. WHITHAM
Biology Department, Museum of Northern Arizona, Flagstaff 86001
and *Department of Biological Sciences, Northern Arizona University*

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7. A standardized corolla color scale was devised with a dilution series of red and white enamel paint. Plants were grouped into one of five color categories: red (100 percent red), dark pink (75 percent red and 25 percent white), pink (50 percent red and 50 percent white), light pink (25 percent red and 75 percent white), and white (100 percent white).
8. Even in neighboring high-elevation areas with populations of *Penstemon barbatus* (a red-flowered, hummingbird-pollinated plant), hummingbirds emigrated during the same period and in the same manner as they did from Fern Mountain. Compared to low-elevation populations of *Penstemon*, high-elevation populations were generally larger and produced similar amounts of nectar per flower, yet hummingbirds still emigrated from them. It is therefore unlikely that pollinators emigrate from Fern Mountain because of local shifts in color, since *Penstemon* (which is found with *Ipomopsis*) provides an abundant and reliable nectar resource during the period of hummingbird desertion. These observations are supported by those of A. Kodric-Brown and J. H. Brown [*Ecology* **59**, 285 (1978)]. In addition, associated with hummingbird emigration is a significant switch in nectar production by red-flowered plants from diurnal to nocturnal secretion (K. N. Paige, unpublished data). Such evidence suggests that plants are responding to local patterns of pollinator abundance and behavior.
9. Since hawkmoths exhibit a negative phototactic response, a red filter was used for conducting nocturnal observations.
10. Pollinator exclosures were switched at sunset and sunrise to exclude hummingbirds and hawkmoths, respectively, throughout the flowering season, restricting overlap to only a few minutes.
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Bovine Leukemia Virus Long Terminal Repeat: A Cell Type–Specific Promoter

Abstract. *The functional activity of the promoter unit contained within the long terminal repeat (LTR) of bovine leukemia virus (BLV) was examined by monitoring transient expression of a heterologous gene placed under its control. Various cell lines were transfected with recombinant plasmids carrying the bacterial chloramphenicol acetyltransferase (CAT) gene coupled to the BLV LTR (pBL-cat). Transient expression of CAT activity directed by the BLV LTR was observed only in the established BLV-producer cell lines derived from fetal lamb kidney (FLK) cells and bat lung cells. The amount of CAT activity transiently expressed in FLK-BLV cells was decreased approximately tenfold by deletion of LTR sequences located within a region 100 to 170 nucleotides upstream of the RNA start site. Surprisingly, removal of the region 50 base pairs downstream of the RNA initiation site to the 3′- end of the LTR reduced the expression of CAT activity by 87 percent. The BLV LTR thus appears to be an unusual promoter unit, functioning in a cell type–specific manner and possessing sequences on both the 5′ and 3′ sides of the RNA start site that influence gene expression.*

Bovine leukemia virus (BLV) is a B-cell lymphotropic retrovirus associated with a disease complex termed "enzootic bovine leukosis" (*1*). Unlike most other avian and mammalian retroviruses, BLV transcripts have not been detected in tumors or lymphocytes of infected animals (*2*). In addition, BLV displays a highly restricted infectivity in vitro (*3, 4*). Nucleotide sequence data have revealed that BLV, like the human T-cell leukemia viruses types I and II (*5*) (HTLV-I and HTLV-II) possesses an unusual long terminal repeat (LTR) structure (*6, 7*). The LTR's bordering retroviral proviruses contain sequences required for viral integration, replication, and expression (*8*), and determine to a large extent the pathological consequences of infection. Because the LTR encompasses a promoter unit analogous to that controlling cellular gene expression, we suspected that the restricted expression and infectivity of BLV were functional manifestations of an atypical LTR. To examine this possibility, we tested the ability of the BLV LTR to promote the expression of a heterologous gene placed under its control in various cellular environments.

The BLV-infected fetal lamb kidney (FLK-BLV) cell line is one of only a few lines characterized that produce significant amounts of BLV (*3*). The four BLV proviruses harbored by FLK-BLV cells were cloned into bacteriophage lambda L47 (*9*). Restriction fragments containing each of the four proviral 5′ LTR's, as well as a single 3′ LTR, were inserted into the plasmid pSV0cat (*10*) as outlined in Fig. 1. The transcriptional utilization of the BLV LTR's were assessed by comparing the levels of CAT activity transiently expressed in cells transfected with pBL-cat and pRSVcat plasmids. The latter plasmid contains a CAT gene controlled by the Rous sarcoma virus LTR (*10*). Results from typical transfection experiments are shown in Table 1 and Fig. 2. In monkey kidney (CV-1), bovine kidney (MDBK), mouse fibroblast (LTK^-), or human rhabdomyosarcoma (RD4) cell lines, CAT activity was not detected after transfection with pBL-9cat or the promoterless pSV0cat. In these same cell lines, CAT activity was easily detected after transfection with pRSVcat, indicating that these cells were competent to take up DNA. Similar results were obtained in transfections of primary fetal lamb kidney (FLK) cells and a bat lung cell line, Tb1Lu (Table 1). In contrast, the BLV LTR directed high levels of CAT activity in the productive-