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Floral colour changes as cues for pollinators

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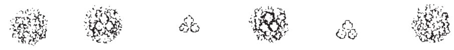
PLANTS have evolved traits that enable them to influence directly the behaviour and movement of their pollinators. Here I show that flowers in at least 74 diverse angiosperm families undergo dramatic, often localized, colour changes which direct the movements of a variety of pollinators to the benefit of both participants. Floral colour change was first noted almost 200 years ago[1] and is known in a variety of species[2–17], but the prevalence and significance of the phenomenon have gone largely unrecognized. I find that retention of older flowers increases a plant's attractiveness to pollinators from a distance, that pollinators discriminate between floral colour phases at close range, and that the discrimination involves learning. The phenomenon of floral colour change is taxonomically widespread, morphologically variable (Fig. 1), and physiologically diverse. It has evolved independently in the angiosperms many times and provides a striking example of functional convergence.

In 1877 Charles Darwin forwarded to *Nature* a letter from his colleague, the naturalist Fritz Müller, in which Müller remarked on a multicoloured *Lantana* growing in the Brazilian forest[3],

> "We have here a *Lantana* the flowers of which last three days, being yellow on the first, orange on the second, purple on the third day. This plant is visited by various butterflies. As far as I have seen the purple flowers are never touched. Some species inserted their proboscis both into yellow and into orange flowers; others, as far as I have hitherto observed, exclusively into the yellow flowers of the first day. This is, I think, a rather interesting case. If the flowers fell off at the end of the first day the inflorescence would be much less conspicuous; if they did not change their colour much time would be lost by the butterflies inserting their proboscis in already fertilized flowers."

I have used *Lantana camara* flowers and two species of nymphalid butterflies, *Agraulis vanillae* and *Junonia coenia*, to examine Müller's ideas. *L. camara* flowers are yellow only on the day they open, turning orange and then red on subsequent days. Red flowers are maintained on the plant for 1 to 9 days, depending on the *Lantana* variety and climatic conditions. Only yellow flowers, which comprise between 9 and 33% of the total floral display, offer nectar and pollen and are receptive (M.R.W., manuscript in preparation).

TABLE 1 Butterfly visits to *L. camara* displays of varying size and reward level



	Large red	vs Large YOR	Small yellow	vs Large YOR	Small yellow	vs Large red
Fraction of trials in which display type received most visits	0.5*/7	6.5*/7	0/7	7/7	1/6	5/6
Total butterfly visits	132	264	137	270	155	205
χ^2 (1 d.f.)	44.00†		43.46†		6.94‡	

Butterflies chose to visit *Lantana camara* inflorescences on the basis of the size of floral display, rather than the level of nectar reward it offered. In 3 replicated sets of trials, *A. vanillae* butterflies were offered different pairs of floral displays which provided various combinations of size and reward level. 'Large yellow-orange-red' (YOR) inflorescences were unmodified, and contained 3–5 yellow, 3–5 orange, and 24–30 red flowers. Flowers were removed from inflorescences as necessary to produce the 2 other types of displays: 'small yellow' consisted of 3–5 rewarding yellow flowers, and 'large red' consisted of 25–35 non-rewarding red flowers. Butterflies were offered large red versus large YOR (7 replicates); small yellow vs large YOR (7 replicates); small yellow vs large red (6 replicates). Inflorescences were picked in the morning before the yellow flowers opened, and were kept in water. In each trial, 3 samples of each of the 2 offerings were placed inside a 2-m^3 field cage, arranged alternately in water-filled jars, 0.5 m apart, in a hexagonal pattern. Ten to fifteen butterflies with previous experience in feeding on *L. camara* were present in the field cage during each half-hour trial. A 'visit' was counted when a butterfly landed on a inflorescence. Placement of each type of offering was rotated 60° between replicates of trials to control for possible position effects. A control trial, in which 6 identical samples were offered, showed no significant differences between positions (*G* test for goodness of fit, $G=1.07$, 1 d.f., n.s.). For each of the 3 sets of trials, I determined the fraction of trials in which a particular display type received more than 50% of the butterfly visits. Additionally, visits to each of the 3 samples of a given display type were grouped, and the total number of visits to each of the 2 offerings was compared using a χ^2 test for goodness of fit[27], with a null hypothesis of equal attractiveness.

* Value of 0.5 indicates that the outcome of the trial was tied, that is, both offerings received equal numbers of visits. † $P<0.001$; ‡ $P<0.01$.

To determine whether large displays are more attractive to pollinators at a distance, I conducted three sets of choice tests inside a field cage, offering experienced butterflies natural or manipulated *Lantana* inflorescences which provided various combinations of display size and nectar reward. The trials included: (1) large displays offering a nectar reward equal to that of small displays; (2) two equally large displays offering different amounts of reward; and (3) large displays offering less reward than small displays. When display sizes differed, butterflies chose to visit the larger offering, regardless of its level of reward; when display sizes were equal, the more rewarding offering was preferred. These results were statistically significant (Table 1). Maintenance of older flowers on an inflorescence thus increases the plant's attractiveness to pollinators at a distance.

To test whether pollinators discriminate between floral colour phases at close range, I presented caged butterflies, experienced in feeding on *Lantana*, with yellow and red *Lantana* inflorescences. These insects discriminated between the colour phases, choosing to feed at the yellow, pre-change flowers significantly more often than would be expected from the abundance of the flowers (Table 2*a*).

The discrimination between *Lantana* colour phases apparently involves associative learning, at least in some insects.

TABLE 2 Insect visits to colour-changing flowers

Plant (family)	Insect or insect family (*n* insects or feeding bouts)	Mean % pre-change flowers	Total visits to pre-change flowers	Total visits to post-change flowers	χ^2 (1 d.f.)
(*a*) Visits of *Agraulis vanillae* and *Junonia coenia* to *L. camara* (caged)					
Lantana camara (Verbenaceae)	*J. coenia* (15) Nymphalidae, Lepidoptera	10.5	347	227	1524.13*
Lantana camara (Verbenaceae)	*A. vanillae* (43) Nymphalidae, Lepidoptera	13.7	436	435	973.77*
(*b*) Insect visits to colour-changing flowers (field)					
Phyla nodiflora (Verbenaceae)	Apidae (3) Hymenoptera	24	100	12	261.71*
Phyla nodiflora (Verbenaceae)	Hesperiidae (1) Lepidoptera	24	28	1	83.69*
Phyla nodiflora (Verbenaceae)	Calliphoridae (1) Diptera	24	20	1	58.43*
Heliotropium anchusifolium (Boraginaceae)	Apidae (8) Hymenoptera	46.5	346	29	315.72*
Androsace lanuginosa (Primulaceae)	Bombyliidae (1) Diptera	42	19	0	26.24*
Androsace lanuginosa (Primulaceae)	Calliphoridae (1) Diptera	42	14	1	16.22*
Lobularia maritima (Cruciferae)	Syrphidae (1) Diptera	7.1	14	1	*
Lobularia maritima (Cruciferae)	Tachinidae (1) Diptera	7.1	23	0	*
Lobularia maritima (Cruciferae)	Calliphoridae (2) Diptera	7.1	57	0	*
Lupinus albifrons (Leguminosae)	Apidae (3) Hymenoptera	38.5	96	8	127.17*

A wide variety of insects discriminate between floral colour phases, visiting pre-change flowers significantly more than would be expected based on the abundances of these flowers in inflorescences. Visits were counted when the insect probed at the flower and attempted to reach the pollen or nectar. The proportion of flowers in each colour phase was determined, and the distribution of the insects' visits was tested against expected values based on abundances of floral colour phases, using a Chi Square test for Goodness of Fit. In cases where the expected value was less than 5, a binomial expansion was used[27]. *a*, Visitation patterns of two species of nymphalid butterflies, *A. vanillae* and *J. coenia*, on *L. camara*; these insects were observed in a flight cage. *b*, Visitation patterns of a variety of insects on a variety of plants, observed in the field, and grouped by family.
* $P < 0.001$.

Newly emerged naive butterflies visit both yellow and red flowers in their initial feeding bouts, but quickly come to concentrate their visits on the rewarding yellow flowers. The plant essentially 'teaches' the insect to focus its attention on sexually viable and rewarding flowers (Table 3).

In a broad survey of flowering plants, I found that colour-changing species were taxonomically and geographically diverse, occurring in at least 214 genera, 74 families and 33 orders worldwide. Conservatively then, colour change occurs in at least 21% of animal-pollinated angiosperm families.

Floral colour change benefits both plant and pollinator. The colour phase of a flower has been shown in several cases to provide an accurate indication of its sexual viability and reward status[9-17]. Comparing nectar volume, stigmatic condition and pollen availability in pre- and post-change flowers of 26 locally available species, I found that pre-change flowers offered nectar rewards in all 26 cases, whereas post-change flowers contained little or no nectar. In addition, pollen was available and stigmas appeared fresh and receptive in the pre-change flowers, but post-change flowers lacked pollen and appeared non-receptive. Thus plants receive efficient pollination service while pollinators are accurately directed to rewarding flowers.

A diverse array of pollinators discriminate between floral colour phases on a variety of colour-changing plants, as butterflies do on *Lantana*. I observed dipteran, hymenopteran and lepidopteran pollinators, representing 15 insect families, foraging on 28 different colour-changing plant species in the field. The insects consistently concentrated their visits on pre-change flowers, visiting them significantly more often than would be expected from the flowers' abundances (Table 2*b*). These insects were not necessarily the plants' natural pollinators: individuals of introduced species discriminated accurately, as did opportunistic nectar or pollen robbers. Thus colour-changing flowers and their pollinators need not have closely coevolved. Rather, the generality and power of the system depend on the plants' plasticity and the insects' ability to learn.

It is often assumed that plants have a largely passive role in plant–animal relationships. But plants are able, through a variety

TABLE 3 Mean preference indices for naive and experienced butterflies

	Junonia coenia	*Agraulis vanillae*
Naive	$\bar{x}=5.8$ $s_x=4.9$ $n=5$	$\bar{x}=3.3$ $s_x=1.0$ $n=8$
Experienced	$\bar{x}=21.6$ $s_x=26.3$ $n=15$	$\bar{x}=30.6$ $s_x=34.0$ $n=15$

Naive butterflies offered bicoloured *Lantana camara* inflorescences chose relatively fewer yellow and relatively more red flowers than did experienced individuals. Naive butterflies, which were not exposed to any flowers before they were offered *L. camara* inflorescences, were tested only once. Experienced butterflies, which had at least 2 days prior experience feeding on *L. camara*, were offered flowers on one or several occasions. For each butterfly, the colour of every flower probed was recorded; to standardize differences in abundances of floral colour phases, a preference index (PI) was calculated as: (number of visits to yellow flowers/number of yellow flowers)/(number of visits to red flowers/number of red flowers). A value of 1 indicates visitation proportional to abundances of colour phases; values above 1 indicate preferential visitation to yellow flowers. For experienced butterflies whose feeding preferences were observed on more than one occasion, mean PI values were used in calculating the overall mean. Mean PIs for naive and experienced butterflies were significantly different within each species: Mann–Whitney *U* test for *Junonia*, 66.5, $P < 0.01$; for *Agraulis*, 120, $P < 0.001$ (ref. 27). *n*, Number of butterflies.

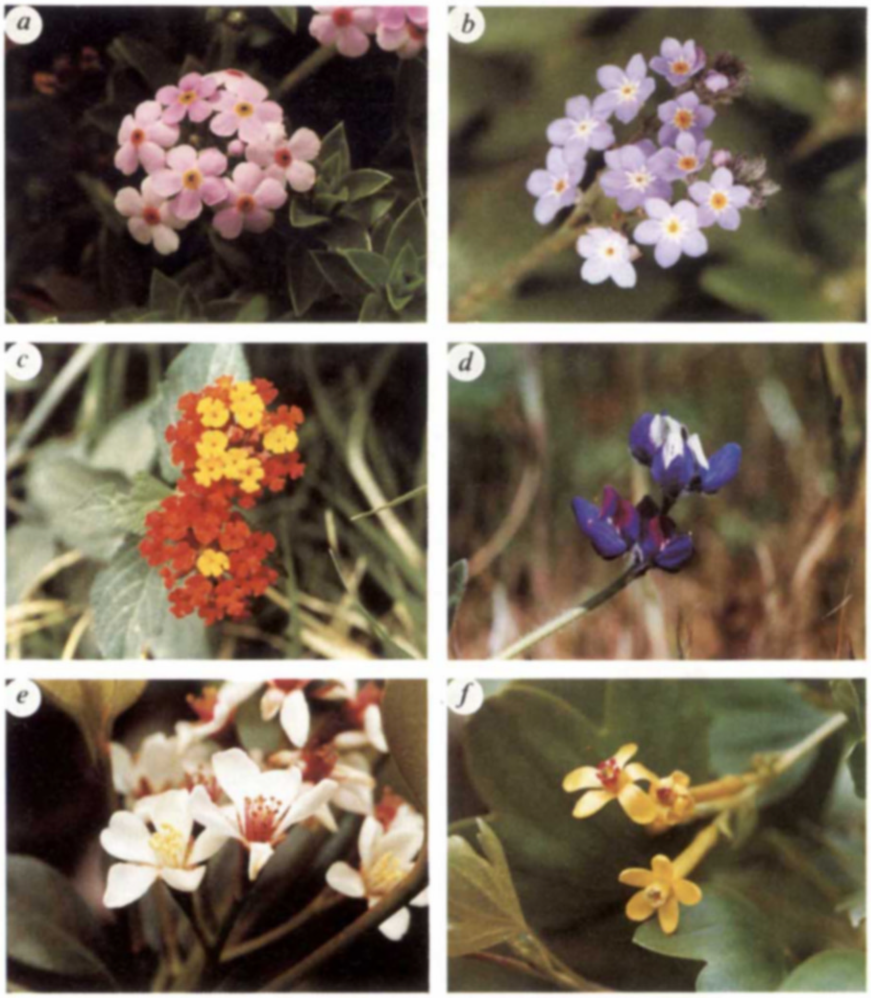


FIG. 1 Examples of floral colour change. Floral colour changes may involve different parts of flowers as well as different physiological processes[18]. Floral parts which change include the corona or eye, corolla throat, nectar guide, banner petal spot, specialized petal, hypanthium, filaments, ovary or whole flower. Physiological processes may involve either appearance or loss of anthocyanin or carotenoid pigments. Appearance of anthocyanin is the most common mechanism of change (M.R.W., manuscript in preparation). In all cases, regardless of the flower part or the physiological processes involved in the colour change, the end result is the same: young, sexually viable and rewarding flowers are visually distinguishable from older flowers. *a*, *Androsace lanuginosa* Wall. (Primulaceae): eye changes from yellow through orange to red; *b*, *Myosotis sp.* (Boraginaceae): corona changes from yellow to white; *c*, *Lantana camara* L. (Verbenaceae): whole flower changes from yellow to red; *d*, *Lupinus nanus* Dougl. (Leguminosae): banner petal spot changes from white to purple; *e*, *Raphiolepis umbellata* Makino (Rosaceae): filaments change from white to red; *f*, *Ribes odoratum* Wendl. (Saxifragaceae): petals change from yellow to red.

of signals, to influence directly the behaviour and movement of the animals on which they depend. Colour signals may be used to indicate to birds which fruits are ripe and ready for dispersal[19–21]; floral nectar volumes can influence the length of time a pollinator will stay on a given plant[22,23]; specific floral odours can attract the right pollinators at appropriate times[24–26]. I have shown that plants in at least 74 different families use colour changes to direct their pollinators to rewarding and sexually viable flowers. Through such signals, plants are able to play a surprisingly active part in their interactions with animals. □

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