

# POLLINATION SYNDROMES AND THE EVOLUTION OF FLORAL DIVERSITY IN *IOCHROMA* (SOLANACEAE)

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Received October 2, 2008

Accepted January 5, 2009

Smith et al. (2008a; hereafter SAB) in their paper, “The role of pollinator shifts in the floral diversification of *Iochroma* (Solanaceae),” combine pollinator observations with a well-supported phylogeny to quantify the relationship between floral trait evolution and pollination system transitions for 15 predominantly Andean species within the Iochrominae, 14 in the genus *Iochroma*, and one from the monotypic genus *Acnistus*. They classified floral visitors into four categories: (1) hummingbirds, (2) bees and wasps, (3) butterflies and moths, and (4) flies. They found that floral nectar volume and size of the floral display was significantly correlated with transitions to hummingbird pollination, whereas corolla tube length and flower color did not significantly correlate with transitions to any of the four major pollinator groups. Because only two of four traits could be directly associated with transitions to specific pollination systems, they conclude that the patterns do not fit those predicted by classical pollination syndromes.

SAB’s efforts to examine the association between pollinator shifts and floral evolution could provide insights into the importance of pollinator-mediated divergence. Correlating trait evolu-

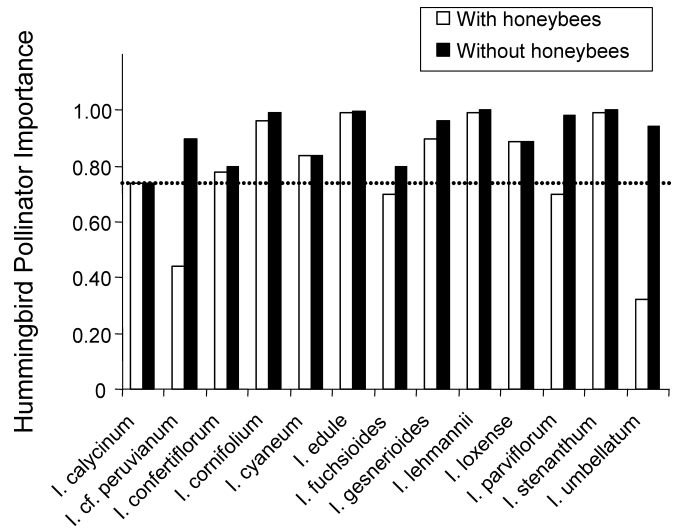
tion with shifts in pollinators is a powerful test of adaptive hypotheses that rely on the pollination syndrome concept. Although a number of studies have used phylogenetic methods to reconstruct the evolution of floral traits and relate these to pollinator transitions (reviewed in Fenster et al. 2004), SAB is exceptional in the use of statistical methods to evaluate floral trait–pollinator relationships (see also Armbruster 1996, 2002). However, we disagree with several of SAB’s approaches and conceptual perspectives, in particular their conclusion that the striking floral diversity of the Iochrominae is inconsistent with predictions of the pollination syndrome concept. Furthermore, we suggest that SAB have insufficient data to conduct a phylogenetically corrected analysis and we question their focus on single floral traits versus viewing the flower as a multivariate adaptation.

Our first and foremost criticism of SAB focuses on their conclusion that floral evolution in Iochrominae is not consistent with predictions of the pollination syndrome concept. Since Darwin considered flowers as adaptations (1859, 1862), pollinators have been viewed as a major selective agent underlying floral evolution (reviewed in Fenster et al. 2004). The pollination syndrome

concept, the convergent evolution of many floral traits in response to the selective pressures exerted by similar pollinators, was developed to predict the pollinators that are the selective agents responsible for the origin of contemporary patterns of floral diversity (Faegri and van der Pijl 1979), in addition to understanding the functional significance of floral trait combinations (Stebbins 1951). In addition to pollinators, pollination syndromes may reflect selection exerted to deter antagonistic visitors, for example, red flower color typically associated with bird pollination may be both attractive to birds and less attractive to bees (Chittka and Waser 1997; Schemske and Bradshaw 1999; Rodríguez-Gironés and Santamaría 2004). According to the classical pollination literature, the pollination syndrome exhibited by a plant should correspond to the important pollinators visiting that plant (Stebbins 1950). Pollinator importance is the variable used by SAB to assess the potential strength of selection exerted by a pollinator.

Pollinator importance is an estimate of the proportion of ovules fertilized in a population by a specific pollinator, and is a product of floral visitation frequency and the amount of pollen deposited on the stigma per visit (Stebbins 1950). Thus, a pollinator importance of 1.0 signifies that a single pollinator taxon (or functional group of pollinators defined by their similarity in behavior on the flower, see Fenster et al. 2004) is responsible for fertilization. For purposes of discussion, we consider a conservative criterion that plants are specialized to their pollinators if pollination by one group accounts for 75% of all pollination (Fenster et al. 2004; also used by Smith et al. 2008b). In other words, the most important pollinator accounts for at least a three-fold greater proportion of ovules fertilized relative to the next important pollinator. Using the 75% threshold for specialization, we conclude from SAB's data that 8 of 15 species were hummingbird specialists, two were exclusively insect pollinated and five had generalized hummingbird and insect pollination. Insect pollination mostly consisted of hymenopteran pollinators.

Given the substantial number of species that displayed a mixed pollination system, SAB concluded that the pollination syndrome concept could not explain floral evolution in this group, because the plants were not specialized to particular pollinators, hence the flowers cannot be regarded "as a set of well-atomized traits that have each been optimized by selection for current pollinators." However, many of the hymenopteran pollinators observed by SAB were honey bees. Honey bees accounted for an average of 0.58 of the hymenopteran flower visits to each of the *Iochrominae* species ( $n = 15$  species, range = 1.0, one SE = 0.11, data kindly provided by S. D. W. Smith from Smith et al. 2008b). Because honey bees were introduced into the New World no earlier than European colonization (early 1500s), it is unlikely that they have effected substantial evolutionary change of floral traits in the relatively long-lived, perennial *Iochrominae*. Although honey bees might contribute to pollination in contemporary *Iochrominae*



**Figure 1.** The importance of hummingbirds as pollinators of *Iochroma*, when including honey bees (open bars) or excluding honey bees (filled bars) as pollinators, respectively. Data are sorted by each *Iochroma* species. Only the 13 species that had any hummingbird pollination are included. The dotted line demarcates specialization (see text for explanation) for hummingbird pollination (above) and generalization (below). *Iochroma calycinum* has a hummingbird pollinator importance of 0.74, and lies just below the dotted line.

populations, they should not be considered as members of the historical pollinator assemblage (or assemblage of selective agents) that gave rise to the floral display of current *Iochrominae* species. Thus, we suggest that nonnative honey bees should be excluded from empirical tests of the pollination syndrome concept.

To this end, using data from Smith et al. (2008b), we excluded honey bees from their dataset of floral visitors and calculated overall pollinator importance for each *Iochrominae* species, following the methods used by SAB. With honey bees excluded, we find that the pollinator importance of hummingbirds increases for most of the 13 species of *Iochroma* previously documented to have hummingbird pollination (Fig. 1). There is a significant increase from 0.788 to 0.910 of the mean importance of hummingbird pollination in these species (Wilcoxon Sign Rank Test statistic = 27.5,  $P < 0.002$ ), and the range of hummingbird pollination importance values is greatly reduced (hummingbird importance = 0.32–0.99 for honey bees included and 0.74–1.00 with honey bees excluded). Of the five hummingbird-visited species that one might consider as having generalized pollination, that is, pollinator importance values  $< 0.75$ , four become classified as hummingbird specialists once honey bees are removed (Fig. 1). Removing honey bees, two species remain exclusively insect pollinated. When the pollinator importance of all 15 species of *Iochrominae* is examined with and without honey bees, the importance of the primary pollinator increases for 12 of the species

(mean pollinator importance of the primary pollinator = 0.75 vs. 0.86, with and without honey bees, respectively) and this difference is also significant (Wilcoxon Sign Rank Test statistic = 33,  $P < 0.001$ ).

After the exclusion of honey bees, and using the  $\geq 0.75$  criterion for evidence of specialization, 12 of the 15 Iochrominae species are specialized for hummingbird pollination, and another species (*I. calycinum*) is classified as generalized hummingbird and insect pollinated, yet this species falls just short of the 0.75 threshold (hummingbird importance = 0.74; Fig. 1). Thus, most Iochrominae are either primarily hummingbird pollinated, or exhibit generalized insect pollination. In light of our reanalysis of SAB, we find that there is little statistical power to evaluate the pollination syndrome concept in Iochrominae using comparative approaches. In contrast, in those systems with a more even mix of insect and hummingbird-pollinated species, and where phylogenetic approaches have revealed the repeated evolution of hummingbird pollination, there is strong evidence of floral evolution conforming to the pollination syndrome concept (Wilson et al. 2004, 2006; Kay et al. 2005).

SAB consider “the pollination system as a continuous trait, where each species receives varying contributions to pollination by different groups of animals,” (p. 794). Although this approach avoids qualitative and subjective assignments of pollination systems, it raises concerns regarding the variance associated with quantifying floral traits, the components of pollinator importance, visitation rates, effectiveness, and finally importance itself. Because the focus of SAB was to associate pollinator importance with floral trait evolution, it is necessary that sample sizes and statistical power are sufficient to adequately discriminate between different levels of pollinator importance. SAB do not statistically compare pollinator importance values for the different Iochrominae, and it is thus uncertain how many levels of importance can be distinguished by statistical criteria. The problem of attaching standard errors or confidence intervals to pollinator importance values is not trivial, because importance is the product of two distributions, frequency, and effectiveness. This has been an obstacle to comparative estimates of pollinator importance (e.g., Sahli and Conner 2007), but there are now approaches that can be adopted (Reynolds and Fenster 2008; published since SAB). We believe that these statistical issues would tend to lessen the distinction in the degree of hummingbird and insect pollination for those species that are predominately pollinated by hummingbirds, reducing their comparative analysis to 13 species with hummingbird pollination and two with insect pollination. Depending upon which pollination system is ancestral to their phylogeny, this is equivalent to perhaps two independent transitions to hummingbird pollination or two independent transitions to insect pollination.

We next consider SAB’s focus on the independent evolution of single floral traits, as opposed to the alternative of treat-

ing the Iochrominae flower as a complex, multivariate structure where traits jointly evolve. The pollination syndrome concept has traditionally been viewed from a multivariate perspective. In other words, an underlying assumption of pollination syndrome evolution is that selection is correlational (Lande and Arnold 1983; Blows 2007), favoring particular combinations of floral traits (Stebbins 1951; Fenster et al. 2004; Wilson et al. 2004; Martén-Rodríguez et al. 2009), where “each part of each [flower] functions in its own way, but functions of various parts within a [flower] are correlated” (Faegri and van der Pijl, 1979, p. 23). We agree with SAB that “a full understanding of floral diversity will require that we move away from viewing a flower as set of well-atomized traits that have each been optimized by selection for current pollinators. . .,” yet SAB do not follow their own admonishments. As a test of the pollination syndrome concept, SAB statistically compare individual floral traits of Iochrominae that differ in their pollination systems. They interpret the lack of a significant difference in corolla tube length between insect- and hummingbird-pollinated species as evidence against the pollination syndrome concept (SAB, p. 802), “In the case of corolla length, the lack of a direct correlation with pollinator groups may be attributable to a combination of phylogenetic inertia and alternative selective forces (such as those imposed by nectar-robbers).” Yet hummingbirds and insects may both select for longer corolla tubes; indeed some of the longest floral nectar containing structures are associated with moth pollination (Nilsson 1987, 1988). Given the variety of hummingbirds in their study system, which range from having short to large beaks and small to large body size, it is not surprising to see a range of corolla tube lengths that overlap the sizes of flowers pollinated by insects. Additionally, it is corolla tube length combined with other traits, such as corolla tube width, reward schedule and composition, color, anthesis, scent production, etc., that distinguishes selection by hummingbirds from, for example, nocturnal moth pollination. Because the pollination syndrome concept is essentially an interpretation of the selective pressures acting on the flower, a complex structure composed of multiple traits, it is an interpretation of selective pressures acting on a multivariate phenotype. Thus, the choice of characters will more or less determine the competency of the test for the relevance of pollination syndromes. We view the choice of traits measured by SAB as limited and we believe that the addition of other traits may have provided better support for pollination syndromes, that is, corolla tube length combined with width and so forth.

SAB make special comment on the variety of colors associated with those species we consider as having specialized hummingbird pollination and speculate that competitive interactions among the plants results in diversifying selection. We agree that this hypothesis warrants further investigation, but note that the lack of association of color with pollination mode is not necessarily in contrast to the pollination syndrome concept. Red color

may be an important feature of hummingbird-pollinated plants in some parts of the world, and not in others, with the particular ecological context dictating the pattern or lack thereof. We also note that a component of flower color, nectar guides (which contrast in color with the petal background), was not reported, yet the evolution of this particular aspect of flower color may be closely associated with pollination type. Deconstructing flower color to its components of chroma, hue and brightness (Endler 1990) may also obscure a signal that depends on the interaction of the three components.

Since Waser et al. (1996) concluded that pollination generalization is much more common than previously thought (in contra Fenster et al. 2004), the utility of pollination syndromes as a conceptual framework for understanding the origins of floral diversity has been increasingly questioned (e.g., Ollerton 2007). However, we wish to convey that the pollination syndrome concept is not a 19th century straw-man, where variation of flower form results only from the outcome of selection pressures exerted by different types of pollinators. At the very inception of considering the flower as an adaptation, Darwin (1862) recognized that at the very least, a flower represents the outcome of selective forces acting on both breeding and pollination systems. Stebbins (1951) viewed the flower as an adaptation for attracting and rewarding pollinators while ensuring efficient pollen transfer, in addition to protecting the ovules and developing seed from predation. Faegri and van der Pijl (1979, p. 23) saw pollination syndromes as a statistical statement, where there is a “certain syndrome of [flower] characteristics corresponding to each pollination type, but in any given case any feature belonging to the syndrome may be missing. . .” Most recently it has been noted that floral traits vary in response to regional variation in the composition of pollinator communities and the strong influence of historical contingency (Fenster et al. 2004), in addition to nonpollinator-mediated selective pressures (Strauss and Whittall 2006). Furthermore, there are many instances where plants have diverged for floral traits while sharing similar pollinators, (e.g., Huang and Fenster 2007). However, the compelling evidence for floral convergence reflecting adaptation to the important pollinators should not be undervalued because there is a lack of universal correspondence between floral traits and floral visitors. We are in agreement with SAB that the diversity of floral form in Iochrominae is noteworthy of study. Although the Iochrominae does not provide an appropriate model system to statistically assess the association between particular groups of floral visitors and pollination syndromes, it appears to be an ideal system to examine the processes underlying floral divergence of species sharing similar pollinators.

#### ACKNOWLEDGMENTS

We are grateful to S. D. W. Smith for providing us with the data for our reanalysis. S. Buzato, J. Conner, K. Kay, and J. Sobel made thoughtful

comments on previous versions of the manuscript. Support was provided by NSF 0108285 to CF and NSF DDIG 0710196 to SM-R and CF.

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Associate Editor: M. Burd