



COMPETITION FOR HUMMINGBIRD POLLINATION SHAPES FLOWER COLOR VARIATION IN ANDEAN SOLANACEAE

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One classic explanation for the remarkable diversity of flower colors across angiosperms involves evolutionary shifts among different types of pollinators with different color preferences. However, the pollinator shift model fails to account for the many examples of color variation within clades that share the same pollination system. An alternate explanation is the competition model, which suggests that color divergence evolves in response to interspecific competition for pollinators, as a means to decrease interspecific pollinator movements. This model predicts color overdispersion within communities relative to null assemblages. Here, we combine morphometric analyses, field surveys, and models of pollinator vision with a species-level phylogeny to test the competition model in the primarily hummingbird-pollinated clade *Lochrominae* (Solanaceae). Results show that flower color as perceived by pollinators is significantly overdispersed within sites. This pattern is not simply due to phylogenetic history: phylogenetic community structure does not deviate from random expectations, and flower color lacks phylogenetic signal. Moreover, taxa that occur in sympatry occupy a significantly larger volume of color space than those in allopatry, supporting the hypothesis that competition in sympatry drove the evolution of novel colors. We suggest that competition among close relatives may commonly underlie floral divergence, especially in species-rich habitats where congeners frequently co-occur.

KEY WORDS: Color vision, interspecific pollen transfer, phenotypic community structure, phylogenetic community structure, phylogenetic signal, reproductive character displacement, signal evolution.

Flower color diversity is one of the most striking features of the angiosperm radiation. Due to variation in pigment production (Tanaka et al. 2008) and cellular structure (Vignolini et al. 2012), flowers can present colors ranging across the UV and visible spectrum (Chittka et al. 1994) and differing in intensity from pale to nearly black (Avishai and Zohary 1980). Moreover, flower color appears to be one of the most evolutionarily labile traits, often differing between sister species (Wesselingh and Arnold 2000; Martén-Rodríguez et al. 2010) or among populations of the same species (Streisfeld and Kohn 2007; Cooley et al. 2011).

Evolutionary transitions in flower color are often attributed to pollinator-mediated selection. One classic explanation for how

pollinators might drive color evolution is the pollinator shift model (Fenster et al. 2004; Whittall and Hedges 2007). Due to differences in preferences, different functional groups of pollinators may select for different flower colors, for example, red for hummingbirds, white for hawkmoths, and blue/purple for bees (Faegri and van der Pijl 1979; Fenster et al. 2004; Whittall and Hedges 2007). Thus, spatiotemporal variation in pollinator assemblages that alters the principal pollinator could drive a shift in color (Stebbins 1970; Campbell 2008; Thomson and Wilson 2008; Kay and Sargent 2009). However, many floral radiations exhibit a remarkable variety of colors despite members sharing the same functional group of pollinators (Armbruster 2002; Cooley et al.

2008; Smith et al. 2008b; Paget-Seekins 2012), suggesting that the pollinator shift model does not fully account for the diversity of colors across angiosperms.

An alternative model involving competition for pollinators can account for color divergence even in the absence of shifts among functional groups of pollinators. When plants co-occur and flower synchronously, fitness can be reduced through competition for pollinator service (Waser 1978; Morales and Traveset 2008; Mitchell et al. 2009). Pollinators that visit multiple flower species can transfer pollen between species, reducing a given plant's fitness when its own pollen is lost during visits to other species (pollen misplacement, Muchhala and Thomson 2012) and/or when foreign pollen arrives on its stigmas (heterospecific pollen deposition, Morales and Traveset 2008). The cost of foreign pollen deposition can be especially high for closely related species, as this pollen is more likely to waste maternal resources through the production of inviable or lower fitness hybrid offspring (Harder et al. 1993; Burgess et al. 2008). Flower color divergence could alleviate competitive costs if it encourages individual or species-level specialization by pollinators on different plant species (Chittka et al. 1999; Gegeard and Laverty 2005; Oyama et al. 2010). A complete shift in pollinator type would eliminate these costs, but even without pollinator shifts, any trait divergence which decreases interspecific pollinator movements will be favored.

One predicted outcome of competitive interactions is phenotypic overdispersion (also termed evenness), wherein co-occurring species are more different than expected due to chance (Webb et al. 2002; Dayan and Simberloff 2005; Kursar et al. 2009). Two processes can contribute to this pattern. First, species that are too similar to existing species may be less able to establish local populations, resulting in communities whose members have divergent phenotypes (termed "ecological sorting"). Second, co-occurring species may evolve differences to minimize competition (termed "character displacement"). Along with favoring phenotypic overdispersion, strong competitive interactions might also be expected to lead to phylogenetic overdispersion because closely related species tend to be phenotypically similar (Sargent and Ackerly 2008; Cavender-Bares et al. 2009; Burns and Strauss 2011). The observation of greater similarity in trait values among closely related species is termed phylogenetic signal and has been documented for a wide range of traits (Blomberg et al. 2003). One potential challenge for studies of competitive interactions is that, for communities that display phylogenetic overdispersion, any aspect of phenotype that has a strong phylogenetic signal will also be overdispersed (Eaton et al. 2012). In this case it becomes difficult to disentangle traits that are actually responding to competition from those that are simply tracking the pattern of phylogenetic overdispersion. Thus, a thorough exploration of the role of competition in driving trait divergence requires analyzing

phenotypic and phylogenetic patterns of community assembly as well as phylogenetic signal for the focal traits.

Despite strong evidence from ecological studies for competitive interactions among co-flowering species (Morales and Traveset 2008), relatively few studies have applied community phylogenetic approaches to test for overdispersion of floral traits (but see McEwen and Vamosi 2010; De Jager et al. 2011; Eaton et al. 2012). These studies focused on temperate communities comprising mostly or entirely bee-pollinated taxa, and provided mixed support for a role of competition in driving local flower color differences, with only one finding evidence for color overdispersion within sites (McEwen and Vamosi 2010). In the present study, we apply community phylogenetic approaches to explore the role of competition in the evolution of color diversity in *Iochrominae* (Solanaceae), a radiation of Andean shrubs principally pollinated by hummingbirds, with lesser contributions by bees and other insects (Smith and Baum 2006). The roughly 33 species of *Iochrominae* present wide variation in flower color (Fig. 1), and changes in flower color are not related to shifts between pollinators (Smith et al. 2008a). The failure of these species to converge on a single color that might be best suited to attract hummingbirds suggests that other factors, such as interspecific competition for pollinators, may have driven color divergence. Moreover, the extensive overlap of pollinator assemblages among sympatric taxa (Smith et al. 2008b) and the potential for producing low-fitness offspring through hybridization (Smith and Baum 2007) suggest that selection for divergent signals in co-occurring taxa may be strong.

Here, we test the competition model of floral diversification by combining morphometric analyses, surveys of species co-occurrence, and models of pollinator color vision with a robust, species-level phylogenetic framework. Although bee vision models have often been employed in studies of flower color variation (e.g., Dyer et al. 2012; Papadopoulos et al. 2013), the implications of the hummingbird visual system for the ecology and evolution of hummingbird-pollinated flowers have yet to be explored. Building on the recently developed model for hummingbird vision (Herrera et al. 2008), we address the following specific questions: (1) Do co-occurring species differ more in flower color (as perceived by hummingbirds and bees) within communities than expected by chance? (2) Do patterns of color variation across communities differ from those for vegetative traits not hypothesized to be linked to competitive interactions? (3) Could the patterns of trait variation across communities be explained by phylogenetic history (phylogenetic signal and phylogenetic community structure) rather than competition? (4) Of the 33 species in the clade, do the subset that co-occur with other clade members (19 species) occupy a larger portion of color space than the subset that only occur in allopatry? If community assembly is shaped by competition for pollinators, we predict that co-occurring species will

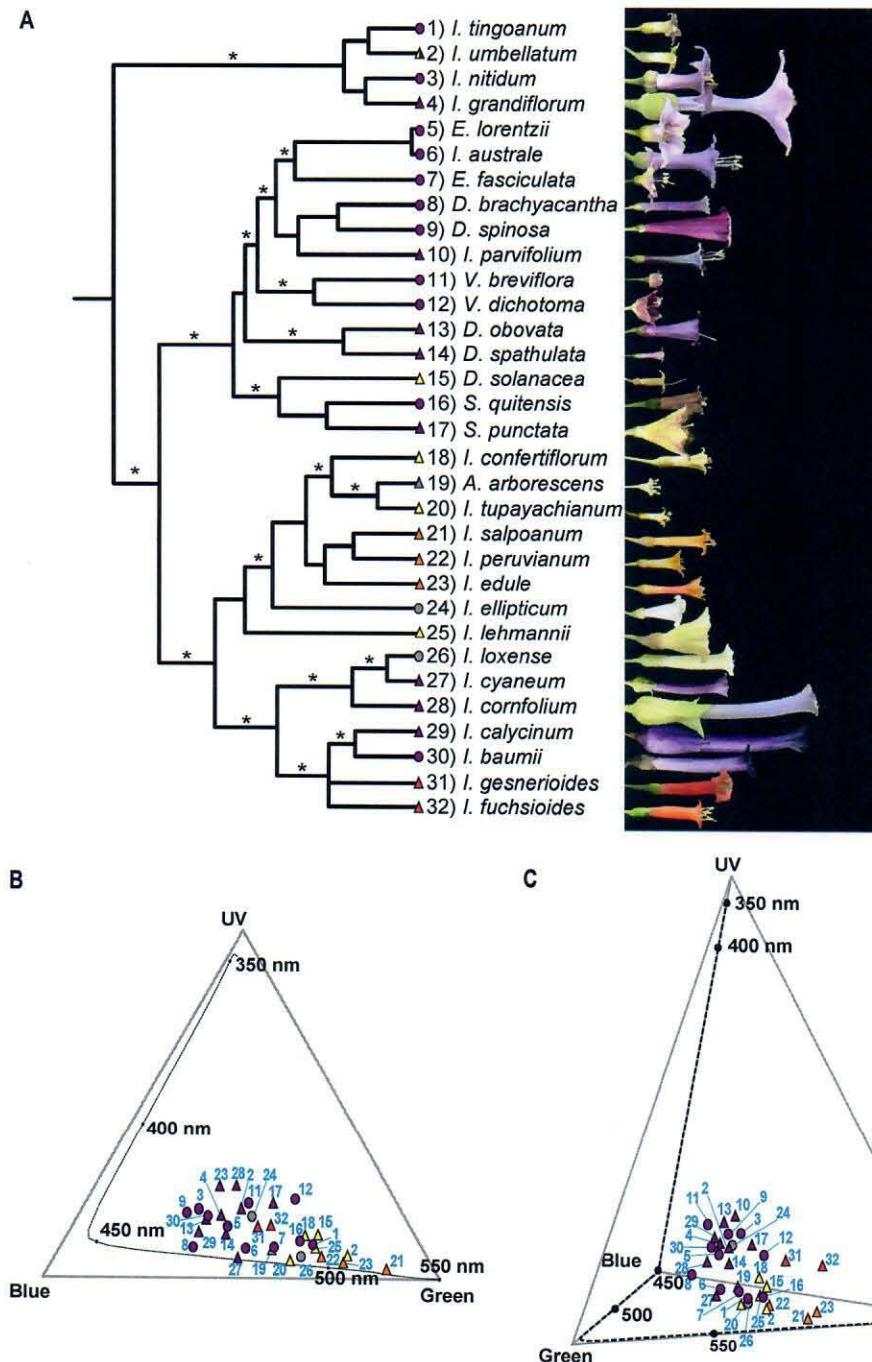


Figure 1. Flower colors and phylogenetic relationships of lochromineae. Triangles correspond to lochromineae species that are known to co-occur with other lochromineae, and circles correspond to species not known to co-occur. (A) Three-gene phylogeny of lochromineae based on Bayesian analysis, with corresponding flowers and the species numbers used in other panels of this figure. Asterisks indicate branches with greater than 95% posterior probability. Abbreviations for genera are as follows: A. = *Acnistus*, E. = *Eriolarynx*, D. = *Dunalia*, I. = *Lochroma*, S. = *Saracha*, and V. = *Vassobia*. (B) and (C) The flower color for each lochromineae species plotted in honey bee (triangle) and hummingbird (tetrahedron) color spaces, respectively. *Lochroma umbellatum* (taxon 2) is represented by two points as it is polymorphic. Vertices (corners) are labeled with the photoreceptor they represent (three for honey bees and four for hummingbirds), and dashed lines correspond to the position of a pure (monochromatic) beam of light as it moves from 350 to 600 nm. See Figure S3 for a stereoscopic (three-dimensional) version of the hummingbird color space.

show significant differences in flower color within communities beyond that observed in other plant traits and not attributable to phylogeny alone. Moreover, if competition has driven the evolution of novel colors, we predict that the 19 co-occurring species will span a greater range of color space than the allopatric species.

Materials and Methods

STUDY SITES

Iochrominae is a largely Andean clade, whose morphologically diverse species have been divided into seven genera (Fig. 1A). Only two species in the group are found outside the Andes: *I. ellipticum* (endemic to the Galapagos) and *Acnistus arborescens*, which has spread to Central America. Of the remaining species, some are relatively widespread across the Andes with ranges up to 50,000 km², whereas others are narrow endemics with ranges as small as 40 km². The areas of highest species richness and endemicity are Ecuador and northern Peru, where the finely dissected Andean valleys are associated with sharp differences in climate and vegetation even over very short distances. For this study, we aimed to sample all Iochrominae species at least once, and widespread species were generally sampled at many sites. Altogether, we identified 71 sites throughout the Andes (Fig. S1), which contain 32 of the 33 species, and include 32 sites with two to four species and 39 sites with one species (Table S1). Some of these sites are in close proximity (2 km apart), but were considered as separate communities because of their distinct vegetation and species composition. Also, the foraging areas of their most common pollinators (hummingbirds and bees) typically span 0.5 km or less (Dick et al. 2008).

TRAIT MEASUREMENTS

Flower color variation was quantified through measurement of reflectance spectra (Johnsen 2005). We measured reflectance in 0.4 nm intervals from 300 to 800 nm using a spectrometer and UV-VIS light source (JAZPX with a built-in pulsed xenon light source, Ocean Optics, Inc., Dunedin, FL). Spectral measurements were taken on fresh corolla tissue using a probe holder to exclude ambient light and fix the fiber-optic probe at a 45° angle relative to the tissue surface. The probe was aimed at the midpoint of the exterior (abaxial) surface of the tubular corolla. Three flowers randomly chosen from different plants were measured for each species, and the average reflectance was used in subsequent analyses. Measurements were taken in the field for 19 of the 32 included species. The remaining 13 samples were from greenhouse-grown individuals, which had been maintained in a lathehouse to simulate field conditions. Raw reflectance spectra were standardized using a Spectralon white standard (Labsphere, North Sutton, NH). One species, *Iochroma umbellatum*, showed intraspecific variation in flower color (purple in one site, greenish-yellow in others); in this

case, we made separate spectral measurements for each form. For four rare species (*I. nitidum*, *Iochroma peruvianum*, *I. tingoanum*, and *Dunalia obovata*), we used data from a previous study (Smith et al. 2008a), in which the reflectance spectra were measured with field equipment not accurate below 375 nm. In these cases, we used data from 300 to 375 nm from a closely related species with similar color (and a similar reflectance spectrum) to approximate the full spectrum. Iochrominae flowers have very little reflectance in this region (typically 1% to 5% of the total reflectance from 300 to 800 nm), so this approximation should not affect the overall results.

We used two approaches to summarize this spectral information and quantify color differences between species pairs. First, we performed a principal components analysis (PCA) on all standardized reflectance spectra using the stats package in R (R Development Core Team 2011). The first four principal components (PCs), which account for 94% of variation, were used in subsequent analyses. Based on the loading of each wavelength onto each PC (Fig. S2), PC1 corresponds roughly to overall brightness, PC2 to blue versus yellow, PC3 to purple versus all others, and PC4 to the presence or absence of ultraviolet reflectance (300–400 nm). This PCA approach allowed us to examine overall differences in raw flower reflectance spectra, independent of how these differences might be perceived. However, different animals will process and perceive these spectra differently. Thus, our second approach to quantify color used models of the visual systems of hummingbirds and bees to examine how the flower color differences are perceived by their two main pollinators. Hummingbirds have four cones (a type of photoreceptor) used to perceive color, with each specialized to detect a different region of the spectrum: ultraviolet, short wavelength (blue), medium wavelength (green), and long wavelength (red). Bees have only three color photoreceptors: ultraviolet, short wavelength, and medium wavelength (Menzel and Backhaus 1991). Colors are perceived using “opponencies,” or the relative amount of light captured by different photoreceptors (Osorio and Vorobyev 2008). The wavelengths of light captured by each photoreceptor have been modeled for a hummingbird, *Sephanoides sephanioides* (Herrera et al. 2008), and for the honey bee, *Apis mellifera* (Menzel and Backhaus 1991). Using these visual system models, we determined the spectral location of each Iochrominae species in a color space for each pollinator as follows. First, we calculated relative quantum catch (q), or amount of light captured, for each photoreceptor (i) as

$$q_i = Q_i / (\text{sum}(Q_i)),$$

$$\text{where } Q_i = \int_{\lambda} R_i(\lambda) S(\lambda) I(\lambda) d\lambda.$$

In the second equation, λ is the wavelength, $R_i(\lambda)$ is the spectral sensitivity of the photoreceptor, $S(\lambda)$ is the spectral

reflectance of the surface (the corolla), and $I(\lambda)$ is the spectral irradiance of the illuminant (the light environment) (Land and Nilsson 2012). We used these relative quantum catches to plot each Iochrominae species in a color space (or chromaticity map) for each pollinator type. For bees this space is an equilateral triangle, with one primary color at each corner (UV, blue, green), corresponding to stimulation of only one of the three photoreceptors, and white in the center corresponding to equal stimulation of all photoreceptors (Fig. 1B). For hummingbirds this space is three-dimensional (3-D), with each of their primary colors (UV, blue, green, and red) at the corner of a tetrahedron (Fig. 1C). The distance between two points in a color space provides an approximation of the perceived color difference (Endler and Mielke 2005). We performed analyses of flower color differences within co-flowering communities using these distances. However, these distances do not account for the fact that photoreceptor noise can render similar colors indistinguishable (Vorobyev and Osorio 1998). Thus, we repeated the analyses using a visual model that incorporates photoreceptor noise, converting distances into the number of “just noticeable differences” (JNDs) between pairs of spectra (Vorobyev and Osorio 1998).

In addition to color, we obtained measurements for five morphological characters for each species: corolla tube length, petiole length, pedicel length, leaf length, and berry width. Tube length, like color, can affect plant–pollinator interactions and thus may respond to pollinator-mediated competition. The other four were included to provide a comparison with floral traits. They are not expected to be involved in competitive interactions and thus should not display significant deviation from random assembly patterns. Measurements were gathered from taxonomic literature (Wiggins and Porter 1971; Hunziker 1984; Leiva 1995; Alvarez 1996; Leiva and Quipuscoa 1998; Shaw 1998; Leiva et al. 2003; Smith and Leiva 2011), with midpoint values used in cases where ranges of values were presented. When not available in the literature, measurements were taken from herbarium collections (using the mean of measurements from three different individuals); this included several instances of berry and pedicel lengths as well as all measurements for *Dunalia solanacea*, *Iochroma gesnerioides*, *Eriolarynx fasciculata*, and *I. peruvianum*. The final data matrix for these traits as well as the color measures was uploaded to Dryad (doi:10.5061/dryad.36v4b). These measurements from naturally occurring populations do not account for variation due to environmental plasticity. For the purposes of this study, however, we note that any environmental effects should tend to lead to a pattern of clustering rather than overdispersion within communities, thus would not be expected to bias results toward the predicted pattern of overdispersion.

PHYLOGENETIC INFERENCE

We expanded a previous phylogeny (Smith and Baum 2006) to include *Iochroma tupayachianum*, a recently described species from

northern Peru. Following the protocols in Smith and Baum (2006), three regions were amplified and sequenced: exons 1 through 6 of LEAFY (*LFY*), exons 3 through 8 of the granule bound starch synthase (*waxy*), and the internal transcribed spacer (ITS). For one gene (*LFY*), *I. tupayachianum* exhibited two divergent alleles, suggesting it might be of hybrid ancestry. Following Smith and Baum (2006), we used a Templeton test (Templeton 1983) to compare the most parsimonious tree to a constrained tree with the two *I. tupayachianum* alleles in a clade. As the test was nonsignificant, we concluded that the *LFY* alleles could form a clade (i.e., could belong to a single lineage), and thus we selected a single allele at random for inclusion in the final dataset. Genbank numbers for these sequences are KC290441 (*LFY*), KC243428 (*waxy*), and KC290442 (ITS). *Iochroma tupayachianum* sequences were added to the existing three-gene alignment (Smith and Baum 2006), which corresponds to TreeBase Study No. S1498 and includes 10 outgroup taxa. Phylogenetic relationships were inferred using MrBayes 3.2 (Ronquist et al. 2012). We applied a GTR + G + I model with a relaxed clock to obtain ultrametric trees for subsequent analyses of trait evolution. Two independent chains were run for five million generations, sampling every 1000 generations. Convergence was judged by the average standard deviation of split frequencies (near 0.01), convergence diagnostics (near 1 for all parameters), and the similarity of trees from the independent runs. The postburnin trees were pruned to contain only the 32 taxa with site and morphology information and were subsampled to provide 100 trees for downstream analyses. For the polymorphic species *I. umbellatum*, we included both color morphs in subsequent analyses by splitting its tip on the trees into two branches of near zero length (0.00001).

COMMUNITY STRUCTURE

To test the prediction that co-occurring Iochrominae differ more in flower color than expected by chance (i.e., are overdispersed), we analyzed the phenotypic community structure in R using functions modified from the package *picante* (Kembel et al. 2010). For the 32 sites with multiple taxa, we summarized phenotypic distance between co-occurring species as the mean nearest-taxon distance (MNTD). We compared the actual mean MNTD, averaged over the 32 sites, to the mean MNTDs from 10,000 randomly assembled communities. These null communities were generated using the independent swap algorithm (Gotelli 2000), which shuffles the site by species presence/absence matrix while maintaining row and column totals. This is a conservative null approach that preserves species richness within each site and frequency of occurrence of each species across sites, both important features of the community structure. Because original *picante* functions did not allow inclusion of sites with a single species, we used code modifications kindly provided by D. Grossenbacher to be able to construct nulls using all 71 of our sites. We included these sites because if competition within areas of sympatry favors local

divergence and extreme phenotypes, then we would predict that species that occur alone would have intermediate phenotypes, and thus should contribute to overall patterns (in Table S2, we also present results using only the sites with multiple species). We calculated P values as the proportion of null models with a mean MNTD more extreme than the actual MNTD. For a two-tailed test with a significance level of 0.05, more than 97.5% of the nulls need to be greater than or less than the actual value. We performed these analyses for all traits, although we only predict overdispersion for color traits (and possibly floral tube length) because of their potential role in mitigating competition for pollinators.

To test patterns of phylogenetic community structure, we used a similar approach to that used for phenotypic community structure. For the 32 sites with multiple taxa, we quantified MNTD for phylogenetic distance; that is the branch length between a species and its closest relative in the community. We then compared actual and null distances using steps described previously. To account for phylogenetic uncertainty, we repeated analyses for each of the 100 trees sampled from the Bayesian analysis.

PHYLOGENETIC SIGNAL

To examine whether patterns of trait variation and community assembly could be attributed to phylogenetic history, it is also necessary to quantify phylogenetic signal, or the extent to which phylogenetic relationships predict trait similarity. Here, we estimated Blomberg's K (Blomberg et al. 2003) for each trait. Values of K near zero indicate a lack of phylogenetic signal, with trait values for closely related species being no more similar than those for randomly selected species. Higher values of K indicate increasing phylogenetic signal, and when $K = 1$, the trait exhibits levels of similarity predicted by Brownian motion evolution along the phylogeny. We computed K using the *picante* package in R (Kembel et al. 2010), and tested whether it was significantly different from zero by comparing it to K values for 10,000 null models that randomly shuffle taxa across the tips of the phylogeny (the "phyllosignal" function in *picante*). Following Revell et al. (2007), we also tested whether each K was significantly different from 1.0 by comparing it to K values for 10,000 simulations of Brownian trait evolution along the phylogeny, implemented using the *fastBM* function in the *phytools* package, with K set to 1.0. We repeated all above analyses for the 100 Bayesian trees.

Although useful for single continuous traits, the K statistic cannot be used to measure signal for multidimensional traits such as the color-space distances (see Harmon and Glor 2010). However, these distances can be expressed as pairwise differences between taxa (in a phenotypic distance matrix) to test for correlations with phylogenetic distance matrices using Mantel tests (e.g., Cubo et al. 2005). Significant correlations indicate phylogenetic signal, comparable to obtaining K values significantly greater than 0. We used Mantel tests to calculate correlations between

phenotypic and phylogenetic distance matrices for bee and hummingbird color space as well as for all other traits (i.e., color PCs and the morphological measurements). Analyses were performed with the *mantel.rtest* function in the *ade4* package (Dray and Dufour 2007), using 1000 permutations to test for significance, and repeated for the 100 trees.

CONVEX HULL ANALYSIS OF COLOR SPACE

We used null models to test the hypothesis that the subset of *Iochrominae* species that co-occur with other clade members (the 19 sympatric species) occupy a larger region of color space than those that occur alone (the 13 allopatric species). The following analysis was performed separately for hummingbird and bee color space. We plotted species in color space (two-dimensional for bees and 3-D for hummingbirds), treating the two morphs of *I. umbellatum* as separate points (thus there are 20 sympatric points), and calculated the convex hull around each of the two groups of points using the *geometry* package in R. For null models, we pooled all 33 species/morphs and created 10,000 nulls in R which randomly drew a group of 13 and a group of 20 from these. We then tested whether the actual difference between hull volume of sympatric and allopatric species in nature is significantly greater than the same difference in the null models.

Results

PHYLOGENETIC INFERENCE

Phylogenetic analyses revealed that the recently described *I. tupayachianum* is a close relative of the monotypic *A. arborescens* (PP = 100%, Fig. 1A). Smith and Baum (2006) found that *Acnistus* is nested within *Iochroma* and is part of a clade containing species with valvate bud aestivation (the "A" clade). *Iochroma tupayachianum* shares this feature, and has white flowers and orange fruit similar to *A. arborescens*. As a whole, the phylogeny is well resolved (with most branches >95% PP, Fig. 1A), providing a strong framework for downstream comparative analyses.

COMMUNITY STRUCTURE

The results of the community phylogenetic analyses were consistent with the competition model for color diversity in *Iochrominae*. When viewed through the visual systems of hummingbirds as well as bees (Fig. 1), flower color is significantly overdispersed within communities; that is, color distances among co-occurring species were significantly greater than the same distances for randomly assembled communities (Table 1). Similar results were obtained when we analyzed the number of "JNDs" (a metric incorporating photoreceptor noise) in color between species pairs (Table S3). Of the other traits, only the color PC3 (purple vs. other colors; Fig. S2) was also significantly overdispersed. All other traits (tube length, leaf length, petiole length, pedicel length, berry

Table 1. Phenotypic and phylogenetic community structure. Shows mean nearest taxon distance (MNTD) for actual and null communities, statistical significance of any deviation from null expectations (with significant *P* values in bold), and pattern detected (overdispersed, clustered, or random). Phylogenetic distance was calculated for all 100 trees; here we report the mean and standard deviation of the 100 values.

		MNTD				
		Actual	Nulls	<i>P</i> value	Pattern	
Morphology	Tube length	1.36	1.26	0.46	Random	
	Leaf length	6.97	6.46	0.66	Random	
	Petiole length	1.57	1.65	0.71	Random	
	Pedicel length	1.03	0.92	0.25	Random	
	Berry width	4.26	3.64	0.11	Random	
Flower color	(1) Hummingbird vision	0.27	0.23	0.02	Overdispersed	
	(2) Bee vision	0.29	0.24	0.01	Overdispersed	
	(3) PCA	PC1	6.61	6.83	Random	
		PC2	4.32	4.84	Random	
		PC3	3.19	2.63	0.047	Overdispersed
		PC4	1.62	1.76	0.47	Random
Phylogenetic Distance		0.032	0.034	0.118	Random	
		(± 0.002)	(± 0.002)	(± 0.032)		

width, and the other color PCs) were not significantly different from random (Table 1). Similarly, phylogenetic distance within sites did not differ significantly from null expectations (Table 1). Averaged over the 100 Bayesian trees, actual MNTD (0.032) was somewhat smaller than null MNTDs (0.034), but this difference was not significant for any of the trees (mean $P = 0.118 \pm 0.032$ SD).

PHYLOGENETIC SIGNAL

Analyses using the K statistic and Mantel tests revealed variation in levels of phylogenetic signal across traits, but consistently low signal for all color traits. Mean values of K (averaged over the 100 trees) ranged from 0.03 to 0.53 (Fig. 2; Table S4). The four PCs of reflectance spectra were all significantly less than 1.0 and not statistically different from zero, indicating an absence of phylogenetic signal for flower color. Similarly, Mantel tests detected no phylogenetic signal for spectral location in bee or hummingbird color space, that is, color-space distance matrices were not correlated with phylogenetic distance matrices (Table S4). The five morphological traits all had higher K values than the color PCs (Fig. 2). Of these, only berry width had a low K value that was statistically indistinguishable from zero. Leaf and petiole lengths had intermediate levels of phylogenetic signal significantly different from both zero and 1.0, whereas tube and pedicel lengths had relatively high signal statistically indistinguishable from 1.0, indicative of Brownian motion evolution.

CONVEX HULL ANALYSIS OF COLOR SPACE

For hummingbird color space, the volume of the convex hull occupied by Iochrominae species was more than threefold greater for

sympatric species (0.0112) than for allopatric species (0.0033). This corresponds to a difference of 0.0079. For the null models, the mean difference between groups was 0.0027, with 95% confidence intervals (CIs) from 0.0014 to 0.0068. The actual difference (0.0079) falls outside of the upper CI, indicating that sympatric species occupy significantly greater color space. For bee color space, the area of the convex hull was also larger for sympatric species (1.538) than for allopatric species (1.126), but the difference (0.412) was not statistically significant according to the null model analysis (95% CIs from 0.085 to 0.692).

Discussion

Our results suggest that competition for pollinators among sympatric taxa was an important driver of flower color diversification in Iochrominae. Although pollinator shifts appear to explain flower color transitions in some clades (Fenster et al. 2004), Iochrominae, like many other groups, exhibits patterns of color variation that do not correspond to differences in pollination systems (Smith et al. 2008a,b). Instead, Iochrominae colors are well predicted by species co-occurrence. Taxa that occur in sympatry tend to differ in flower color, with color space distances greater than would be predicted by chance. This pattern of overdispersion cannot be attributed to phylogenetic history alone, as species are randomly assembled into communities with respect to their phylogenetic placement. Moreover, traits other than color follow the pattern of the phylogeny, showing random community assembly. This contrast between flower color and other plant traits reinforces the notion that color differences emerged in

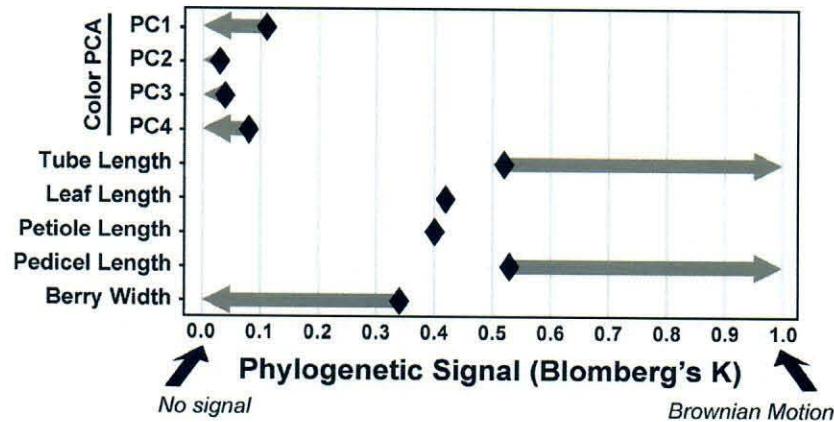


Figure 2. Phylogenetic signal for flower color (PC variables) and five other traits in Iochrominae. Diamonds show the estimated Blomberg's K value. Gray arrows show when traits are statistically indistinguishable from zero (no signal) or one (Brownian motion).

response to interspecific competition, whereas the other traits are not principally shaped by competition.

The color overdispersion we detected within sites could be due either to competition sorting species with preexisting color differences into communities, or to competition selecting for the evolution of novel color variation (i.e., character displacement). Although it is difficult to separate these possibilities (and indeed both may have played a role), three lines of evidence lend support to the latter hypothesis. First, color (unlike other traits) does not follow a neutral Brownian motion evolutionary model, as indicated by its low phylogenetic signal (Fig. 2), and such lack of signal is predicted for a trait under selection (Revell et al. 2008). Second, if patterns of overdispersion were solely due to ecological sorting rather than the evolution of novelty, we would expect a similar range of flower colors in the pool of species that occur in allopatry compared to the pool that occur in sympatry with other Iochrominae. In fact, all allopatric species (see circles in Fig. 1B and C) have either purple or white corollas, and occupy less color space than sympatric species for both hummingbird and bee vision. This difference is particularly large and statistically significant for hummingbird vision (although not for bee vision) according to our null model analyses of convex hull size. The unusual derived colors in the clade (the red, orange, and yellow outliers in color space; Fig. 1C) are only found among the species that co-occur with relatives. Third, intraspecific patterns of color variation within one polymorphic species follow predictions of the character displacement scenario. We sampled both purple and yellow-green races of *I. umbellatum* and found that the yellow-green form occurs in areas of overlap with purple and red species (*I. parvifolium*, *I. edule*, *D. obovata*) whereas the purple form occurs in areas of overlap with yellow and white species (*I. salpaeum* and *I. tupayachianum*). Together, this evidence sup-

ports the interpretation that the color shifts across Iochrominae evolved via character displacement in zones of sympatry.

Our analyses of the principal components of color allow some insight into which aspects of floral reflectance contribute to the observed patterns of overdispersion in animal color space. Although most PC axes are randomly distributed across sites, PC3 is significantly overdispersed (Table 1). PC3 has positive loadings from 400 to 500 nm (the blue region of the spectrum) as well as from 650 to 700 nm (red) and negative loadings elsewhere (Fig. S2); thus it divides purple species (which reflect both blue and red wavelengths) from all others. Interestingly, this axis of variation corresponds well to the production of major classes of floral pigment compounds. Purple flowers are colored by delphinidin-derived anthocyanin pigments, whereas other colors arise from the lack of any pigments (white), the presence of pelargonidin-derived anthocyanins (red), or a lack of these anthocyanins combined with the presence of carotenoid pigments (yellow, orange). Given that purple is the ancestral state in Iochrominae, color divergence can thus be achieved either by changes in the anthocyanin pathway to produce pelargonidin rather than delphinidin (Smith and Rausher 2011) or by loss of anthocyanins (possibly followed by gains of carotenoid expression).

The color differences among sympatric taxa may be favored because they encourage individual pollinators to specialize, restricting their foraging bouts to fewer angiosperm species. Increased specialization would reduce the competitive costs associated with interspecific pollen transfer (Waser 1978; Fenster et al. 2004; Muchhala et al. 2010). Color differences have been shown to increase individual specialization in bees, in that they are more likely to visit the same color flower as their previous visit (Heinrich 1975; De Jager et al. 2011). Hummingbirds are not known to display such flower constancy, but do have strong social

hierarchies that can serve to limit access to nectar resources. Aggressive hummingbirds dominate rewarding flowers, relegating less aggressive hummingbirds to less rewarding flowers (Wolf et al. 1976; Feinsinger and Colwell 1978; Gutierrez-Zamora 2008). If flowers produce divergent color signals, hummingbirds would be able to readily detect nectar quality from a distance, and thus reduce aggressive interactions by preferentially visiting the angiosperm species appropriate to their position in the social hierarchy. Initial observations of *Iochroma* visitation provide support for this hypothesis; where two species of *Iochroma* co-occur, hummingbirds visit one or the other and were not observed to switch between species even when individual shrubs were located within meters of each other (Smith et al. 2008a). Additional observational and experimental work would be useful to clarify the proposed role of pollinator behavior in selecting for color differences among Iochrominae.

Given that corolla tube length varies widely across the clade (Fig. 1A), much like corolla color, it is interesting that this trait does not show similar patterns of overdispersion within sites. It would be reasonable to expect that competition for pollination would also select for tube length differences in co-occurring taxa. We speculate that this selection may in fact be taking place, but two factors obscure its detection in our analyses of overdispersion. First, corolla tube length may be slower to respond to this selection than floral color. Whereas flower color shows no evidence of phylogenetic signal, tube length has an elevated K -value that is not statistically distinguishable from 1 (Fig. 2). This signal in tube length is reflected in the clustering of taxa with similar tube lengths in particular clades (e.g., *A. arborescens* and *I. tupaianum* or *V. breviflora* and *V. dichotoma*). A slow response to selection could be due to a lack of standing variation, a more complex genetic architecture, and/or pleiotropic constraints. A second complicating factor may be selection on tube length imposed by nectar robbers, which are common visitors to many Iochrominae (S. D. Smith, pers. obs.). Nectar robbers, including flower-piercing birds and many Hymenoptera, preferentially attack species with longer tubes (Lara and Ornelas 2001; Urcelay et al. 2006), and thus might counter selection imposed by pollinators for longer tubes. Some long-tubed *Iochroma* species have evolved thickened or inflated calyces (e.g., *I. calycinum* and *I. grandiflorum*) that are posited to act as deterrents for nectar robbers (Lagerheim 1891), further complicating the predicted evolutionary dynamics for corolla length variation. Collectively, these historical and ecological factors suggest that it may be challenging to elucidate the forces responsible for the diversity of flower sizes in Iochrominae.

Although the idea that competition for pollinators could lead to overdispersion in floral phenotype was first proposed over a century ago (Robertson 1895), statistical tests have only been undertaken relatively recently, and results have been mixed. One

interesting pattern that emerges is the contrast between studies at different scales. Community-based approaches that focus on multiple evolutionary lineages within sites often fail to detect overdispersion (Fleming and Partridge 1984; Wheelwright 1985; Motten 1986; Murray et al. 1987; Rathcke 1988a,b; Aizen and Vázquez 2006; Boulter et al. 2006; but see McEwen and Vamosi 2010) whereas clade-based approaches like ours often do detect overdispersion, for example, for *Stylidium* (Armbruster et al. 1994), *Acacia* (Stone et al. 1998), *Burmeistera* (Muchhal and Potts 2007), orchids (Waterman et al. 2011), and *Pedicularis* (Eaton et al. 2012). We suggest that this contrast may be due to the especially severe costs of pollen transfer between close relatives, and in some cases may represent the reinforcement of species boundaries in response to gene flow. For Iochrominae, receipt of heterospecific pollen likely often leads to wasting maternal resources on fruits containing less-fit hybrid offspring. Hybrid seed can be formed between many taxa (Smith and Baum 2006), and these hybrid and backcross individuals show low pollen viability and low survivability in the greenhouse (Smith and Rausher 2011). We expect they would fair even worse in their harsh natural habitats, an idea supported by their rarity in zones of sympathy (S. D. Smith, pers. obs.).

Several previous studies suggest that our findings for Iochrominae are likely to extend to other clades and communities. For example, multiple shifts to red flowers occurred in a tropical clade of *Mimulus* despite all being pollinated by bumblebees (Cooley et al. 2008; Cooley and Willis 2009). These color differences were found to increase individual-level specialization by the bumblebees (Cooley et al. 2008), consistent with the competition model for color divergence. Similarly, although temperate hummingbird flowers tend to be red, tropical species are highly variable, as first pointed out by Grant (1966) and demonstrated in subsequent community-wide studies (Dziedzioch et al. 2003; Gutierrez-Zamora 2008). We propose that the high levels of biodiversity in the tropics leads to frequent sympatry among relatives, which in turn promotes such competition-driven selection for color diversification.

In summary, results of this study show that co-occurring Iochrominae, which possess similar pollination systems, are significantly more divergent in flower color than expected based on random assemblages. This suggests that the remarkable diversity of flower colors observed across the clade evolved in response to competition for pollinators, an idea bolstered by the lack of color variation among allopatric members of the clade. Although interspecific competition has received relatively little attention as a mechanism for divergence in floral cues, it has commonly been invoked to explain signal evolution in animals (Hobel and Gerhardt 2003; Rundle et al. 2005; Lemmon 2009). We expect that additional work in plant systems will reveal divergence due to interspecific competition explains a substantial portion of the

remarkable breadth of flower diversity that Darwin famously termed an abominable mystery.

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DATA ARCHIVING

The doi for our data is 10.5061/dryad.36v4b.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. A map of South America showing the distribution of study sites.

Figure S2. The loading of spectral wavelengths (from 300 to 700 nm, in 0.4 nm increments) onto the first four PCs of the principal components analysis of flower color spectra.

Figure S3. Paired stereoscopic images of Iochrominae flower colors in hummingbird color space.

Table S1. List of study sites with the Iochrominae species that occur in each and corresponding collection numbers, sorted in alphabetical order within each country.

Table S2. Phenotypic community structure for the subset of sites which contain multiple Iochrominae species (32 of our original 71 sites).

Table S3. Phenotypic community structure for color distance calculated using models of vision that incorporate photoreceptor noise.

Table S4. Phylogenetic signal for *lochroma* traits, including four components of flower color and four morphological measurements.