Floral Color Change in Response to pollination in the Genus *Encyclia* Hook

Ruben P. Sauleda
22585 SW 187 Ave., Miami, Fl. 33170. USA.
rpsauleda@gmail.com

Abstract: The phenomenon of floral color change in response to pollination is reviewed and floral color changes are reported in six species of *Encyclia* Hook.

Keywords: Colombia, Orchidaceae, functional convergence, pollinator preference *Encyclia*.

Introduction

Changes in flower color after anthesis are common in the angiosperms. Approximately 78 taxonomically, morphologically and geographically diverse families of both monocot and dicot angiosperms exhibit a floral color change (Weiss, 1995). This color change is distinct from the color change associated with floral senescence. Many of the floral color changes are pollination induced and all function to provide important information for pollinators. These changes are an adaptive trait benefiting both the plant and the insect pollinator, by indicating to the insect when the flower is at the optimal reproductive stage and offers the greatest reward (Weiss, 1991). Visitors to color changing flowers are from 15 insect families and four bird families (Weiss, 1995). These visitors can discriminate between flowers in different color phases, a preference for a given color phase is always exhibited.

The prechange flower at anthesis is always preferred because it offers more reward to the pollinator than the post-change flower. However, the retention and maintenance of older flowers increases the size of the floral display providing a long distance attractant for pollinators. Investigations of a number color changing species shows that plants that retain flowers receive more pollinator visits (Weiss, 1991). The location and scale of the color change in flowers is correlated with the type of pollinator that visits the flower (Weiss, 1995). Color changes in flowers pollinated by moths, birds or bats usually affect the whole flower, while flowers pollinated by bees, butterflies or flies usually affect only a localized part of the flower. Localized color changes do not affect floral parts that are not part of the reproductive or reward structures, such as the sepals and petals in *Encyclia* Hook. (Orchidaceae). Bee pollinated flowers usually have a color pattern that functions as a nectar or pollen guide and it is these parts that change color such as the labellum in *Encyclia*. With bee pollinated flowers, retention of older flowers is likely to contribute to the showy visual display that will attract pollinators from a distance although these old flowers do not contribute to the olfatory attraction. In many cases a change in floral odor correlates with the color change. Retention of the older flowers results in increased visitation by pollinators from a distance, while at close range, color change directs visitors towards the rewarding and sexually viable flowers (Weiss and Lamont, 1997).

There are many different mechanisms used by color changing flowers to attract visitors. Ojeda *et. al*. (2013) found that four putatively bird-pollinated Macaronesian *Lotus* (Leguminosae) evolved within a group of entomophilous species, which have the capacity to produce anthocyanin cyanidin, and delphinidin derivatives. The shift in flower color between the two pollination syndromes only involved a redirection of pigment production toward anthocyanin, rather the activation or inactivation of new branches within the anthocyanidin pathway. Pigment changes by a redirection of pigment production after flower anthesis evolved independently at least three times in bee pollinated Macaronesian *Lotus*, to deter bees from visiting already pollinated flowers, and so increase pollinator foraging efficiency.

Ram and Mathur (1984) report that *Lantana camara* L. (Verbenaceae) flowers undergo a color change subsequent to anthesis. In the color variant selected for their study, pink buds, yellow newly opened flowers and ageing orange, scarlet and magenta flowers are found in the same inflorescence. Pollination was identified as the trigger for the color change by rapid anthocyanin synthesis. Even the presence of one pollen grain on the stigma of a yellow flower was sufficient to cause color change. The post-pollination shift in petal coloration is caused by the masking of carotenoids by differential amounts of anthocyanin. Thrips, the pollinators, are attracted to only yellow flowers; chromatic changes play a role in conserving pollinator energy.

Farzad *et. al.* (2002), found that *Viola cornuta* L. (Violaceae) cv. Yesterday, Today and Tomorrow (YTT) undergoes a striking color change from white to purple. The appearance of color is easily triggered, and only one anthocyanin is involved. Comparison of emasculated and intact flowers shows that the presence of pollen on the stigma is necessary to produce the color change.

A study by Lippi *et. al.* (2011) on the reproductive biology of *Boswellia sacra* Flueck (Burseraceae), demonstrated that the nectary disc changes its color from yellow to orange and red during flower development. The colors are related to the main period of the stigmatic receptivity, to the dehiscence of anthers with pollen presentation and the nectar secretion. Pollinators preferentially visit the flowers in the "yellow" phase and neglect the "red phase". This suggests a sophisticated dialogue between the plant and its pollinators. The color change from yellow to red occurs in a very short time (less than 24 h) and it is due to the accumulation of anthocyanins. These color changes are not dependent on pollination but are signals to the pollinators.

Pereira *et. al*. (2011) observed that floral color changes are common among Melastomataceae and have been interpreted as a warning mechanism for bees to avoid old flowers. The flowers lasted three days or more, and the floral color changed from white in the 1st day to pink in the following days. Pollen deposition on stigma induced the floral color change. During experiments on floral fragrance and color, the bees visited both natural and mimetic 1st-day flowers and 2nd-day flowers with 1st-day flower scents. The bees avoided natural 2nd-day flowers, and seldom visited modified 2nd-day flowers. The attractiveness of the flowers cannot be attributed exclusively to the color or the fragrance separately; both factors seemingly act together in this case.

For a flower to change color and then partly reverse that change is unique. Farzad, *et. al.* (2003) reported that in *Viola cornuta* L. (Violaceae), a color change from white to purple occurs after pollination. Three genes responsible for anthocyanin production increase their expression at that time, probably activated via signals from ethylene and gibberellic acid. Willmer (2009) observed that a reverse gene switch is triggered by inadequate pollen tube growth and reduced hormone levels. In *Desmodium setigerum* (E. Mey) Harv. (Fabaceae) a rapid color change from all lilac to white flag/turquoise keel occurred after pollination, along with a folding down of the flag petal over the anthers and stigma. Whereas these initial shape and color changes in *Desmodium* are automatic signals to pollinators that visitation has occurred, subsequent reverse changes occur when a given flower has detected that pollination has been inadequate, thus signaling that further visits are needed to improve pollination effectiveness (Willmer *et. al*., 2009). The plant thereby achieves two separate opportunities for pollination, if a morning bee visit is not fully effective; the reversal of flower color and shape provides a "safety net" later in the day. This is an ideal strategy for a plant, because it can explicitly signal when it has had the necessary visit. When 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.

Yan *et. al*. (2016) found that *Quisqualis indica* L. flowers change color from white to pink to red for a shift from moth to butterfly pollination. Flowers secreted nectar continuously from the evening of anthesis until the following morning, then decreased gradually with floral color change. The scent compounds in the three floral color stages were similar; however, the scent composition was different, and the scent emission rate decreased from the white to red stage. Different pollinators were attracted in each floral color stage; mainly moths at night and bees and butterflies during the day. Observations of open-pollinated inflorescences showed that white flowers had a higher fruit set than pink or red flowers, indicating the high contribution of moths to reproductive success. Yen *et. al*. (2016) concluded that the nectar and scent secretion are related to floral color change in *Q. indica*, in order to attract different pollinators and promote reproductive fitness. Kunze and Gumbert (2001) found that scent cues change flower choice by *Bombus terrestris* L. and the presence of scent enhances color discrimination by improving attention towards visual cues.

In *Cattleya* Lindl. species sepals and petals fold over to cover the column shortly after pollination. This early closure immediately following pollination was observed by Gartner (1844), Fitting (1909) and Faegri and van der Pijl (1979). Fitting (1910) also found that in *Lycaste skinneri* Lindl. and *Anguola uniflora* Ruiz & Pav. pollination had no visual effect on petal color or on flower closure but doubled the time to wilting. Faegri and van der Pijl (1979) also reported that some species wilt within a day or two after pollination but some species can stay turgid for several months. Species of *Anacheilium* (*Anancheilium chimborazoense* [Schltr] Withner & Harding) undergo a swelling of the column to prevent additional pollinia from being deposited. Additionally, a floral color change occurs in segments of the flower to signal that pollination has been realized.

Cleisostoma koordersii Rolfe and *Cleisostoma latifolium* Lindl. have a green perianth, which becomes darker green following pollination and has a considerable delay in petal wilting (Fitting, 1910). Pollinators are able to distinguish these shades of green. In *Listera ovata* (L.) R. Br. (Hildebrand, 1863) and *Dendrobium antennatum* Lindl. (Fitting, 1910), pollination also results in greener petals and is accompanied by a slight closing movement.

Many *Phalaenopsis* Blume species (stauroglottis), the sepals and petals turn green and photosynthetic after pollination. They become leaf-like and photosynthesize producing extra energy for the developing ovule and the embryos subsequent to fertilization for many months until the capsule matures. In *Phalaenopsis violacea* H. Witte the petals change from white to yellow within 2-3 days after pollination. Subsequently, the petals close, wilt and become glassy. Two to three days later they recover turgor, start to become green and, unlike unpollinated flowers, subsequently stay green and fresh for several months (Fitting, 1909, 1910). Similar petal yellowing and flower closure, followed by greening and persistence of the petals, is found in *Phalaenopsis cornu-cervi* (Breda) Blume & Rchb. f. (Fitting, 1910), but in this species the petals do not wilt prior to becoming green. It has been found that many plants in poor physical condition due to environmental stress will show this phenomenon of not wilting and the floral segments photosynthesizing to provide extra energy for the process of seed production. This is an adaptive mechanism to insure survival of the species.

The physiological and molecular mechanisms of pollination-induced senescence have been studied in several orchid species, such as *Phalaenopsis* and *Dendrobium*, in terms of ethylene sensitivity. The enhanced sensitivity to ethylene following pollination is the initial event triggering an increase in ethylene production and the consequent physiological changes in the flower (Porat *et al.*, 1995). In *Phalaenopsis* spp., there is an abundant production of ethylene in the perianth up to 72 hours after pollination and has been shown to have a critical role in ovary maturation and ovule differentiation (Yu and Goh, 2001). Doorn (1997) also found that endogenous ethylene has a role in changing perianth form and color to signal the occurrence of pollination. The number of flowers that show high ethylene-sensitivity with regard to flower closure and color, abscission, or withering of the petals seems much larger than the number of species recorded to show changes in pollinator attraction shortly after pollination. Ethylene production has also been shown to be a signal of the presence of pathogens in the petals, enabling the host to inhibit further pathogen entry as the petals will be shed or wither as a response to increased ethylene production (Doorn, 1997).

Angraecum Bory normally visited by moths (Strauss and Koopowitz, 1973) rotates the whole flower by 180 degrees after pollination so that normal pollinators can no longer access the tube. Gori (1983) also reported that some orchid species change the flower orientation after pollination.

Fitting (1910) reports that in a few orchids, pollination produces flower closure, but increases floral longevity. In pollinated *Zygopetalum mackaii* Hook. and *Zygopetalum crinitum* Lodd., for example, the perianth shows a slight closing movement, which may be noticeable to the pollinators. When the flowers remain unpollinated, the petals wilt in about a month, but remain for three months if pollinated. In these flowers no visible color change was observed. The slight wilting signals the pollinator to avoid the flower.

Schiestl *et. al.* (1997) found that pollinated flowers of *Ophrys sphegodes subsp. sphegodes* Mill. (Orchidaceae) produced significantly different odor bouquets, and the total amount of scent emitted two to four days after pollination was significantly lower compared with unpollinated flowers. In addition, behavioral tests with *Andrena nigroaenea* (Kirby) (Apoidea, Andrenidae) males showed that flowers faded and were significantly less attractive three days after pollination. This reduced attractiveness is hypothesized to guide pollinators to the unpollinated flowers within an inflorescence, and thus increase the reproductive success of the plant. Schiestl *et. al*. (1997) clearly confirmed the theoretical assumption that flowers change their odor bouquets and their color after pollination and are thus less attractive to potential pollinators.

Artificial removal of pollinia in *Cymbidium floribundum* Lindl. (Orchidaceae) induced labial color change. Labia in color-changed flowers showed a decreased reflectance of wavelengths less than 670 nm compared to control intact flower. Both reflectance irradiance spectra and ultraviolet photographs showed that only the nectar guide in white (unchanged) flowers reflected ultraviolet light, and that this reflectance decreased with labial color change. Dual choice experiments showed that the honeybee (*Apis cerana japonica* Fabricus) foragers preferentially visited flowers having white labia rather than reddish brown. Japanese honeybees discriminate between the floral color phases using color vision.

Suetsugu and Tanaka (2013) report that pollinated flowers of *Sedirea japonica* (Rchb. f.) Garay and Sweet (Orchidaceae) changed color from white to bright yellow to enhance the pollination success of the plants.

Methodology - Discussion

Floral color changes in the visible light range in *Encyclia* (Orchidaceae) are common in most species, but have not been adequately investigated. Two different plants of *Encyclia replicata* (Lindl. & Paxt.) Schltr., a common Colombian species were artificially pollinated and the resulting visual floral color changes recorded. The floral color change only affected the labellum of the flower. A comparison was made of flowers at anthesis and flowers artificially pollinated. The first day after pollination a color change was not visually discernable. Two days after pollination a slight color change was visible. The change involved a faint yellowing gradually changing to reddish-purple during a five-day period. Five days after pollination the labella reached the maximum color change. These changes in floral color are a definite signal to the pollinator that the flower had been pollinated. The floral fragrance turned off during the first day after pollination.

Five additional species of *Encyclia* were also studied; all exhibited a floral color change after being artificially pollinated. The Colombian species *Encyclia microtos* (Rchb. f.) Hoehne, *Encyclia cordigera* (HBK) Dressler and *Encyclia stellata* (Lindl.) Schltr., the Cuban species, *Encyclia phoenicea* (Lindl.) Nueman and a Mexican species, *Encyclia nematocaulon* (A. Rich.) Acuña demonstrated a slow floral color change starting on the second day and reaching maximum color change after 5 days. Floral fragrance turned off during the first day after pollination in all species.

Conclusions

In all species of *Encyclia* studied there was retention of older flowers. The older flowers increase a plant's attractiveness to pollinators from a distance, but at close range pollinators could discriminate between floral color phases. Observation of inflorescences confirmed the pollinator preferences. The phenomenon of floral color change was first reported over 200 years ago (Sprengel, 1792) and has been documented for a variety of species but has gone mostly unrecognized in the Orchidaceae. The occurrence of floral color change in so many different families including the Orchidaceae is an excellent example of functional convergence.

Changes in floral attraction, in relatively long-lived flowers like orchids, can be interpreted as an adaptation to low effective pollinator activity, partly due to high pollinator specificity, resulting in saving pollinator energy. Many orchid flowers have a long period of floral attraction, modulated by pollination. The morphological changes resulting from pollination are varied but all result in signals to pollinators to avoid flowers that are already pollinated.

In light of all the research that has been done on floral color changes very little is actually known about all the flower signals meaningful to insects that are invisible to the human eye or not obvious to the human nose. It is not known how many flowers react to pollination by changes in ultraviolet reflection or by changes in scent production that convey a special message to pollinators. ■

BIBLIOGRAPHIC REFERENCES AND PHOTO CREDIT

See spanish version.