Current Biology *19*, 919–923, June 9, 2009 DOI 10.1016/j.cub.2009.03.070

Report

Bidirectional Flower Color and Shape Changes Allow a Second Opportunity for Pollination

Pat Willmer,[1,*] Dara A. Stanley,[2] Karin Steijven,[3] Iain M. Matthews,[1] and Clive V. Nuttman[4]
[1]School of Biology
University of St Andrews
Fife KY16 9TS
Scotland
UK
[2]Department of Botany
Trinity College Dublin
Dublin 2
Ireland
[3]Wageningen University
6700 HB Wageningen
The Netherlands
[4]Tropical Biology Association
Department of Zoology
Cambridge University
Downing Street
Cambridge CB4 3EJ
UK

Summary

Flowers act as "sensory billboards" with multiple signals (color, morphology, odor) attracting and manipulating potential pollinators [1]. Many use changing signals as indicators that visitation and/or pollination have occurred [2, 3]). Floral color change is commonly used to transmit this information [3–7] (often correlated with reduced nectar reward [8, 9]) and can be specifically triggered by pollination or visitation. By retaining color-changed flowers, plants benefit from larger floral displays but also indicate at close range which flowers are still rewarding (and still unpollinated), so that visitors forage more efficiently [5, 6]. However, the legume *Desmodium setigerum* shows a unique ability, if inadequately pollinated, to reverse its flowers' color and shape changes. Single visits by bees mechanically depress the keel and expose stigma and anthers (termed "tripping"); visits also initiate a rapid color change from lilac to white and turquoise and a slower morphological change, the upper petal folding downwards over the reproductive parts. But flowers receiving insufficient pollen can partially reopen, re-exposing the stigma, with a further color change to deeper turquoise and/or lilac. Thus, most flowers achieve pollination from one bee visit, but those with inadequate pollen receipt can reverse their signals, earning a "second chance" by eliciting attention from other potential pollinators.

Results and Discussion

Desmodium setigerum E. Mey. (Fabaceae) is a herbaceous, scrambling perennial bearing typical leguminous flowers (see Figure 1), each 10–12 mm from calyx base to petal tip. These last for just one day, and like many legumes, they require "tripping" when a visitor lands on the keel petal and probes for nectar, so depressing the keel and explosively exposing the reproductive parts. A single visit produces a tripped flower (Figure 1B), which then stays in its "open" state with exposed anthers and stigma. In many other legumes, a tripped flower closes again after the visitor leaves, leaving its "visited" status undetectable; specialized irreversible explosive tripping occurs in only a few genera such as *Genista*, *Indigofera*, *Mucuna*, and *Desmodium*. When tripped, pollen is ejected away from the flower and the central stigma, so the flower does not normally self-pollinate, and untripped and artificially tripped flowers do not set seed (D.A.S. and K.S., unpublished data). *D. setigerum* was studied in late August and September 2008 along forest edges in Kibale National Park, Uganda (0°13′N–0°41′N, 30°19′E–30°32′E), a region of moist semideciduous and evergreen rainforest at an altitude of 1500 m. During the study, heavy rain occurred at various times each afternoon, which affected timings of flower and visitor behaviors.

In this species, a rapid color change from all lilac to white flag/turquoise keel (Figures 1A and 1C) occurred, along with a folding down of the flag petal over the anthers and stigma. These changes were triggered solely by the mechanical effects of visitation, irrespective of amounts or types of pollen deposited (Figure 2). Flowers tripped early in the day generally completed their initial color change and flag-to-keel closure within 2 hr, but occasionally much sooner, the period of change depending in part on ambient temperature and thus being rather longer on cooler days (range 30–180 min). Untripped unvisited flowers changed color only very slowly, with persistent lilac upright flags throughout the daylight hours on their first day that became a uniform dark turquoise by the following morning (flowers that were physically damaged during handling also tended to become dark turquoise). Thus, induced color change in *D. setigerum* is a reaction to visitation, which greatly accelerates the normal age-related floral color change (and because the flowers last for only one day, the slow, purely age-related change occurs near the end of the life span of the flowers, after visitation has ceased, and is normally irrelevant to their reproductive success).

The course of color change after tripping is complex and somewhat variable, but the key stages are shown in the photographs and in Figure 3, with an overall change from lilac (L) to white/turquoise (WT), followed by gradual wilting. But crucially, a small proportion of flowers remained turgid and showed some reversal of color after about 1200 hr (depending on the timing of the daily rains; color reversal was not observed until 1500–1600 hr when rain fell at midday). The keel (and often also the flag) either darkened to a stronger turquoise (Figure 1D) or returned to a partly lilac color (Figure 1C). All flowers with this secondary color change (but also a few that did not change color again) showed an additional morphological change, with the flag lifting away from the stigma. Thus, by 1600 hr, some previously WT closed flowers had reopened (scored as WTO), with the flag-to-keel angle increasing and the opening to the flower widening (Figure 4), so that anthers and stigma were again accessible (Figure 1D).

Note that color change was assessed here by using only the human visual system with direct comparisons in the field

*Correspondence: pgw@st-and.ac.uk

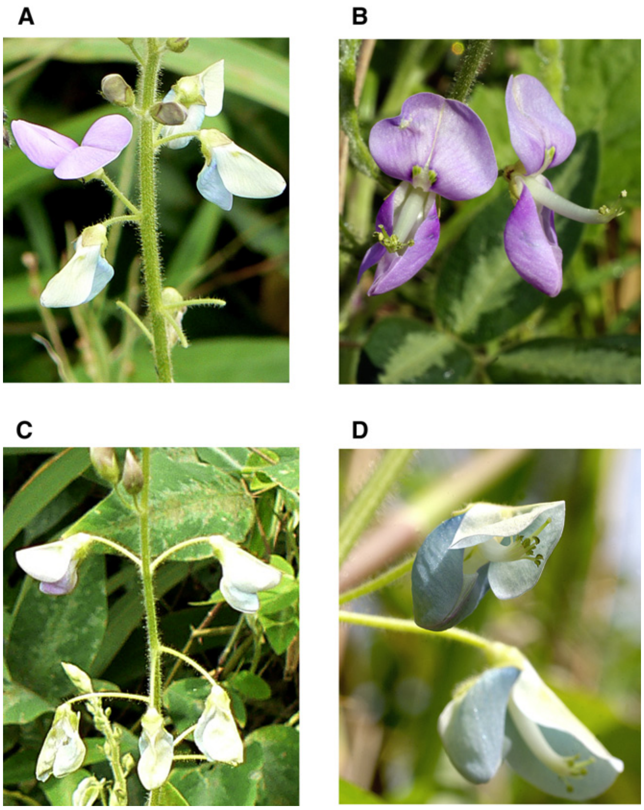

Figure 1. Photographs of *Desmodium setigerum* Flowers Showing the Key Phases Identified

(A) Upper left: open but untripped (L) flower. Lower left: flower with white flag depressed over reproductive organs and turquoise keel (WT). Middle right: flower reopening with some recurrence of lilac color (WTO).
(B) Open and tripped lilac flowers (LV), with plentiful pollen visible on the right stigma and little on the left stigma.
(C) Two reopening WTO flowers, the left flower substantially lilac, plus three WT flowers from the same day, already wilting.
(D) Two WTO flowers in close-up, showing access to re-exposed stigma (with little pollen visible) and anthers, turquoise/lilac keel, and white/turquoise flag.
A brief phase of pale lilac (PLV, not shown) occurred after visitation but before the transition to WT. Photographs were taken at 1205, 0910, 1117, and 1145, respectively.

(e.g., pale lilac as intermediate between lilac untripped flowers and white flags on older visited flowers), but the changes reported may appear rather differently to insect visitors. Indeed, interpreted in a bee-subjective color space, where receptors occur with peak sensitivities for ultraviolet, blue, and green wavelengths [e.g., 10], white and turquoise may appear rather similar, with the white petals likely to be UV absorbing [11], both petal colors thus giving a strong blue-green response. Pre- and postchange flowers are both likely to be easily detectable for bees against a green background [12], but the prechange flowers perceived by humans as lilac are likely to be preferred by bees when set against the green foliage because bees commonly have an innate preference for shades of blue/mauve [e.g., 13].

The proportion of flowers showing secondary change varied on different days. Early in the flowering season, when flowers and visitors were both rare, reversal of floral state was more common (maximum reopening 22% on September 2, 2008, with 12% showing secondary color change), and some of the reversed flowers persisted on the plant in a functional state the following morning (e.g., on September 4, 2008, 11 unwilted WTO flowers remained on 20 observed flower spikes at 0930 hr, these spikes also having 33 new L flowers opened). However, by mid-September, when visitation rates were much higher, the color and shape reversals were absent on some days.

Inspection of pollen amounts on the stigmas of tripped flowers provided an explanation for the reversal of floral changes later in the day (Table 1). Flowers that had received little or no pollen from their first tripping visit changed color and closed down like all other visited flowers (confirming that this was a purely mechanical effect) but were then highly likely to show some reopening. Therefore, the secondary floral changes appear to be induced facultative responses to poor pollination early in the day, in cases where the incoming visitor either had just started a flower-visiting trip and bore no pollen to deposit on the stigma or possibly carried and deposited only heterospecific pollen.

Visitation overall was almost entirely to the untripped intact young flowers (Figure 5A) and thus inevitably to lilac flowers (Figure 5B). Bee visits predominated (at least 15 species caused tripping, ranging from small megachilid to large *Xylocopa* bees, though a few small halictid and megachilid bees visited without eliciting the tripping mechanism). Bees were the only visitors capable of tripping flowers, while inserting their tongues at the base of the keel during visits commonly lasting less than 2 s. Bees were also especially adept at inspecting but avoiding the already visited/tripped flowers and showed good floral constancy, often visiting many *Desmodium* flowers (up to 25 seen in succession). Visits by non-bees (butterflies, hoverflies, bee flies, and other small flies and bugs) increased

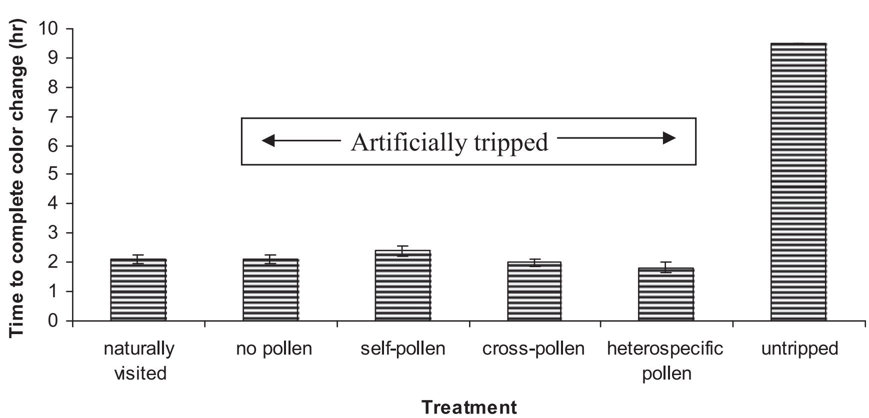

Figure 2. Time to Complete the Main Color Change in *Desmodium setigerum* Flowers after Natural Visitation and Varying Artificial Treatments

Values are means ± SEM; n = 18 for all groups except naturally visited, where n = 53. Untripped flowers were protected with fine muslin to prevent visitation. Time to complete color change varied significantly among pollen treatments (one-way ANOVA: $F_{5,107}$ = 428.83, $p < 0.001$). Only the untripped flowers took significantly longer (Tukey's post hoc test), remaining unchanged 10 hr after opening and finally changing color overnight.

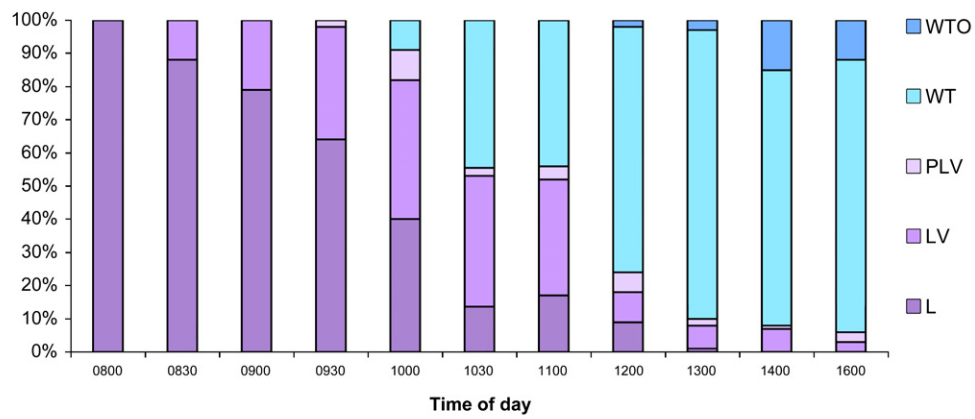

Figure 3. Patterns of Color Change in *Desmodium setigerum* Flowers

Data are averaged from 9 days of observation for a total of 117 flowers. L, lilac, untripped; LV, lilac, visited/tripped; PLV, pale lilac, tripped; WT, white flag over turquoise keel, flower closed; WTO, white/turquoise flag over turquoise/lilac keel, flower partially reopened.

in the afternoon (Figure 5C) but were never common; their visits were usually substantially longer, and these insects rarely visited more than 2–3 flowers in succession. In the mornings, such visitors often probed sideways into the keel of an untripped flower but thereby achieved no reward and triggered no trip response. However these non-bee visitors did visit the WTO flowers in the afternoons and would thus potentially be effecting some further pollen transfer and seed set. Specifically, afternoon visits by a small megachilid bee and by two *Melanostoma* hoverflies were seen to produce some additional pollen deposition on the stigmas of the flowers.

D. setigerum is therefore most unusual in several respects. It exhibits both color and morphology changes, triggered mechanically by visitation. And its extremely rapid color transition contrasts with Van Doorn's suggestion that induced floral color change does not occur in flowers that last only one day [4]. Furthermore, most leguminous tripped flowers do not remain in a conspicuously "already visited" state, but *Desmodium*'s morphological trait of persistently exposed tripped anthers is a potent signal that further visits are not appropriate. From a long distance, insects appear unable to distinguish between tripped and untripped flowers, and the larger display produced by both attracts more visitors, but the flower tripping is recognized at close range so that already-visited flowers are rarely revisited. This long-range attraction together with distinguishing close-up signals is commonly found in plants that change color, increasing pollinator attraction without losing pollinator efficiency and reducing geitonogamy [3, 4]. Finally, and above all, *Desmodium* can undergo change reversals in both morphology and color.

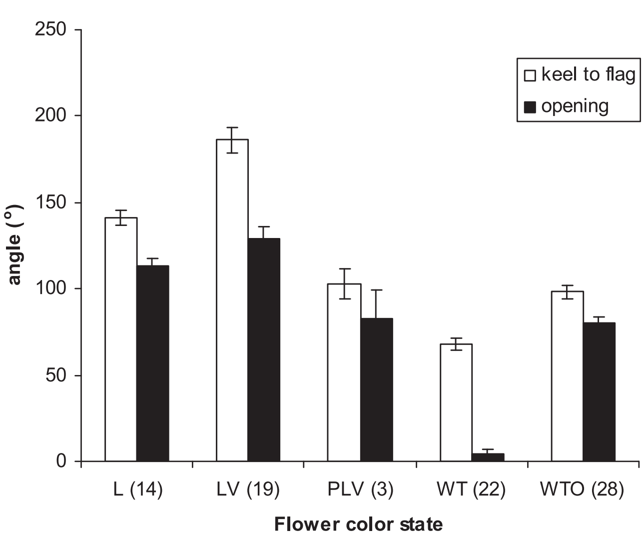

Figure 4. Morphological Changes in Flower Opening

Angle between back of keel and back of flag (white bars) and angle of corolla opening (between upper side of keel and underside of flag) (black bars), in degrees, for the five color phases shown in Figure 3. WT floral organs are therefore inaccessible to visitors, but the keel lifts in the WTO phase to restore access. Values are means ± SEM; sample sizes are given in parentheses with x axis labels.

For a flower to close and reopen or otherwise change shape is not unknown. Some flowers show diurnal changes in shape and accessibility, for example opening in daylight and closing at night or during rain (e.g., *Crocus*, *Hepatica*, and *Nemophila*) or more specifically opening in response to sun and closing fully or partly during cloudy periods (e.g., *Adonis*, some *Gentiana*, and some *Mesembryanthemum*). Indeed, gentians show two kinds of flower closure, one temporarily in response to environmental conditions (e.g., cooling temperatures or approaching thunderstorms [14]) and a second more permanently in direct response to being pollinated [15]; in the latter case, the closed flowers may be retained on the plant to add

Table 1. Effects of Pollen Receipt on Flower Reopening and Secondary Color Change

	Number of Pollen Grains on Stigma		
Flower Features	~25–50	~10–20	~0–8
WT: flag fully down, covering reproductive organs; keel turquoise	38	9	3
WTO: flag partly raised, stigma accessible; keel dark turquoise and/or lilac	1	2	19

Morphological and color status of *Desmodium* flowers examined between 1300 and 1700 hr, compared with their estimated pollen status in grains per stigma in the morning (1000–1230 hr) after visitation and tripping; both from direct observations (36 flowers, on August 30, September 2, and September 3) and based on photographs of 36 flowers tracked from other days. Presence of negligible pollen is highly correlated with partial reopening and secondary color change (G test against equal frequencies WT/WTO, G = 62.55, degrees of freedom = 5, $p < 0.0001$).

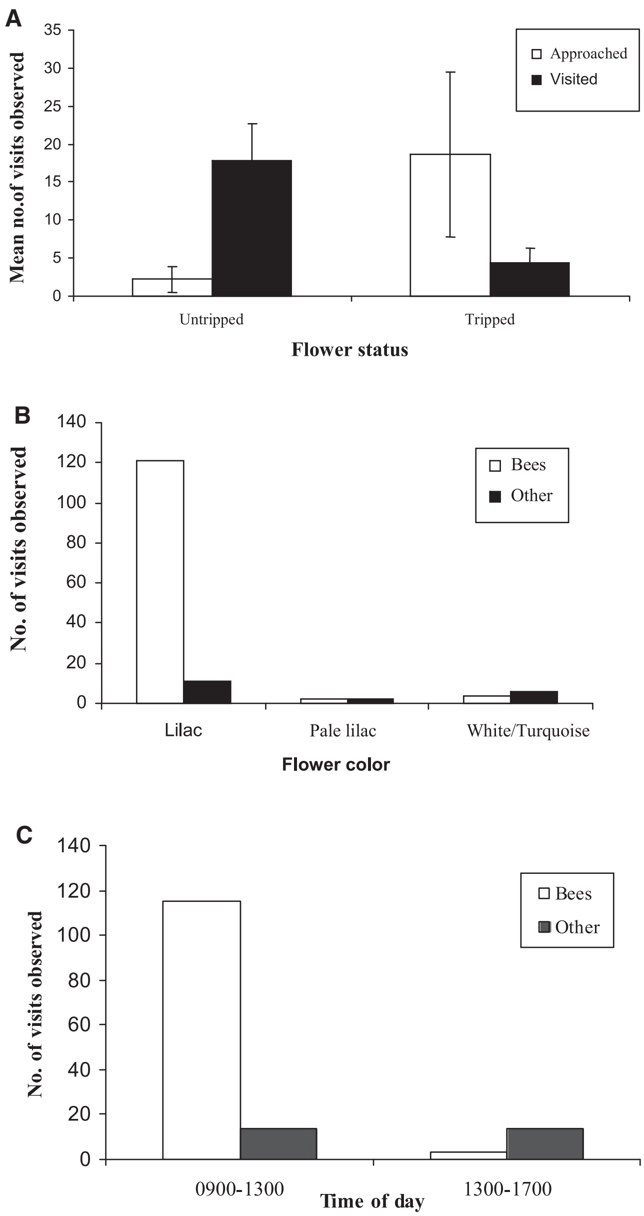

Figure 5. Visitation to Different Flower Phases

(A) Untripped flowers (n = 100) received significantly more visits than tripped flowers (n = 115) (χ^2 = 104.56, degrees of freedom = 1, $p < 0.001$), although the latter were approached more often. The visitors distinguished flower status close up, approaching to within 10 mm of a tripped flower but not subsequently landing. Data are presented as means ± SEM.

(B) Visits by bees were greatly skewed to flowers in the lilac color phase; other insects did not discriminate between colors.

(C) Bee visits predominated in the morning, but other visitors were more common in the afternoon (when they were more likely to encounter white/turquoise flowers).

to the display effect. Fireweed flowers (*Chamerion angustifolium*) exhibit a similar pollination-induced closure mechanism within 4 hr of pollen receipt [16], the time to close being reduced when pollen loads are larger and perhaps increased when self-pollen is deposited, again indicative of an appropriately adaptive response. In orchids, morphological changes are particularly common, some species showing petals folding over to cover the column, others undergoing a swelling of the column, and still others rotating the whole flower by 180° so that normal pollinators can no longer access the tube (e.g., *Angraecum* orchids normally visited by moths [17]). A more spectacular effect occurs in *Quisqualis indica*, a climber whose long tubular flowers are white and horizontal on their first night of opening and are visited by hawkmoths but change over the next 2 days through pink and red and become pendulous with somewhat larger corolla diameters, attracting daytime visits from bees, flies, and sunbirds [18]. This phenomenon of different color phases attracting different visitors is reasonably common—for example, different species of butterfly show different preferences for *Lantana* color phases [19]; honeybees and bumblebees only visit lupins with prechange banner petals [6]; and *Anthophora* bees choose yellow (but not white) *Alkanna* [7, 20].

However, for a flower to change color and then facultatively partly reverse that change is, we believe, unique. Mechanisms for this can readily be envisaged. Color change can be specifically triggered by pollination (as pollen tubes grow into the style and elicit a wound response, often mediated by ethylene [21]) or by visitation (mechanical stimuli from the animal alighting on or probing the flower, again producing wounding). In *Viola cornuta*, a color change from white to purple occurs after pollination, and three genes responsible for anthocyanin production increase their expression at this time, probably activated via signals from ethylene and gibberellic acid [22]. A reverse gene switch triggered by inadequate pollen tube growth and reduced hormone levels could be initiated similarly.

Whereas the initial shape and color changes in *Desmodium* are automatic signals to pollinators that visitation has occurred, the subsequent reverse changes occur only when a given flower has detected that pollination has been inadequate, thus signaling that further visits are invited to improve pollination effectiveness. The plant thereby achieves two separate opportunities for pollination, where a morning bee visit is normally fully effective but where the reversal of flower color and shape provides a "safety net" later in the day. This may be an ideal strategy for a plant that so classically demonstrates the ability of a single pollinator visit to achieve both male and female function for its flowers, because it can explicitly signal when it has had the necessary visit. When that one visit has proved inadequate, with too few pollen grains germinated, the flower reopens and increases its visual attraction. Thus, it has a "second shot" at pollination later in the day, utilizing the more generalist insect visitors then available.

Acknowledgments

We are extremely grateful to the Tropical Biology Association for funding and the opportunity to work at Kibale Field Station (Makerere University, Uganda), and to all of the staff there for excellent facilities. K.S. is funded by the Alberta Mennega Stichting and Wageningen Universiteits Fonds. The photos in Figures 1B and 1D were kindly taken by Karen Sarkisyan, and Luke Rendell gave statistical advice. We thank three anonymous referees for particularly helpful comments.

Received: December 18, 2008
Revised: March 30, 2009
Accepted: March 31, 2009
Published online: April 30, 2009

References

1. Schaefer, H.M., Schaefer, V., and Levey, D.J. (2004). How plant-animal interactions signal new insights in communication. Trends Ecol. Evol. *19*, 577–584.

2. Weiss, M.R. (1995). Floral color change: A widespread functional convergence. Am. J. Bot. *82*, 167–185.
3. Raguso, R.A. (2004). Flowers as sensory billboards: Progress towards an integrated understanding of floral advertisement. Curr. Opin. Plant Biol. *7*, 434–440.
4. Van Doorn, W.G. (1997). Effects of pollination on floral attraction and longevity. J. Exp. Bot. *48*, 1615–1622.
5. Weiss, M.R., and Lamont, B.B. (1997). Floral color change and insect pollination: A dynamic relationship. Isr. J. Plant Sci. *45*, 185–200.
6. Nuttman, C., and Willmer, P.G. (2003). How does insect visitation trigger floral colour change? Ecol. Entomol. *28*, 467–474.
7. Nuttman, C.V., and Willmer, P.G. (2008). Hoverfly visitation in relation to floral colour change. Entomol. Gen. *31*, 33–47.
8. Ollerton, J., Grace, J.N., and Smith, K. (2007). Adaptive floral colour change in *Erysimum scoparium* (Brassicaceae) and pollinator behaviour of *Anthophora alluadii* on Tenerife (Hymenoptera: Apidae). Entomol. Gen. *29*, 253–268.
9. Lunau, K. (1996). Unidirectionality of floral colour changes. Plant Syst. Evol. *200*, 125–140.
10. Briscoe, A.D., and Chittka, L. (2001). The evolution of color vision in insects. Annu. Rev. Entomol. *46*, 471–510.
11. Daumer, K. (1958). Blumenfarben: Wie sie der Bienen sehen. Z. Vergl. Physiol. *41*, 49–110.
12. Dyer, A.G., Whitney, H.M., Arnold, S.E.J., Glover, B.J., and Chittka, L. (2007). Mutations perturbing petal cell shape and anthocyanin synthesis influence bumblebee perception of *Antirrhinum majus* flower colour. Arthropod-Plant Interactions *1*, 45–55.
13. Giurfa, M., Nuñez, J., Chittka, L., and Menzel, R. (1995). Color preferences of flower-naïve honeybees. J. Comp. Physiol. A *177*, 247–259.
14. Bynum, M.R., and Smith, W.K. (2001). Floral movements in response to thunderstorms improve reproductive effort in the alpine species *Gentiana algida* (Gentianaceae). Am. J. Bot. *88*, 1088–1095.
15. He, Y.P., Duan, Y.W., Liu, J.Q., and Smith, W.K. (2005). Floral closure in response to temperature and pollination in *Gentiana straminea* Maxim (Gentianaceae), an alpine perennial in the Qinghai Tibetan plateau. Plant Syst. Evol. *256*, 17–33.
16. Clark, M.J., and Husband, B.C. (2007). Plasticity and timing of flower closure in response to pollination in *Chamerion angustifolium* (Onagraceae). Int. J. Plant Sci. *168*, 619–625.
17. Strauss, M., and Koopowitz, H. (1973). Floral physiology of *Angraecum*. I. Inheritance of post-pollinator phenomena. Am. Orchid Soc. Bull. *42*, 495.
18. Eisikowitch, D., and Rotem, R. (1987). Flower orientation and color change in *Quisqualis indica* and their possible role in pollinator partitioning. Bot. Gaz. *148*, 175–179.
19. Ram, H.Y.M., and Mathur, G. (1984). Flower colour changes in *Lantana camara*. J. Exp. Bot. *35*, 1656–1662.
20. Willmer, P.G., Gilbert, F.S., Ghazoul, J., Zalat, S., and Semida, F. (1994). A novel form of territoriality: Daily paternal investment in an anthophorid bee. Anim. Behav. *48*, 535–549.
21. Van Doorn, W.G. (2002). Does ethylene treatment mimic the effects of pollination on floral lifespan and attractiveness? Ann. Bot. (Lond.) *89*, 375–383.
22. Farzad, M., Griesbach, R., Hammond, J., Weiss, M.R., and Elmendorf, H.G. (2003). Differential expression of three key anthocyanin biosynthetic genes in a colour-changing flower, *Viola cornuta* cv. Yesterday, Today and Tomorrow. Plant Sci. *165*, 1333–1342.