MACROEVOLUTIONARY TESTS OF POLLINATION SYNDROMES: A REPLY TO FENSTER ET AL.

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ABSTRACT

Studies of floral ecology and evolution are often centered on the idea that particular floral trait combinations, or syndromes, represent adaptations for particular pollinators. Despite the conceptual importance of pollination syndromes, few macroevolutionary studies have statistically examined the relationship between pollinators and floral traits. Using 15 species of Iochroma, Smith et al. (2008a) applied phylogenetically-structured correlation analyses to test the relationship between floral variation and pollination system, quantified in terms of the importance of major pollinator groups. This study revealed that pollinator shifts are tied to changes in nectar reward and floral display but are not significantly correlated with changes in corolla length or color, contrary to what might be predicted from classical pollination syndromes. Fenster et al. (2009) question these findings because our pollinator importance estimates included recently-introduced honeybees. To address this concern, we re-calculated importance values excluding honeybees and repeated the analyses. We found the same patterns as in our original study with significant correlations between pollinators and nectar reward and display. We conclude that phylogenetic approaches provide essential tools for testing macroevolutionary predictions of pollination syndromes and, by applying these approaches to other radiations, we can refine our understanding of the role of pollinators in floral diversification.

Pollination syndromes are combinations of floral traits that have arisen many times independently across flowering plants and are thought to reflect adaptation for particular pollinators (Faegri and van der Pijl 1966; Stebbins 1970). This idea of a close correspondence between flower types and pollination mode has had a major impact on research in plant ecology and evolution. Floral traits comprising syndromes have been targeted in studies of phenotypic variation and selection (e.g, Johnston 1991; Schueller 2007), and quantitative and molecular genetic studies have begun to identify the loci which underlie differences in these traits (Bradshaw et al. 1998; Stuurman et al. 2004; Hobollah et al. 2007). Pollination syndromes are also used to categorize species and describe diversity in clades or communities (Cadotte and Lovett-Doust 2001; Beardsley et al. 2003; Roalson et al. 2003). Despite their broad influence, pollination syndromes have rarely been tested statistically on a macroevolutionary scale using phylogenetic comparative methods (but see Armbruster 1996, 2002). Although Fenster et al. (2009) agree that phylogenetic approaches offer a powerful complement to other studies of pollination syndromes, they raise objections to the approach and conclusions we presented in Smith et al. (2008a).

Our study aimed to test a key prediction of pollination syndromes, that shifts in pollination system are correlated with changes in floral traits. Taking a phylogenetic approach, we studied the pollination ecology and floral trait variation in a core group of Iochrominae, a clade of Andean shrubs in the Solanaceae. By sampling nearly all the species within this core clade, we sought to determine the extent to which pollinator shifts could explain the observed floral variation. We chose to focus our analysis on four floral traits: flower length, nectar reward, display size, and flower color. Fenster et al (2009) suggest that studying this limited set of floral traits prevented us from detecting pollination syndromes. We recognize that syndromes involve

many aspects of floral morphology (Wilson et al. 2004) and that the four traits we have chosen

are not likely to encompass of all the variation that influences plant-pollinator interactions.

Nonetheless, the traits we selected are all important components of syndromes and collectively

account for most of the floral diversity in *Iochroma*. Other characters, like nectar guides or style

exsertion that are included in some pollination syndromes, are largely invariant in *Iochroma*.

In terms of characterizing pollination system, we chose to use the continuous variable,

pollinator importance, which takes into account both visitation and pollen deposition.

Importance was calculated separately for four major groups of pollinators (hummingbirds,

Hymenoptera, Lepidoptera and Diptera) and was directly compared to the continuous floral trait

variables using single (pairwise) and multiple phylogenetic correlation analyses. Fenster et al.

(2009) suggest that instead of considering pollination as a continuous variable, the species should

have been grouped into discrete categories based on whichever pollinator group accounted for

most (>75%) of their pollination. While we find such categorization useful for discussion (Smith

et al. 2008b), we consider the continuous variable preferable for quantitative analyses.

Pollination systems range from more to less generalized (Waser et al. 1996), and we see no

reason to assume that a single "dominant" pollinator that provides 75% or more of the

fertilization for a particular species is uniquely effective in influencing the selective regime.

Additionally, whatever statistical power one might have to detect a real correlation is likely to be

reduced by converting continuous measures into discrete alternative states. For both these

reasons, we consider it preferable to use a quantitative estimate over a rather arbitrary

assignment of species to pollination system.

Fenster et al. (2009) point out that the honeybee *Apis mellifera*, a common pollinator of

Iochroma species, was introduced relatively recently to the Americas, and they suggest that it

should not have been included in our pollinator importance estimates. Although the composition

of pollinator faunas fluctuates naturally over time (Herrera 1988), we agree that dealing with

recently introduced species presents a challenge for these sorts of comparative ecological studies.

In the case of honeybees, it is impossible to know how their arrival might have changed the

pollination system of native plants (reviewed in Goulson 2003). If the honeybees had displaced

non-hymenopteran pollinators, then it would seem appropriate to remove them from the

analyses. However, if they displaced or outcompeted native bees as pollinators of *Iochroma*,

then pollinator importance values including the honeybees would provide the best estimate of the

importance of Hymenoptera prior to the arrival of honeybees.

In order to determine the potential effect of including honeybees in our comparative

analyses, we recalculated the importance values with the honeybees omitted and re-ran the single

correlation analyses. Because we used *relative* importance values for each pollinator group,

changing the importance of hymenoptera by removing honeybees changes the relative

importance of other groups of pollinators (Suppl. Table 1). While Fenster et al. (2009) correctly

re-calculated hummingbird importance after removing bees, they did not re-calculate the

importance of the other groups (Hymenoptera, Lepidoptera and Diptera) nor did they examine

how the adjusted values would affect the correlations with floral traits. Using the importance

values excluding honeybees, we re-ran correlation analyses for all four groups of pollinators.

Comparing with table 3 in Smith et al. (2008a), we found that the exclusion of honeybees had

little quantitative effect and resulted in no change in terms of significant correlations (Suppl.

Table 2). As in Smith et al. (2008a), reward and display were significantly positively correlated

with hummingbird importance and negatively correlated with dipteran importance.

Hymenopteran importance was again positively correlated with display, and lepidopteran

importance was negatively correlated with reward. All other correlations were non-significant.

Thus, contrary to the inference of Fenster et al. (2009), inclusion or exclusion of honeybees has

no effect on our conclusions.

Fenster et al. (2009) note that in our paper, we discussed each of the four floral traits

separately, and argue that we should consider the evolution of the flower as a complex,

multivariate structure. We fully agree that the evolution of floral traits should be approached

using multivariate statistical methods. Indeed, we included these methods in our paper (Smith et

al. 2008a, table 4), making our study one of the very few to develop and implement multivariate,

phylogenetically-structured analyses (see also Ives et al. 2007, Lavin et al. 2008). We pursued

multivariate analyses specifically to determine which suites of floral traits were evolving jointly

with pollination systems. These analyses indicated that nectar reward and display evolve in a

correlated fashion with pollination system, whereas flower color and corolla length evolve

largely independently of changes in pollination system. Our interpretation of these results is that

reward and display are components of the suite of traits that respond to shifts between pollinator

types in *Iochroma*. In contrast, the lack of correlation between pollinator shifts and flower color

and length suggests that these traits are shaped by other evolutionary forces.

Fenster et al. (2009) argue that this observed lack of correlation does not necessarily

contradict the pollination syndrome concept because the concept does not invoke a "universal

correspondence" but rather a general tendency (p. 10). For example, the presence of a large

nectar reward may be considered part of the hummingbird syndrome even though some

hummingbird-pollinated species do not produce large rewards. We agree that pollination

syndromes predict general trends as opposed to a perfect correspondence between traits and

pollinators, and our study was designed to test these predicted trends. We found significant

relationships between pollinator shifts and nectar reward and display because they *tend* to evolve together across the phylogeny. Although the correspondence is not perfect, species with more bird pollination, for instance, tend to have larger nectar rewards and larger displays. Conversely, species with less bird pollination tend to have smaller rewards and smaller displays. Such strong patterns did not appear with flower color and corolla length.

In the case of corolla length, Fenster et al. (2009) suggest that the lack of a correlation might reflect different *Iochroma* species being pollinated by hummingbirds with different beak lengths. However, this cannot easily explain the observed pattern because many *Iochroma* species are pollinated by multiple hummingbird species with different beak lengths and conversely, several hummingbird species visit multiple *Iochroma* species with different corolla lengths (Smith et al. 2008b). Instead, considering the significant phylogenetic autocorrelation in corolla length, we suggested that the variation in tube length is explained not so much by current pollinator type but by a combination of phylogenetic inertia and other selective forces, like nectar-robbers. Although we cannot exclude the possibility that adding more taxa might elevate the non-significant correlation between pollinator shifts and corolla length (Fig. 1A), it appears that this relationship is much weaker than those found with nectar reward and display.

Regarding flower color evolution in *Iochroma*, we found that the trait was highly labile across the phylogeny (as indicated by the low phylogenetic autocorrelation, Smith et al. 2008a) and that the frequent color shifts were not related to changes in the importance of any group of pollinators. Particularly striking was the variation of flower colors among the mostly hummingbird-pollinated taxa, which include white, blue, yellow, red and purple-flowered species (Fig. 1B). Fenster et al. (2009) state that these findings do not contrast with the predictions of pollination syndromes. However, flower color has been a central component of

nearly every study describing pollination syndromes (e.g., Wilson et al. 2004; Whittall and Hodges 2007) and is often used as a predictor of pollination mode (Harrison 1999; Tripp and Manos 2008). Thus, the lack of a correlation between flower color and pollination system runs counter to at least some conceptions of pollination syndromes. That being said, we agree with Fenster et al. (2009) that the relationship between flower color and pollination system may vary across geographic regions (Grant 1966), and we consider this an interesting question for future study.

In conclusion, while it would certainly be desirable to increase the number of species studied so as to obtain more statistical power, we stand by our conclusion that two commonly-cited elements of pollination syndromes, flower length and color, are not tightly linked to pollination mode in *Iochroma*. But it would be a mistake to extrapolate from this result to conclude that these traits are never correlated with pollination system. If one sets up the pollination syndrome concept not as a "19th century strawman" (Fenster et al. 2009) but as a set of testable phylogenetic hypotheses, then we surely should expect cases in which particular traits are <u>not</u> shaped by pollination system in certain groups of plants. We do not imagine that future phylogenetic comparative studies will either prove or disprove the pollination syndrome concept *in toto*. Instead we hope that, through multiple phylogenetic comparative studies, we will acquire a better understanding of which traits in which clades and communities are most tightly correlated with pollination system, and why.

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Figure 1. Hummingbird importance vs. corolla length and hue for 15 Iochroma species. Data

from Smith et al. (2008a). Hue can be described as the type of color (e.g. red, blue). The dots

representing each species in (B) are colored according to flower color by sampling the flower

photographs in Smith et al. (2008a, Fig. 1).