doi:10.1111/j.1558-5646.2008.00327.x

THE ROLE OF POLLINATOR SHIFTS IN THE FLORAL DIVERSIFICATION OF *IOCHROMA* (SOLANACEAE)

Stacey DeWitt Smith,[1,2,3] Cécile Ané,[1,4,5] and David A. Baum[1,6]

[1]*Department of Botany, University of Wisconsin, 430 Lincoln Drive, Madison, Wisconsin 53706*

[2]*E-mail: sds21@duke.edu*

[4]*Department of Statistics, University of Wisconsin, 1300 University Avenue, Madison, Wisconsin 53706*

[5]*E-mail: ane@stat.wisc.edu*

[6]*E-mail: dbaum@wisc.edu*

Received August 21, 2007
Accepted December 23, 2007

Differences in floral traits among plant species have often been attributed to adaptation to pollinators. We explored the importance of pollinator shifts in explaining floral divergence among 15 species of *Iochroma.* We examined four continuously varying floral traits: corolla length, nectar reward, display size, and flower color. Pollinator associations were characterized with a continuously varying measure of pollinator importance (the product of visitation and pollen deposition) for four groups of pollinators: hummingbirds, Hymenoptera, Lepidoptera, and Diptera. A phylogenetic generalized least squares approach was used to estimate correlations between pollinator groups and floral traits across a sample of Bayesian trees using different models of trait evolution. Multivariate analyses were also employed to identify suites of traits associated with each pollinator group. We found that nonphylogenetic models typically fit the data better than phylogenetic models (Brownian motion, Ornstein–Uhlenbeck), and thus results varied little across trees. Our results indicated that species with high nectar reward and large displays are significantly more likely to be pollinated by hummingbirds and less likely to be pollinated by all groups of insects. Corolla length and flower color did not show any consistently significant associations with pollinator groups. For these two traits, we discuss alternative evolutionary forces, including phylogenetic inertia and community-level factors.

KEY WORDS: **Floral display, flower color, nectar, PGLS, pollinators, quantitative convergence index.**

Botanists have long offered adaptive explanations of floral diversity in terms of biotic pollination (Faegri and van der Pijl 1966; Stebbins 1970). Differences in floral traits among closely related species have been explained as adaptations to different pollinators (e.g., Schemske and Bradshaw 1999; Muchhala 2003; Streisfeld and Kohn 2007), whereas floral convergence has been tied to parallel adaptation to the same pollinators (e.g., Schemske 1981; Patterson and Givnish 2004; Whittall et al. 2006). The latter phenomenon has been formalized as pollination syndromes, wherein suites of floral characters are associated with different modes of pollination (Faegri and van der Pijl 1966). Despite ongoing debate over the relevance of pollination syndromes (Herrera 1996; Ollerton 1996; Waser et al. 1996), the concept continues to serve as the overarching framework for many studies of floral diversity (Johnson and Steiner 2000; Kay and Schemske 2003; Fenster et al. 2004).

The basic assumptions underlying the pollination syndrome framework have been largely supported by empirical studies. Pollinators have been shown to exert selection pressure on a wide array of floral traits such as flower color, corolla shape, and nectar reward (Galen 1989; Cresswell and Galen 1991;

[3]Current address: Department of Biology, Box 90338, Duke University, Durham, North Carolina 27708

Melendez-Ackerman et al. 1997). Moreover, different functional groups of pollinators (sensu Fenster et al. 2004) can select in different directions, theoretically generating fitness trade-offs (Aigner 2001). Although pollinator-mediated trade-offs for floral traits have been hard to demonstrate (Wilson and Thomson 1996; Castellanos et al. 2004; Aigner 2005), several studies provide compelling evidence for the importance of pollinators in adaptive divergence of flower color and form (Schemske and Bradshaw 1999; Muchhala 2007).

The role of pollinators in floral evolution has been explored to a lesser extent with phylogenetic approaches. Several studies have reconstructed the evolution of floral traits and discussed their relationship to pollinators (e.g., Hapeman and Inoue 1997; Johnson et al. 1998; Kay et al. 2005; Perez et al. 2006), but these failed to include any statistical analyses to quantify the relationship. To our knowledge, the series of *Dalechampia* studies by Armbruster (1996, 2002) represents the only previous attempt to use direct observations of pollinators (rather than inferences from floral biology) and phylogenetically corrected statistical analyses to examine this association. Although the *Dalechampia* studies provide an excellent example of using a phylogenetic approach to test hypotheses about floral adaptation, the system is somewhat limited in that all species are bee-pollinated and thus may not exhibit the range of variation thought to be associated with different pollination syndromes.

Here we take a comparative approach to estimate correlations between several groups of pollinators and floral traits using 14 species of *Iochroma* and the nested monotypic genus *Acnistus*. Previous phylogenetic studies in Iochrominae, a clade of around 35 predominantly Andean species, have shown that members of *Iochroma* are divided among three clades (Smith and Baum 2006). Our sampling was concentrated in the "ACLF" subclade (sensu Smith and Baum 2006), a group of 16 species that exhibits dramatic diversity in flower form and color (Fig. 1). The combination of a moderate number of species with a well-resolved phylogeny and a wide diversity of floral traits makes this an ideal group for testing the role of pollinators in floral diversification.

We focused on four floral traits: corolla length, flower color, nectar reward and display size. Variation in corolla tube length, which affects both access to reward and pollen deposition/receipt, has been related to differences in the length of animal feeding apparatuses (e.g., Nilsson 1988; Schemske and Horvitz 1989). Shallower flowers are often associated with pollination by bees and flies whereas deeper flowers can only be accessed by pollinators with comparably long mouthparts, such as hummingbirds and moths (Faegri and van der Pijl 1966; Whittall and Hodges 2007). Flower color is involved in signaling to pollinators, with the classic prediction being that bird-pollinated flowers reflect longer wavelengths (especially red) than most insect-pollinated flowers (Stiles 1976; Altshuler 2003). Although the importance of particular colors in attracting different pollinators remains a source of contention (Chittka and Waser 1997), the assumption that red flowers indicate bird pollination remains common (e.g., Harrison et al. 1999; Dressler et al. 2004). Reward type and size are important in determining and maintaining interactions between plants and their pollinators (Armbruster 1993; Goldblatt and Manning 2006), with the most consistent pattern being a positive correlation between the energetic needs of pollinators and the energy content of the reward (Heinrich and Raven 1972). Although display size is not a trait commonly included in pollination syndromes, it has well-documented effects on pollinator visitation rate (Brody and Mitchell 1997; Galloway et al. 2002), and recent work reveals that different pollinator groups respond differently to variation in display (Thompson 2001). Here we were particularly interested in the possibility that species with low reward flowers may attract hummingbirds by producing large displays (Feinsinger 1976). Although there are clearly many additional traits that could have been chosen for study, these four have all been featured in the pollination literature, and together they encompass much of the floral diversity in *Iochroma*.

We consider pollination system as a continuous trait, where each species receives varying contributions to pollination by different groups of animals. Visitors to *Iochroma* were classed into four functional groups: hummingbirds, Hymenoptera (bees and wasps), Lepidoptera (moths and butterflies), and Diptera (flies); the importance of each group was estimated from field studies (S. D. Smith, S. J. Hall, P. R. Izquierdo, and D. A. Baum, unpubl. ms.). We estimate single and multiple correlations between the continuously varying pollinator importance values and the floral traits using phylogenetic generalized least squares (PGLS) with an Ornstein–Uhlenbeck (OU) model of trait evolution (Garland et al. 1993; Martins and Hansen 1997). As the amount of observed phylogenetic signal (similarity due to shared history) varies across traits and lineages, so does the need for phylogenetic correction in comparative analyses (Blomberg et al. 2003). We chose the PGLS approach because it allows us to directly compare analyses assuming different levels of phylogenetic signal by varying the parameters of the OU model. Using the Akaike Information Criterion (AIC) as a measure of model fit, we simultaneously determine the appropriate level of phylogenetic signal and the best estimates of the parameters of interest, namely the correlations between pollinator groups and floral traits. Applying this process to multivariate analyses, we identify suites of floral traits significantly associated with different pollination systems across *Iochroma*.

Materials and Methods

STUDY TAXA AND PHYLOGENETIC RELATIONSHIPS

Fifteen species within Iochrominae were selected for this study. The taxon sampling encompasses 13 of 16 species in the core clade of the iochromas (ACLF clade sensu Smith and Baum 2006),

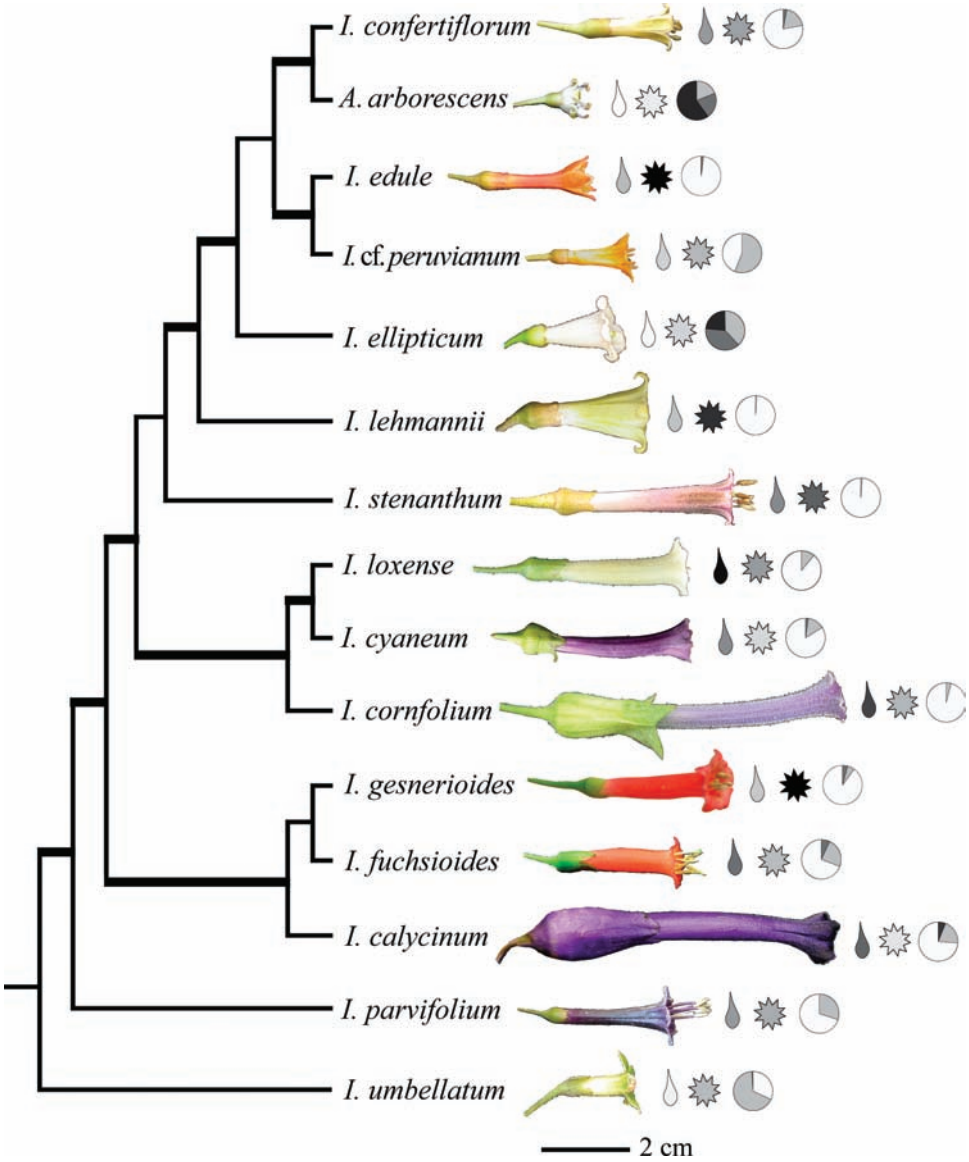

Figure 1. **Phylogenetic relationships and floral trait diversity in *Iochroma*. The topology shown is a consensus tree from Bayesian analysis of the three-gene dataset (Smith and Baum 2006) pruned to show only the 15 taxa studied here with all compatible groupings included. Branches with greater than 95% posterior probability are bolded. Flowers for each species are shown to scale. The droplet and star symbols indicate reward per flower and display size, respectively, with darker shading showing higher values. The pie-graphs show proportion of pollinator importance from each of the four groups: hummingbirds (white), hymenopterans (light gray), lepidopterans (dark gray), and dipterans (black) (See Table 1).**

which includes the nested monotypic *Acnistus arborescens*. One of these species, *I. peruvianum*, was previously known only from the type specimen, and its determination remains tentative. Another species, *I. stenanthum*, is suspected to have some hybrid ancestry based on its morphological features. However, we chose to include it because its phylogenetic position was not strongly contested by independent loci (Smith and Baum 2006). We also sampled one representative of the sister clade (*I. parvifolium*) and one more distantly related species (*I. umbellatum*) to serve as an outgroup.

Phylogenetic relationships among the 15 study taxa follow the analysis of Smith and Baum (2006), which used three nuclear genes: the internal transcribed spacer (ITS), the second intron of *LEAFY* (*LFY*), and exons 2–9 of the granule-bound starch synthase gene (*waxy*). For this study, we extracted 500 trees from Bayesian analysis of the combined three-gene dataset, by sampling every 500 generations after burn-in. Adequate mixing during the runs and convergence among independent runs (Smith and Baum 2006) suggest that these post–burn-in trees represent a reasonable sample of the posterior distribution of trees. The trees

were pruned to include only the 15 studied species while retaining the total path length between each species in each tree. The 500-tree sample was used in two sets of analyses. First, we used the trees to estimate the Quantitative Convergence Index (QVI) for our characters of interest (see *Analysis of trait convergence*). Second, we conducted PGLS analyses on the 500 trees to examine the sensitivity of the single and multiple correlations to phylogenetic uncertainty (see *Correlation analyses*).

POLLINATION ECOLOGY

Field studies were conducted in Ecuador and Peru to characterize the importance of groups of pollinators to each species (S. D. Smith, S. J. Hall, P. R. Izquierdo, and D. A. Baum, unpubl. ms.). Pollinators were clustered in four major groups (hummingbirds, hymenopterans, lepidopterans, and dipterans), which seem to encompass the principal functional groups (sensu Fenster et al. 2004) of visitors to *Iochroma* species. Hummingbird visitors probed *Iochroma* flowers for nectar, accumulating pollen on their beaks as they probed. The hymenopteran pollinators were mainly honey bees (*Apis mellifera*) and wasps, which crawled around the opening of the corolla and sometimes down into the tube, foraging for pollen and nectar. Most dipteran visitors were syrphid flies, which landed at the mouth of the corolla, probing for nectar, and consuming pollen. Moths and butterflies both occasionally forage, for nectar on *Iochroma* species although, for the two species with substantial lepidopteran pollination, *A. arborescens* and *I. ellipticum*, all of the lepidopteran visitors were moths.

The visitation rate for each pollinator group was estimated from field observations, in which we recorded the number of flower visits per flower per hour (Dafni 1992). Pollinator importance was quantified as the product of the visitation rate of a pollinator group and the proportion of ovules potentially pollinated per visit by a member of that group (maximum = 1.0). This proportion was calculated by comparing the average number of ovules per flower of each species to the amount of pollen deposited on a single visit by a given pollinator to that species. Although the relationship between pollen deposition and seed set is not known in *Iochroma*, studies in other taxa indicate that at least as many pollen grains as ovules are required to achieve maximal seed set (Silander and Primack 1978; Snow 1982). Choosing higher thresholds for effective pollination (e.g., twice the number of ovules) as observed in some species (Kohn and Waser 1985) would have little effect on the estimated importance because most legitimate pollinators deposited several times more pollen grains than there were ovules present (S. D. Smith, S. J. Hall, P. R. Izquierdo, and D. A. Baum, unpubl. ms.).

FLORAL TRAITS

Nectar measurements were collected in the plant populations used for pollinator observations. Nectar was sampled from 10 to 40 bagged, first- and second-day flowers. Reward per flower was calculated as the product of nectar volume and sugar concentration measured in the field (S. D. Smith, S. J. Hall, P. R. Izquierdo, and D. A. Baum, unpubl. ms.). Display size, the number of open flowers on a plant, was also estimated in the field by averaging across the individuals used for pollinator observations.

Corolla length data for each species was collated from herbarium specimens and the taxonomic literature (Leiva 1995; Leiva et al. 1998; Shaw 1998; Hunziker 2001). When a range of values was presented in the taxonomic descriptions, the midpoint value was used. For *I. peruvianum*, a poorly known species, the mean corolla length was obtained by averaging across herbarium collections from the study site and nearby localities ($n = 6$).

Flower color was characterized through analysis of standardized reflectance spectra. Raw reflectance measurements, in 3.3 nm segments from 400 nm to 700 nm were collected with a Unispec spectrometer (PP Systems, Inc., Amesbury, MA) with a built-in 7.0 W halogen light source (shorter wavelengths could not be collected by this equipment). Fresh corolla tissue to be measured was placed in a standard-clip, which holds the fiber-optic cable (light output and input) at a fixed 60° angle and excludes ambient light. The tissue was sampled from the midpoint of the corolla tube, and measurements were taken with the exterior (abaxial surface) oriented toward the fiber optic. One first-day flower from the plant sampled as the voucher specimen for each study population (Smith 2006) was used for the measurements. Each measurement was done three times to ensure that the reading was repeatable. In the rare case that it was not repeatable, a new flower was used. Standardized reflectance spectra were calculated by dividing the amount of light reflected by corolla tissue in each 3.3 nm segment by that reflected by a Spectralon white standard (Labsphere, North Sutton, NH).

Variation in reflectance spectra across species was summarized using segment classification (Endler 1990). This system divides color into brightness, chroma, and hue. Brightness is the total visible light reflected, that is the total area under the reflectance curve from 400 to 700 nm. Chroma is $\sqrt{(R-G)^2 + (Y-B)^2}$, where B is the proportion of total brightness occurring between 400 and 475nm, G is the proportion between 475 and 550 nm, Y is the proportion between 550 and 625, and R is the proportion between 625 and 700 nm. Hue is calculated as arcsine $(\frac{Y-B}{C})$,where C is chroma.

COMPARATIVE ANALYSES

Analysis of trait convergence

As an initial assessment of phylogenetic signal in the floral traits and pollinator importance values, we computed their QVI (Ackerly and Donoghue 1998). QVI provides a measure of homoplasy in a continuous character across a phylogeny, analogous to the retention index for discrete characters (Farris 1989). It ranges from

0 to 1, with higher values signifying higher convergence, that is, greater similarities among distantly related species. We used the program CACTUS (Schwilk and Ackerly 2001) to compute QVI for the pollinator importance values and floral traits on 500 trees extracted from the Bayesian posterior of the combined three-gene analysis. To test the hypothesis that the observed QVI is less than expected by chance (i.e., that the traits exhibit some phylogenetic autocorrelation), we permuted the character values for each trait across taxa 1000 times to produce null distributions for each tree, and compared the observed to the null distributions using a one-tailed test of significance, as implemented in CACTUS.

Correlation analyses

A variety of comparative methods have been proposed to accommodate the fact that species cannot be viewed as independent datapoints due to their shared evolutionary history. Here we used a PGLS approach with an Ornstein–Uhlenbeck (OU) model of trait evolution to estimate correlations between pollinators and floral traits while accounting for phylogeny (see Appendix). First proposed by Grafen (1989), PGLS generalizes the independent contrasts approach and can be used to incorporate a variety of models of evolutionary change (Martins and Hansen 1997). Unlike the Brownian motion model, which assumes that trait variation increases linearly along the phylogeny (Felsenstein 1985), the OU model assumes that trait evolution is constrained as might be expected for traits under stabilizing selection, and the level of constraint is determined by the OU parameter α (Martins and Hansen 1997). As in an increasing number of comparative studies (e.g., Ives and Godfray 2006; Ord and Martins 2006), we used the OU model here as a flexible approach for exploring the effects of assuming different levels of phylogenetic signal (Blomberg et al. 2003). When the OU parameter α is zero (assuming strong phylogenetic signal), the model is equivalent to Brownian motion and covariance in trait values between any two species is linearly related to their shared branch length. With higher values of α (lower phylogenetic signal), the expected similarity between any pair of taxa exponentially decreases with increasing phylogenetic distance. As α approaches infinity, the OU model reduces to a nonphylogenetic "TIPS" model in which the trait values are independent of the tree.

In phylogenetic regression and correlation analyses with the OU model (Martins and Hansen 1997), a selected value of α and a phylogeny with branch lengths are used to produce a variance–covariance matrix that describes the expected similarity in trait values due to phylogenetic relatedness (see Appendix). The observed trait values can then be transformed with this matrix before analyses to account for phylogenetic dependence. Because we do not know a priori how strongly the trait values depend on phylogeny, we explored a range of values of α (0, 10, 100, ∞). To make the results fully comparable across models, we used a fixed parameter value at the root (see Appendix). Because α acts as a transformation of the branch lengths, the effect of a chosen value of α will depend on the phylogenetic tree. Based on the branch lengths in the *Iochroma* phylogeny, α values of 10 and 100 were chosen as intermediates between Brownian motion ($\alpha = 0$) and TIPS ($\alpha = \infty$) (see Appendix).

Correlation analyses incorporating the OU variance–covariance matrix were used to estimate single and multiple correlations between each group of pollinators and floral traits. Analyses were conducted using the APE (Paradis et al. 2004) and MASS (Venables and Ripley 2002) packages in R (R Development Core Team 2005). We estimated correlations among variables instead of regression coefficients because all of the variables under consideration were random, and we did not want to assume that any is causally dependent on any other (Sokal and Rohlf 1995). As standard parametric statistical analyses, including regression and correlation, assume that data are normally distributed, we created quantile–quantile plots for each variable and assessed the need for transformation before undertaking any analyses. The relative pollinator importance values, estimated as proportions, were arcsine-square root transformed to produce a more normal distribution, display size and chroma were log transformed, and reward per flower was square-root transformed. The remaining variables were analyzed without transformation.

To explore the effect of phylogenetic uncertainty on the correlations, we repeated these analyses on the sample of 500 trees from Bayesian analysis of the combined three-gene dataset (Smith and Baum 2006). For the pairwise analyses, we computed the correlation coefficient between each pollinator group and each trait separately using the four values of α for each of the 500 trees. We used AIC scores (Akaike 1974) to examine the model fit across trees and values of α. The value of α that resulted in the lowest AIC score on the majority of trees was judged to be optimal, and the mean correlation with this α across all trees was taken as the best estimate of the correlation. We removed the tails of the distribution (highest and lowest 2.5% of trees) to produce a 95% interval around this correlation estimate. Although this interval accounts for uncertainty in the tree topology and branch lengths, it does not account for other possible sources of error (measurement error, stochastic variation).

Next, we used multiple correlation analyses to determine which sets of floral characters jointly explain the largest amount of variance in pollinator importance values. We used a two-step procedure to eliminate floral traits from the model for each pollinator group for each value of α. First, we used stepwise model selection (stepAIC function in R) to eliminate variables from the full set (a pollinator group regressed on all floral traits), repeating for each of the 500 trees. Those variables (floral traits) that were retained by the majority of trees for a given value of α were kept, and the multiple correlation analysis was repeated, using

the same reduced set of variables for all trees. Because variables with intermediate partial correlations (0.20–0.40) were not consistently retained or lost across all the trees during stepwise model selection, this two-step procedure ensured that the same parameters were computed and summarized across all trees. However, it remains possible that different sets of variables will be retained when different values of α are used.

Comparing across values of α, we chose the value that gave the lowest AIC score for the majority of trees, and we considered the mean partial correlations across the trees with this value of α as the best estimates of these correlations. The partial correlations we present are marginal, that is, corrected for all other floral traits retained in the model. Again we pruned the extremes of the distribution across trees to produce a 95% interval for each partial correlation. Although these sets of analyses entail many correlation estimates, the selection of a single multivariate model per pollinator group reduces the potential issue of multiple tests.

Results

FLORAL AND POLLINATION DATA

Pollinator variables and floral traits are given in Table 1. The only missing data were the reflectance spectra for the Galapagos endemic *I. ellipticum*. Because the methods of analysis used here are not equipped to deal with missing data, the creamy flowers of *I. ellipticum* were given intermediate color values between the white flowers of *A. arborescens* and the more yellowish-white flowers of *I. confertiflorum*. Because both species are closely related to *I. ellipticum*, this extrapolation should have minimal effect on phylogenetic correlations. The standardized reflectance curves are shown in online Supplementary Figure S1.

Variation was observed in all traits of interest (Table 1). Pollination systems vary from entirely insect to entirely bird, with many species tending toward the latter. Average corolla length showed approximately sixfold variation across the sampled species (0.95 cm in *A. arborescens* to 5.75 cm in *I. calycinum*), display size (number of flowers per plant) varied 24-fold, and reward per flower (nectar volume × concentration) nearly 80-fold. The wide range in chroma, hue, and brightness reflected diversity of flower colors among the studied taxa (Fig. 1).

TRAIT CONVERGENCE

Table 2 summarizes the observed QVIs for each trait across the 500 Bayesian trees and the expected QVIs if the trait values were unrelated to the phylogeny. Average QVI values for pollinator importance ranged from 0.78 to 1.00 and were indistinguishable from the expected (random) distribution ($P = 0.17$–0.81), suggesting little phylogenetic autocorrelation. Most of the floral traits had similarly high QVI values that were not significantly different from that expected by chance (Table 2). Only corolla

Table 1. Pollinator importance and floral traits values for study taxa. Details of data collection described in the methods.

Species	Hummingbird importance	Hymenopteran importance	Lepidopteran importance	Dipteran importance	Corolla length (cm)	Nectar reward per flower	Display size	Chroma	Hue	Brightness
A. arborescens	0.00	0.19	0.21	0.60	0.95	0.11	21.4	0.13	69.58	0.24
I. calycinum	0.74	0.20	0.00	0.06	5.75	5.68	29.8	0.09	9.10	0.06
I. confertiflorum	0.78	0.19	0.03	0.00	2.25	3.78	112.2	0.17	79.49	0.42
I. cornifolium	0.96	0.04	0.00	0.00	3.50	6.91	87.0	0.12	59.65	0.16
I. cyaneum	0.84	0.14	0.02	0.00	3.25	4.09	41.5	0.17	46.96	0.16
I. edule	0.99	0.01	0.01	0.00	2.10	2.26	311.2	0.35	50.78	0.23
I. ellipticum	0.00	0.38	0.38	0.23	2.75	0.20	50.0	0.15	74.53	0.33
I. fuchsioides	0.70	0.24	0.07	0.00	2.50	5.24	79.2	0.50	24.52	0.25
I. gesnerioides	0.90	0.06	0.04	0.00	3.25	1.67	505.0	0.18	24.42	0.10
I. loxense	0.89	0.11	0.00	0.00	3.50	8.71	120.3	0.18	80.77	0.28
I. parvifolium	0.70	0.30	0.00	0.00	2.25	3.50	98.0	0.05	5.59	0.04
I. cf. *peruvianum*	0.44	0.56	0.00	0.00	1.57	1.44	85.0	0.13	44.90	0.18
I. lehmannii	0.99	0.01	0.00	0.00	2.75	1.87	275.0	0.20	69.26	0.34
I. stenanthum	0.99	0.01	0.00	0.00	3.95	4.21	209.3	0.07	10.70	0.19
I. umbellatum	0.32	0.67	0.01	0.00	1.75	0.39	80.5	0.11	96.02	0.11

Table 2. Summary of trait convergence analysis. The quantitative convergence index (QVI) was calculated for all traits for each of 500 Bayesian trees. The mean value across trees is listed below as the mean observed QVI, with a 95% interval across trees in brackets. Tip values were randomized 1000 times on each tree, and QVI was recalculated. The mean QVI from randomized data across all trees is given, with a 95% interval around this mean across trees given in brackets. The mean *P*-value indicates the average proportion of the 1000 permutated datasets whose QVI was greater than the unpermuted data. A 95% interval across trees is provided in brackets.

Character	Mean observed QVI	Mean QVI for randomized data	Mean *P*-value
Hummingbird importance	0.86 [0.83–0.87]	0.84 [0.83–0.85]	0.49 [0.39–0.54]
Hymenopteran importance	0.78 [0.73–0.80]	0.84 [0.83–0.85]	0.26 [0.17–0.32]
Lepidopteran importance	0.87 [0.82–0.92]	0.87 [0.86–0.88]	0.59 [0.35–0.81]
Dipteran importance	1.00 [1.00–1.00]	0.94 [0.92–0.95]	0.72 [0.69–0.74]
Corolla length	0.55 [0.53–0.60]	0.81 [0.81–0.82]	0.03 [0.01–0.05]
Reward per flower	0.74 [0.68–0.83]	0.80 [0.80–0.81]	0.30 [0.14–0.59]
Display	0.95 [0.83–1.00]	0.86 [0.86–0.87]	0.78 [0.33–0.99]
Chroma	0.86 [0.80–0.90]	0.83 [0.83–0.84]	0.59 [0.31–0.78]
Hue	0.68 [0.67–0.71]	0.81 [0.80–0.82]	0.15 [0.11–0.21]
Brightness	0.65 [0.62–0.69]	0.82 [0.81–0.82]	0.09 [0.05–0.15]

length had an observed QVI that was significantly different from (and lower than) permuted data in a large majority (451/500) of trees, meaning that only this trait showsed significant phylogenetic autocorrelation.

CORRELATIONS BETWEEN POLLINATORS AND FLORAL TRAITS

For each pairwise analysis (a pollinator group plus a floral trait), we used AIC scores to select the best fitting value of α among 0 (Brownian motion), 10, 100, and ∞ (TIPS). In all cases (Table 3) the best fitting values were either $\alpha = 100$ or ∞, indicating low phylogenetic signal of the pairs of traits. This is expected given the low phylogenetic autocorrelation for all traits except corolla length, as indicated by the QVI values (Table 2).

Based on the optimal models, only two floral traits, reward per flower, and display size, showed consistently significant associations with pollinator groups in pairwise analyses. Reward and display were positively correlated with hummingbird importance and negatively correlated with dipteran importance (Table 3). Also, lepidopteran importance was negatively correlated with reward, and hymenopteran importance was negatively correlated with display. These significant relationships were found for all trees under the best fitting model (value of α) except in the case of display and hymenopteran importance, where 5% of trees produced a nonsignificant correlation. Also, these correlations typically remained significant regardless of the value of α, although the estimated correlations varied by as much as 0.1 (see online Supplementary Table S1). In these pairwise analyses, corolla length, chroma, hue,

Table 3. Pairwise correlation coefficients for traits and pollinator groups. Before analysis, the pollinator importance variables were arcsine-square root transformed, reward per flower was square-root transformed, and display size and chroma were $\log_{10}$ transformed. For each pair of variables, the estimated correlation (corr) is given above the optimal value of α (based on AIC). For phylogenetically structured models ($\alpha < \infty$), the 95% interval across the 500 Bayesian trees is provided in brackets. Significant correlation coefficients ($P < 0.05$) are bolded. Correlation coefficients and AIC scores for all models are provided in online Supplementary Table S1.

Pollinator group		Corolla length	Reward	Display	Chroma	Hue	Brightness
Hummingbird	Corr	0.46	**0.71**	**0.69** [0.67, 0.71]	0.15 [0.12, 0.19]	−0.33	−0.10
	α	∞	∞	100	100	∞	∞
Hymenoptera	Corr	−0.37	−0.41	**−0.53** [−0.55, −0.51]	−0.22	0.23	−0.18
	α	∞	∞	100	∞	∞	∞
Lepidoptera	Corr	−0.34 [−0.31, −0.36]	**−0.63** [−0.64, −0.61]	−0.38	0.27 [0.24, 0.30]	0.28	0.39
	α	100	100	∞	100	∞	∞
Diptera	Corr	−0.23	**−0.57**	**−0.63**	−0.10	0.19	0.15
	α	∞	∞	∞	∞	∞	∞

Table 4. **Partial correlation coefficients between pollinator groups and retained traits from multivariate analyses. Variables were transformed before analysis as described in Table 3. Variables were retained in the models if they lowered the AIC score across the majority of the 500 sampled trees. When phylogenetic models fit the data better than $\alpha = \infty$ for a majority of the trees, a 95% interval across the 500 trees is provided in brackets. The correlation coefficients and AIC scores for all values of α for each pollinator group are given in online Supplementary Table S2.**

Pollinator	Best model (α)	Corolla length	Reward	Display	Chroma	Hue	Brightness
Hummingbird	∞		0.85	0.67			
Hymenoptera	∞	−0.42		−0.51			
Lepidoptera	100		−0.63	−0.45			0.44
			[−0.65, −0.61]	[−0.48, −0.42]			[0.41, 0.47]
Diptera	∞		−0.64	−0.63			

and brightness were not significantly correlated with any group of pollinators under the best model nor under any of the poorer fitting models for any tree (see online Supplementary Table S1).

Before undertaking the multiple correlation analyses, we determined the extent of correlation among the floral traits (with $\alpha = \infty$), as collinearity may confound the estimation of individual effect sizes (McCullagh and Nelder 1989). We found that corolla length had a significant positive correlation with reward per flower ($r = 0.63$), as in Ornelas et al. (2007), and that brightness was positively correlated with hue ($r = 0.58$). Because corolla length, hue, and brightness were not significantly tied to any pollinator groups in pairwise analyses, these correlations may not have serious implications for interpreting the multivariate analyses.

Multiple correlation analyses generally produced similar results to pairwise analyses. For hummingbirds, reward and display, both significant in pairwise analyses, were retained with positive partial correlations in the best fitting model ($\alpha = \infty$, Table 4). For Hymenoptera, the best model ($\alpha = \infty$) showed negative partial correlations for display and corolla length, the latter of which was not significant in pairwise analyses. Models including some phylogenetic signal ($\alpha = 100$) were a better fit for multivariate analyses with Lepidoptera, and the results indicated that reward and display were negatively correlated whereas brightness was positively correlated. The latter two traits did not show significant correlations in the pairwise analyses (Table 3). For Diptera, reward and display were retained with negative partial correlations in the best fitting model ($\alpha = \infty$, Table 4), in accordance with the pairwise correlation results (Table 3). As with pairwise analyses, results varied somewhat across values of α (online Supplementary Table S2). However, better-fitting models (typically $\alpha = 100, \infty$) retained the same subset of the floral trait variables, varying only in the magnitude of the correlation. Also, the rank order of the correlations did not change with different α values: the floral trait with the highest partial correlation with a given pollinator group remained the highest regardless of the value of α.

Discussion

Our goal in this study was to identify the extent to which the diversification of floral traits in *Iochroma* can be associated with differences in pollination system. Using phylogenetically corrected pairwise and multiple correlation analyses, we tested the relationship between four floral traits (corolla length, nectar reward, display size and flower color) and each group of pollinators (hummingbirds, hymenopterans, lepidopterans, and dipterans). Under the pollination syndrome framework, we would expect to see certain trait values associated with particular pollinators (e.g., high reward and long corollas with hummingbirds) and deviations from those trait values leading to decreases in the importance of that pollinator. Across all analyses, we found that although some floral traits appeared closely tied to changes in pollinator importance, other traits appeared to evolve independently of relationships with pollinator groups. These results were robust to differences in the phylogenetic model used to compute correlations and to variation in topology and branch lengths across a Bayesian sample of trees.

FLORAL TRAITS THAT CORRELATE WITH POLLINATORS

Among the floral traits examined, reward and display showed the most consistent association with differences in pollinator importance. The relationship between pollinator shifts and reward evolution has been found in many plant groups (reviewed in Fenster et al. 2004). In *Iochroma*, species with high amounts of reward per flower were significantly more likely to be pollinated by hummingbirds and less likely to be pollinated by dipterans and lepidopterans (Tables 3 and 4). Given their larger body size and high energy requirements, particularly in high elevation habitats (Altshuler et al. 2004), hummingbirds might be expected to require more rewarding flowers than most insects. The lack of correlation between reward per flower and hymenopterans is perhaps not surprising because these insects consume both nectar and pollen as rewards, and we measured only nectar rewards.

The importance of display size in attracting pollinators has been confirmed by many studies although few have looked at how different pollinator groups respond to variation in display (Thompson 2001). We found that species with larger displays (more flowers per plant) were more likely to be pollinated by hummingbirds, and species with small displays were more likely to be pollinated by insects (Tables 3 and 4; Fig. 1). This effect could result partly from enhanced signaling to visually foraging and wide-ranging pollinators such as hummingbirds and less so to local, possibly olfactorily foraging, animals such as flies and moths. This pattern is illustrated by *A. arborescens* and *I. ellipticum*, two species with small displays, which produced a sweet scent and attracted only insect pollinators (Fig. 1). The only other scented species, *I. confertiflorum*, has a larger display and more reward per flower and attracted a mix of hummingbirds and insects. The observed correlations between pollinator groups and display size could also relate to differences in energetic needs. The presentation of a large number of flowers on a single plant may render the plant a more attractive resource to a high-energy visitor. This is particularly important for territorial hummingbirds, like those observed in this study, because large display sizes increase the energy obtained per guarded plant.

FLORAL TRAITS THAT DO NOT CORRELATE WITH POLLINATORS

In contrast to the strong relationship between reward and display and pollinators, we found little evidence for correlations between any group of pollinators and corolla length or flower color. Although the mostly insect-pollinated species tended to be small (e.g., *I. umbellatum*, *A. arborescens*), some insect-pollinated species (*I. ellipticum*) have longer flowers than some hummingbird-pollinated species. Also, there is substantial corolla length variation among the mostly hummingbird-pollinated species, from the small *I. edule* to the very long *I. cornifolium* (Fig. 1). A similar lack of correlation is observed between pollination system and flower color. Lepidopterans tended to be associated with white-flowered species but also pollinated red and purple flowers. Dipterans visited white- and purple-flowered species, and hymenopterans visited all colors. Interestingly, hummingbirds pollinated flowers with a wide array of colors, including classic "bee" colors such as blue and yellow (Fig. 1). These results contrast with the close association between color and pollinator identity found in some taxa (e.g., Schemske and Bradshaw 1999; Wilson et al. 2004) but corroborate the findings of several ecological surveys of plant–pollinator interactions in the tropics (Feinsinger 1976; Snow and Snow 1980; Momose et al. 1998; Dziedzioch et al. 2003).

The lack of a consistent correlation between either corolla length or flower color and pollination mode has several possible explanations: (1) these traits do not represent adaptations for different pollination systems (nonadaptation), (2) these traits do represent adaptations, but shifts in pollination syndrome have been frequent relative to the rate of corolla length and color evolution (nonequilibrium), or (3) these traits are evolving in response to factors other than (or in addition to) simple pollinator group identity, for example community composition. We will consider how well each of these hypotheses, in turn, explains the observed patterns of variation in *Iochroma*.

Nonadaptation

The adaptive significance of corolla length variation has been extensively investigated at both the micro- and macroevolutionary scales. Intraspecific studies have demonstrated pollinator-mediated selection on corolla length (Nilsson 1988; Galen and Cuba 2001; Engel and Erwin 2003) whereas a growing number of macroevolutionary studies have tied differences in corolla length among species to differences in pollination system (Grant and Temeles 1992; Whittall and Hodges 2007). Also, the rapid evolutionary diminution of corolla size in selfing lineages (Ornduff 1969; Wyatt 1988) underscores the presumed cost of building large corollas and the importance of pollinators as selective agents on floral morphology. Considering the energetic cost associated with large corollas and evidence of an interaction between flower depth and pollinator mouthpart length (Nilsson 1988; Schemske and Horvitz 1989), it is hard to imagine that corolla length is not primarily evolving by selection.

Because of its role in signaling, flower color has also been seen as an important trait in plant–pollinator relationships. Many studies have reported associations between pollination mode and particular flower colors (Grant and Grant 1965; Melendez-Ackermann et al. 1997; Wilson et al. 2004). These associations have often been attributed to an innate ability of particular animal groups (e.g., bees, birds) to more easily distinguish certain colors (Raven 1972; Bleiweiss 1990), although such functional explanations for flower color diversity remain the subject of debate (Chittka and Waser 1997). Unlike the case of corolla tubes, however, the energetic costs of a "mismatching" color may be minimal, and thus nonadaptive evolution is a possible explanation for a lack of correlation between pollinator group and color in *Iochroma*.

Nonequilibrium

Another explanation for the lack of a significant correlation between pollination groups and flower color or tube length is phylogenetic inertia. That is, shifts in pollination system cause changes in the selective regime acting on flower color and tube length, but these traits have been slow to respond to repeated and/or recent pollinator shifts. This hypothesis would predict that color and tube length would show stronger phylogenetic autocorrelation than pollination system.

Corolla length had a lower QVI (0.55; $P = 0.03$), in other words, greater phylogenetic autocorrelation, than pollinator importance values (0.78–1.00; $P = 0.26$–0.99). This implies that corolla length tends to evolve more slowly than pollinator identity. This may seem at odds with quantitative genetic studies that have demonstrated significant heritable variation in corolla length, implying great evolvability of this trait, in a wide variety of plant taxa (Conner and Via 1993; Mitchell and Shaw 1993; Caruso 2004). However, there could be less genetic variation in corolla length in *Iochroma* populations and/or the genetic architecture in *Iochroma* could be such that genes influencing corolla length tend to have pleiotropic effects, resulting in a reduced rate of adaptive transitions. Consequently, it remains plausible that the weak correlation between corolla length and pollinator identity could be due to a delay in the response of floral morphology to a rapidly changing pollination environment.

Color evolution, on the other hand, cannot easily be explained by phylogenetic inertia. Flower color variables (chroma, hue, brightness) had mean QVI measures that were nearly as high as those for pollinator identity and that were statistically indistinguishable from those expected by chance. This suggests that color is sufficiently labile to produce a response to a change in the selection regime that might accompany a pollinator shift. Thus, the lack of an association between flower colors and pollinator groups is not well explained by phylogenetic constraints on flower color.

Alternative drivers of adaptation

The lack of association between floral traits and pollination mode has often led to the exploration of alternate selective agents (reviewed in Strauss and Whittall 2006). In the case of corolla length many other selective factors may have contributed to interspecific differences in *Iochroma*. For example, larger flowers may be more likely to be attacked by herbivores (Ashman et al. 2004), and plants with longer corollas experience increased nectar robbing (Lara and Ornelas 2001; Urcelay et al. 2006). Although *Iochroma* flowers are not typically consumed by herbivores, they do suffer from nectar-robbing by bees and flower-piercing birds (S. D. Smith, pers. obs). Thus, although the corolla may need to be sufficiently long to hold an attractive reward, the benefit of additional increases in corolla length may vary depending on the composition and abundance of the nectar robber guild. It is noteworthy that the two longest species, *I. cornifolium* and *I. calycinum*, appear to have evolved additional protection from nectar-robbers in the form of greatly inflated calyces (Fig. 1).

Selective forces other than pollinators may also affect flower color evolution. Several authors have suggested that differences in flower color within and between species may be influenced by indirect selection because many flower pigments are derived from the flavonoid pathway, which also produces compounds important for defense and UV protection. For example, Armbruster (1996, 2002) found that although blossom color in *Dalechampia* did not covary with pollinators, the species with pigmented blossoms tended to also have pigmented vegetative tissue. Thus, he suggested that colored blossoms may have evolved as an indirect response to selection for pigmented stems and leaves. *Iochroma* species do not produce the same pigments in vegetative tissue as in the corolla (S. Smith, unpubl. data), making this hypothesis an unlikely explanation for its radiation of flower colors.

Community-level factors are another potential selective agent. Grant (1966) proposed that the convergence of North American hummingbird-pollinated species on red coloration is a form of Müllerian mimicry, related to the birds' migratory lifestyle. By sharing a common signal (red flowers), the migrating birds quickly learn to associate the color with reward and do not have to learn new signals as they move to new areas. In Central and South America where hummingbirds are resident, she suggested that such selection for a shared signal would be absent, and that a wider diversity of colors would be found among the hummingbird-pollinated species. Subsequent studies have largely supported this prediction (Feinsinger 1976; Snow and Snow 1980; Dziedzioch et al. 2003; but see Stiles 1975), suggesting that selection for local convergence among hummingbird flowers may be relaxed when hummingbirds are resident. This raises the question: Could community-level factors actively drive divergence?

In the case of *Iochroma*, the cooccurrence of hummingbird-pollinated taxa and the variety of colors present in these taxa raise the possibility of competitive interactions as drivers of diversifying selection. The principally hummingbird-pollinated iochromas, which comprise most of the flower color diversity (Fig. 1), typically occur in mid- to high-elevation Andean communities, often containing multiple hummingbird-pollinated taxa (e.g., members of *Fuchsia*, *Macleania*, *Salvia*) and in some areas, multiple species of *Iochroma*. In such mixed communities, successful reproduction is aided by the targeted movement of pollen between members of the same species, which will be facilitated by the presence of distinct signals. Indeed, individual hummingbirds were found to exhibit markedly biased patterns of visitation toward particular *Iochroma* species in areas of sympatry (S. D. Smith, S. J. Hall, P. R. Izquierdo, and D. A. Baum, unpubl. ms.). It is, therefore, plausible that color differences facilitate resource partitioning and the resulting assortative pollen targeting.

Conclusions

Although we have considered the various factors that may have influenced flower color and corolla length evolution independently, it is certainly possible that several factors are at play. 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). Although we cannot rule out the possibility of

nonadaptive flower color evolution, the patterns of diversification seem most easily explained by community-level selection for diversified signals, particularly among the species that share hummingbird pollinators. The latter hypothesis can potentially be examined by testing the importance of color in assortative pollen transfer and by surveying flower color diversity in communities containing *Iochroma* species.

This study, along with many others (Herrera 1996; Ollerton 1996; Waser et al. 1996; Waser 2001), suggests that the role of pollination syndromes in floral diversification is in need of ongoing reassessment. We have shown that *Iochroma* does not fit the patterns predicted by the classical pollination syndromes because only a few traits (reward and display) can be directly associated with selection by functional groups of pollinators whereas other traits (size and color) cannot. A full understanding of floral diversity will require that we move away from viewing a flower as a set of well-atomized traits that have each been optimized by selection for current pollinators, and toward a view that includes the possibility of functional trade-offs, nonadaptive evolution, phylogenetic inertia and complex interactions among cooccurring plant species and the animals with which they live. Because of their relative ease of study and the occurrence of both allopatric and sympatric species, *Iochroma* may prove to be an excellent system in which to further explore these interesting topics.

ACKNOWLEDGMENTS

The authors would like to thank J. W. Boughman, A. R. Ives, T. J. Givnish, B. Larget, and K. J. Sytsma for discussion; M. A. Streisfeld, P. Wilson, J. B. Whittall, and associate editor Jon Ågren for useful comments on the manuscript; and T. J. Givnish for use of his spectrometer. The authors acknowledge financial support from the National Science Foundation grant DEB-0309310 and the UW Department of Botany O. N. Allen Memorial Fund.

LITERATURE CITED

Ackerly, D. D., and M. J. Donoghue. 1998. Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). Am. Nat. 152:767–791.

Aigner, P. A. 2001. Optimality modeling and fitness trade-offs: when should plants become pollinator specialists? Oikos 95:177–184.

———. 2005. Variation in pollination performance gradients in a *Dudleya* species complex: can generalization promote floral divergence? Funct. Ecol. 19:681–689.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Control AC-19:716–723.

Altshuler, D. L. 2003. Flower color, hummingbird pollination, and habitat irradiance in four Neotropical forests. Biotropica 35:344–355.

Altshuler, D. L., R. Dudley, and J. A. McGuire. 2004. Resolution of a paradox: hummingbird flight at high elevation does not come without a cost. Proc. Natl. Acad. Sci. USA 101:17731–17736.

Armbruster, W. S. 1993. Evolution of plant pollination systems—hypotheses and tests with the neotropical vine *Dalechampia*. Evolution 47:1480–1505.

———. 1996. Evolution of floral morphology and function: an integrated approach to adaptation, constraint, and compromise in *Dalechampia* (Euphorbiaceae). Pp. 241–272 *in* D. Lloyd and S. Barrett, eds. Floral biology. Chapman & Hall, New York, NY.

———. 2002. Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. J. Evol. Biol. 15:468–486.

Ashman, T-L., D. H. Cole, and M. Bradburn. 2004. Sex-differential resistance and tolerance to herbivory in a gynodioecious wild strawberry. Ecology 85:2550–2559.

Bleiweiss, R. 1990. Spectral confusion by hummingbirds and the evolution of red coloration in their flowers: a new hypothesis. Wisc. Acad. Sci. Arts. Lett. 78:33–38.

Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal and comparative date: behavioral traits are more labile. Evolution 57:717–745.

Brody, A. K., and R. J. Mitchell. 1997. Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. Oecologia 110:86–93.

Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. Am. Nat. 164:683–695.

Caruso, C. M. 2004. The quantitative genetics of floral trait variation in *Lobelia*: potential constraints on adaptive evolution. Evolution 58:732–740.

Castellanos, M. C., P. Wilson, and J. D. Thomson. 2004. 'Anti-bee' and 'pro-bird' changes during the evolution of hummingbird pollination in *Penstemon* flowers. J. Evol. Biol. 17:876–885.

Chittka, L., and N. M. Waser. 1997. Why red flowers are not invisible to bees. Israel J. Pl. Sci. 45:169–183.

Conner, J., and S. Via. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. Evolution 47:704–711.

Cresswell, J. E., and C. Galen. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations—the pollination of *Polemonium viscosum*. Am. Nat. 138:1342–1353.

Dafni, A. 1992. Pollination ecology: a practical approach. Oxford Univ. Press, Oxford.

Dressler, R. L., W. M. Whitten, and N. H. Williams. 2004. Phylogenetic relationships of *Scaphyglottis* and related genera (Laeliinae:Orchidaceae) based on nrDNA ITS sequence data. Brittonia 56:58–66

Dziedzioch, C., A. D. Stevens, and G. Gottsberger. 2003. The hummingbird plant community of a tropical montane rain forest in southern Ecuador. PLOS Biol. 5:331–337.

Endler, J. A. 1990. On the measurement and classification of color in studies of animal color patterns. Biol. J. Linn. Soc. 41:315–352.

Engel, E. C., and R. Irwin. 2003. Linking pollinator visitation rate and pollen receipt. Am. J. Bot. 90:1612–1618.

Faegri, K., and L. Van Der Pijl. 1966. The principles of pollination ecology. Pergamon Press, New York.

Farris, J. S. 1989. The retention index and homoplasy excess. Syst. Zool. 38:406–407.

Feinsinger, P. 1976. Organization of a tropical guild of nectarivorous birds. Ecol. Monogr. 46:257–291.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Ann. Rev. Ecol. Syst. 35:375–403.

Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine skypilot, *Polemonium viscosum*. Evolution 43:882–890.

Galen, C., and J. Cuba. 2001. Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. Evolution 55:1963–1971.

Galloway, L. F., T. Cirigliano, and K. Gremski. 2002. The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. Int. J. Plant Sci. 163:133–139.

Garland, T., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. Syst. Biol. 42:265–292.

Goldblatt, P., and J. C. Manning. 2006. Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. Ann. Bot. 97:317–344.

Grafen, A. 1989. The phylogenetic regression. Phil. Trans. R. Soc. Lond. B 326:119–157.

Grant, K. A. 1966. A hypothesis concerning the prevalence of red coloration in California hummingbird flowers. Am. Nat. 100:85–97.

Grant, V., and K. A. Grant. 1965. Flower pollination in the Phlox family. Columbia Univ. Press, New York, NY.

Grant, V., and E. J. Temeles. 1992. Foraging ability of rufous hummingbirds on hummingbird flowers and hawkmoth flowers. Proc. Natl. Acad. Sci. USA 89:9400–9404.

Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. Evolution 51:1341–1351.

Hapeman, J. R., and K. Inoue. 1997. Plant-pollinator interactions and floral radiation in *Platanthera* (Orchidaceae). Pp. 433–454 *in* T. J. Givnish and K. J. Sytsma, eds. Molecular evolution and adaptive radiation. Cambridge Univ. Press, Cambridge.

Harrison, C. J., M. Moller, and Q. C. B. Cronk. 1999. Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. Ann. Bot. 84:49–60.

Heinrich, B., and P. H. Raven. 1972. Energetics and pollination ecology. Science 176:597–602.

Herrera, C. M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. Pp. 88–111 *in* D. Lloyd and S. C. H. Barrett, eds. Floral biology. Chapman and Hall, New York, NY.

Hunziker, A.T. 2001. Genera of Solanaceae. A. R. G. Ganter Verlag K. G., Konigstein, Germany.

Ives, A. R, and H. C. J. Godfray. 2006. Phylogenetic analysis of trophic associations. Am. Nat. 168:E1–E14.

Johnson, S. D., and K. E Steiner. 2000. Generalization versus specialization in plant pollination systems. Trends Ecol. Evol. 15:140–143.

Johnson, S. D., H. P. Linder, and K. E. Steiner. 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). Am. J. Bot. 85:402–411.

Kay, K. M., and D.W. Schemske. 2003. Pollinator assemblages and visitation rates for 11 species of Neotropical *Costus*. Biotropica 35:198–207.

Kay, K. M., P. A. Reeves, R. G. Olmstead, and D.W. Schemske. 2005. Rapid speciation and the evolution of hummingbird pollination in neotropical *Costus* subgenus *Costus* (Costaceae): evidence from nrDNA ITS and ETS sequences. Am. J. Bot. 92:1899–1910.

Kohn, J. R., and N. M. Waser. 1985. The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. Am. J. Bot. 72:1144–1148.

Lara, C., and J. F. Ornelas. 2001. Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. Oecologia 128:263–273.

Leiva, S. 1995. Una nueva especie de *Iochroma* (Solanaceae: Solaneae) del norte del Perú. Arnaldoa 3:41–44.

Leiva, S., V. Quipuscoa S., and N. W. Sawyer. 1998. *Iochroma stenanthum* (Solanaceae: Solaneae), una nueva especie del norte de Perú. Arnaldoa 5:77–82.

Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. Am. Nat. 149:646–667.

McCullagh, P., and J. A. Nelder. 1989. Generalized linear models. Chapman and Hall, London.

Melendez-Ackerman, E., D. R. Campbell, and N. M. Waser. 1997. Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. Ecology 78:2532–2541.

Mitchell, R. J., and R. G. Shaw. 1993. Heritability of floral traits for the perennial wild flower *Penstemon cetranthifolius* (Scrophulariaceae)—clones and crosses. Heredity 71:185–192.

Momose, K., T. Yumoto, T. Nagamitsu, M. Kato, H. Nagamasu, S. Sakai, R. D. Harrison, T. Itioka, A. A. Hamid, and T. Inoue. 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. Am. J. Bot. 85:1477–1501.

Muchhala, N. 2003. Exploring the boundary between pollination syndromes: bats and hummingbirds as pollinators of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae). Oecologia 134:373–380.

———. 2007. Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. Am. Nat. 169:494–504.

Nilsson, L. A. 1988. The evolution of flowers with deep tubes. Nature 334:147–149.

Ollerton, J. 1996. Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. J. Ecol. 84:767–769.

Ord, T. J., and E. P. Martins. 2006. Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behavior. Anim. Behav. 71:1411–1429.

Ornduff, R. 1969. Reproductive biology in relation to systematics. Taxon 18:121–133.

Ornelas, J. F., M. Ordano, A. J. de Nova, M. E. Quintero, and T. Garland. 2007. Phylogenetic analysis of interspecific variation in nectar of hummingbird-visited plants. J. Evol. Biol. 20:1904–1917.

Paradis, E., J. Claude and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 17:662–663.

Patterson, T. B., and T. J. Givnish. 2004. Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): evidence from a cpDNA phylogeny. New Phytol. 161:253–264.

Perez, F., M. T. K. Arroyo, R. Medel, and M. A. Hershkovitz. 2006. Ancestral reconstruction of flower morphology and pollination systems in *Schizanthus* (Solanaceae). Am. J. Bot. 93:1029–1038.

R Development Core Team. 2005. R: a language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria.

Raven, P. H. 1972. Why are bird-visited flowers predominantly red? Evolution 26:674.

Schemske, D. W. 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. Ecology 62:946–954.

Schemske, D. W., and H. D. Bradshaw, Jr. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. USA 96:11910–11915.

Schemske, D. W., and C. C. Horvitz. 1989. Temporal variation in selection on a floral character. Evolution 43:461–465.

Schwilk, D. W., and D. D. Ackerly. 2001. Flammability and serotiny as strategies: correlated evolution in pines. Oikos 94:326–336.

Shaw, J. M. H. 1998. *Iochroma*—a review. New Plantsman 5:153–191.

Silander, J. A., and R. B. Primack. 1978. Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). Am. Midl. Nat. 100:213–216.

Smith, S. D. 2006. Floral diversification and pollination biology of the Andean clade Iochrominae (Solanaceae). Ph.D. Thesis, Univ. of Wisconsin, Madison.

Smith, S. D., and D. A. Baum. 2006. Phylogenetics of the florally diverse Andean clade Iochrominae (Solanaceae). Am. J. Bot. 93:1140–1153.

Snow, A. A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. Oecologia 55:231–237.

Snow, D. W., and B. K. Snow. 1980. Relationships between hummingbirds and flowers in the Andes of Colombia. Bull. Br. Mus. Nat. Hist. Zool. 38:105–139.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. W. H. Freeman and Co., New York.

Stebbins, G. L. 1970. Adaptive radiation of reproductive characters in angiosperms I: pollination mechanisms. Ann. Rev. Ecol. Syst. 1:307–326.

Strauss, S., and J. B. Whittall. 2006. Non-pollinator agents of selection on floral traits. Pp. 120–138 *in* L. Harder and S. Barrett, eds. Ecology and evolution of flowers. Oxford Univ. Press, Oxford.

Streisfeld, M. A., and J. R. Kohn. 2007. Environment and pollinator-mediated selection on parapatric floral races of *Mimulus aurantiacus*. J. Evol. Biol. 20:122–132.

Stiles, F. G. 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. Ecology 56:285–301.

———. 1976. Taste preferences, color preferences, and flower choice in hummingbirds. Condor 78:10–26.

Thompson, J. D. 2001. How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? Oecologia 126:386–394.

Urcelay, C., C. L. Morales, and V. R. Chalcoff. 2006. Relationship between corolla length and floral larceny in the South American hummingbird-pollinated *Campsidium valdivianum* (Bignoniaceae). Ann. Bot. Fennici 43:205–211.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Springer, New York.

Waser, N. M. 2001. Pollinator behavior and plant speciation: looking beyond the "ethological isolation" paradigm. Pp. 318–335 *in* L. Chittka and J. D. Thomson, eds. Cognitive ecology of pollination: animal behavior and floral evolution. Cambridge Univ. Press, Cambridge.

Waser, N. M., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology 77:1043–1060.

Whittall, J. B., and S. A. Hodges. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. Nature 447:406–409.

Whittall, J. B., C. Voelckel, D. J. Kliebenstein, and S. A. Hodges. 2006. Convergence, constraint and the role of gene expression during adaptive radiation: floral anthocyanins in *Aquilegia*. Mol. Ecol. 15:4645–4657.

Wilson, P., and J. D. Thomson. 1996. How do flowers diverge? Pp. 88–111 *in* D. Lloyd and S. C. H. Barrett, eds. Floral biology. Chapman and Hall, New York, NY.

Wilson, P., M. C. Castellanos, J. N. Hogue, J. D. Thomson, and W. S. Armbruster. 2004. A multivariate search for pollination syndromes among penstemons. Oikos 104:345–361.

Wyatt, R. 1988. Phylogenetic aspects of the evolution of self-pollination. Pp. 109–131 *in* L. D. Gottlieb and S. K. Jain, eds. Plant evolutionary biology. Chapman and Hall, London, UK.

Associate Editor: J. Ågren

Appendix

Ornstein–Uhlenbeck (OU) models of trait evolution were first developed by Hansen (1997), and have been incorporated into a variety of comparative methods (e.g., Blomberg et al. 2003; Butler and King 2004). Here we used OU models that included the root state as a parameter instead of assuming equilibrium at the root, so that OU with $\alpha = 0$ corresponds to Brownian motion (BM) exactly. In other words, the covariance of a trait between two species separated by a distance d_{ij} and sharing time t_{ij} from the root to their common ancestor was uniformly taken to be $\sigma^2 V_\alpha(i,j)$ where

$$V_\alpha(i,j) = e^{-\alpha d_{ij}} * (1 - e^{-2\alpha t_{ij}})/2\alpha$$

(Hansen 1997). With small α, $e^{-\alpha} d_{ij}$ is about 1 and the dominant term is $(1 - e^{-2\alpha} t_{ij})/2\alpha$, which is about t_{ij}, the amount of branch length shared by the two species, as in the BM model. With large α, the dominant term is $e^{-\alpha} d_{ij}$, making distantly related species largely independent. As α approaches infinity, the OU model reduces to a nonphylogenetic TIPS model. In this study, we sought to explore intermediate models between BM and TIPS model. The appropriate values of α to serve as intermediate points depend on branch lengths in the phylogeny because α appears as a multiplicative factor to path lengths d_{ij} and t_{ij}. Values for d_{ij} and t_{ij} among the sampled taxa range from 0.01 to 0.04 (in terms of average substitutions per site). Thus, values of α under 5 produce results very similar to BM because e^{-5d} and $e^{-5\times 2t}$ are close to 1, whereas values of α above 200 quickly approach the TIPS model because e^{-200d} is almost zero. Values of 10 and 100 were selected as intermediates in our study.

Using the phylogenetic covariance matrix obtained with the above formula, traits were linearly transformed to remove phylogenetic correlation, as in PGLS (Martins and Hansen 1997). Pairwise and partial correlations were obtained based on the transformed traits. In the BM model ($\alpha = 0$), the results are equivalent to those using independent contrasts. The PGLS transformation was preferred over independent contrasts because it generalizes to the OU model and provides a likelihood framework to assess the fit of each model. Partial correlations were obtained from multiple linear regressions with the signs derived from the regression coefficients. The maximum log-likelihood of a regression model assuming phylogenetic covariance matrix V_α is

$$\log L = -\frac{n}{2} - \frac{n \log(2\pi\hat{\sigma}^2)}{2} - \frac{\log(\det(V_\alpha))}{2},$$

where n is the number of species and $\hat{\sigma}$ is the ML estimate of the variance component. The log likelihood obtained from the linear regression on the PGLS-transformed traits in R includes the first two terms only, so the last term, which depends on α only, was added to it. We used these likelihood values to calculate AIC scores (Akaike 1974) for use in model selection where

$$AIC = -2 \ln L + 2p,$$

and p is the number of free parameters in the model, which here included the effects of the predictors and the intercept. A script written for the R program, which calculates the single and multiple correlations with varying α values and performs AIC model selection is available from the authors upon request.

Supplementary Material

The following supplementary material is available for this article:

Figure S1. Standardized reflectance curves for study taxa.
Table S1. Pairwise correlations between pollinator groups and floral traits.
Table S2. Partial correlation coefficients for individual traits with pollinator groups from multivariate analyses.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1558-5646.2007.00327.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.