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Comparative Pollination Biology of Sympatric and Allopatric Andean *Iochroma* (Solanaceae)

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COMPARATIVE POLLINATION BIOLOGY OF SYMPATRIC AND ALLOPATRIC ANDEAN *IOCHROMA* (SOLANACEAE)[1]

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ABSTRACT

Field studies were conducted for 15 species of *Iochroma* Benth. and the nested genus *Acnistus* Schott to quantify the diversity of pollination systems and to assess the potential contribution of pollinator behavior to the persistence of closely related species in sympatry. We combined measures of pollinator visitation and pollen deposition to estimate the importance of major groups of pollinators for each species, and we calculated proportional similarity in the pollinator assemblage among species. We found that 12 species of *Iochroma*, encompassing a range of flower colors and sizes, were principally pollinated by hummingbirds and, in many cases, by the same hummingbird species. The remaining species were either pollinated by a mix of hummingbirds and insects (two species) or exclusively by insects (two species). Based on proportional similarity values, the overlap in pollinator assemblages was found to be higher for sympatric species than for allopatric ones, reflecting sharing of local pollinator fauna. However, observations of individual pollinator fidelity, perhaps related to territorial interactions among hummingbirds, suggested that pollinators may still contribute to the reproductive isolation of sympatric congeners. Nonetheless, because interspecific pollen flow does occur, the maintenance of species boundaries in sympatry probably requires postmating reproductive isolating mechanisms.

Key words: *Acnistus*, flower color, hummingbird pollination, *Iochroma*, pollen deposition, pollinator importance, pollinator visitation, reproductive isolation, sympatry.

The rich floristic diversity of the Neotropics has often been attributed to the complex and specialized interactions between plants and animals, typically in the form of herbivory or pollination (Faegri & van der Pijl, 1966; Janzen, 1973; Johnson & Steiner, 2000). For example, Gentry (1982) noted that the Andean-centered families, such as the Ericaceae, Gesneriaceae, and Campanulaceae, which account for a large proportion of the Neotropical species diversity, are biotically pollinated and often appear to be specialized for particular groups of animals such as hummingbirds or bats (Perret et al., 2001; Luteyn, 2002; Muchala, 2006). While geographical patterns in pollinator specialization have become the subject of debate (Ollerton & Cranmer, 2002; Olesen & Jordano, 2002), the largest barrier to understanding the role of pollinators in the diversification of Neotropical taxa remains the paucity of detailed studies of pollination ecology, particularly those that catalog not only the range of visitors but their effectiveness as pollinators (Kay & Schemske, 2004).

Here, we investigate the pollination biology of *Iochroma* Benth., an Andean genus of approximately 25 species of Solanaceae (Smith & Baum, 2006). Several authors (e.g., Lagerheim, 1891; Cocucci, 1999) have speculated that the showy tubular flowers of *Iochroma* species are pollinated by hummingbirds; however, no previous field studies of pollination exist for this group. While several *Iochroma* species (e.g., *I. fuchsioides* (Humb. & Bonpl.) Miers and *I. gesnerioides* (Kunth) Miers) are indeed a close fit to the classic hummingbird syndrome flower, namely red, scentless, and tubular, most species vary from this suite of traits, suggesting pollination by other groups

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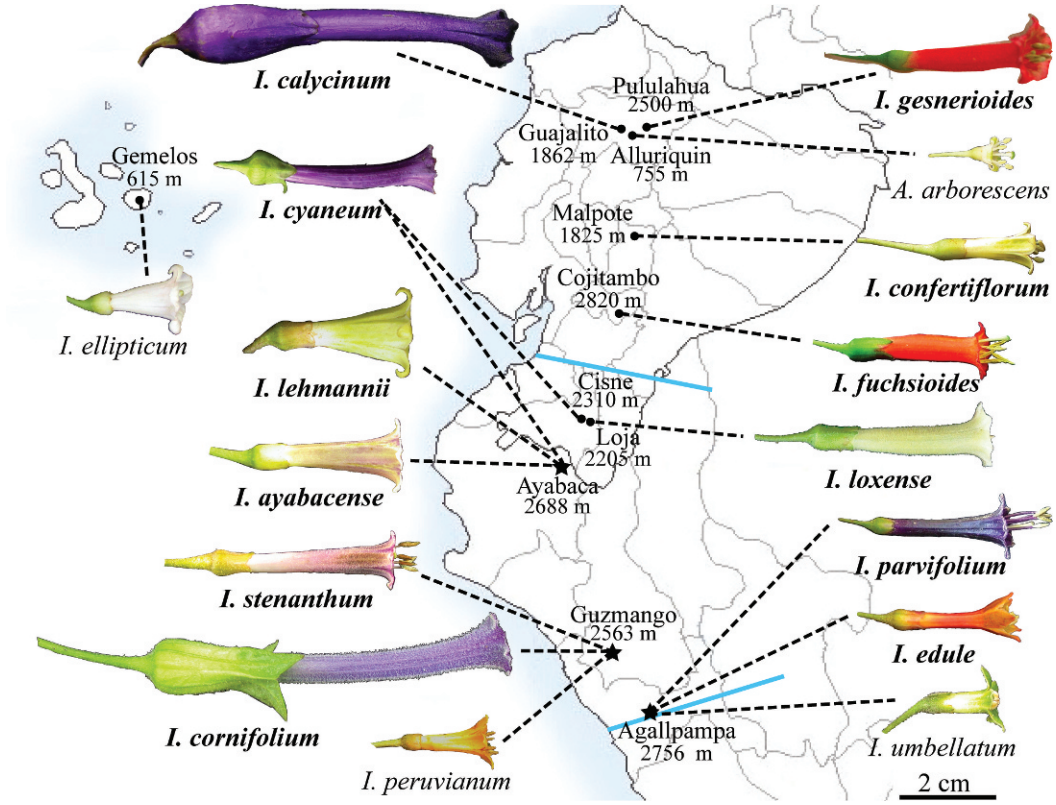

Figure 1. Study sites in Ecuador and Peru in northwestern South America. Country borders are in dark grey, and provinces or departments are bounded in light grey. Approximate boundaries of the Amotape–Huancabamba zone (from Weigend, 2002) are indicated with blue lines. Galápagos Islands (upper left) are not to scale. Study species at each site are indicated with dashed lines, and flowers are shown to scale (bottom right). Sites with sympatric taxa are marked with stars, and the names of the principally hummingbird-pollinated taxa (as determined by this study) are in boldface.

of animals (Fig. 1). For instance, the flowers of *I. confertiflorum* (Miers) Hunz. are greenish white, tubular, and scented, traits more commonly associated with moth pollination (Faegri & van der Pijl, 1966). Also, many species exhibit the peculiar combination of long, tubular, blue or purple flowers, a combination that does not correspond to any known syndrome (Faegri & van der Pijl, 1966). Given the diversity of floral morphologies present in *Iochroma*, we predicted that the composition of visitors and their effectiveness as pollinators would vary substantially between species.

Differences in pollination system among *Iochroma* species may carry important implications for the maintenance of species boundaries. In some parts of the Andes, *Iochroma* species occur in sympatry and flower together during the rainy season, creating the potential for interspecific pollen flow. Although some hybrids have been documented in zones of sympatry (Smith & Baum, 2006), interspecific hybridization is not rampant, and many taxa coexist with no observed hybrid formation (Smith, 2006). Pollinators may contribute to reproductive isolation in areas of sympatry either by forming specialized relationships with particular *Iochroma* species or by exhibiting constancy during foraging bouts, such that interspecific gene flow is limited (Jones, 1978; Campbell & Motten, 1985; Waser, 1986).

In the present study, we assessed the specialization of pollinators on 15 *Iochroma* species and the nested genus *Acnistus* Schott, and, in areas of sympatry, we examined the importance of pollinator behavior in interspecific pollen flow. In order to characterize the pollination system for each species, we measured pollinator visitation rates and pollen deposition for four major groups of pollinators (hummingbirds [Trochilidae], as well as hymenopteran, lepidopteran, and dipteran insects), and we calculated a composite variable, pollinator importance, for each group. Using the visitation rates for each pollinator, we compared the similarity in pollinator assemblage for allopatric and sympatric species pairs to determine if sympatric

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taxa tend to show more divergence in their pollinator use than is typical for allopatric taxa. Finally, we compiled observations of individual pollinator movements in sympatric areas to examine the possibility of preferential visitation by individual pollinators.

Materials and Methods

Study taxa

Our taxon sampling of *Iochroma* represented the range of floral variation within the genus. *Iochroma* flowers may be red, orange, yellow, white, green, blue, and purple, and all of these colors were included in our study group. Flower form in *Iochroma* ranges from campanulate to narrowly tubular. Here, the campanulate form is represented by *Acnistus arborescens* (L.) Schltdl., the sole member of the genus *Acnistus* (Hunziker, 2001), which is nested within the core clade of *Iochroma* (Smith & Baum, 2006). Corolla tube length varies more than eight-fold in *Iochroma*, and in our study taxa ranges from less than 1 cm to more than 6 cm (Fig. 1). Only two *Iochroma* species (*I. ellipticum* (Hook. f.) Hunz. and *I. confertiflorum*) produce any noticeable scent, and both were included here. Their light, sweet scent is very similar to that of their close relative *A. arborescens*, whose scent apparently derives from a mixture of 3,5-dimethoxytoulene, jasmine, anisaldehyde, and methyl anthranilate (Kaiser, 2000).

The selection of study taxa also took into account the recent phylogenetic analysis of Iochrominae (Smith & Baum, 2006), which indicated that the genus *Iochroma* is not monophyletic, but is instead divided among three major clades. Eleven of the studied taxa fall into the large core clade of 13 *Iochroma* species (Smith & Baum, 2006), which also includes *Acnistus arborescens*. Outside of this core group of iochromas, we sampled two additional species, *I. parvifolium* (Roem. & Schult.) D'Arcy and *I. umbellatum* (Ruiz & Pav.) Hunz. ex D'Arcy (Smith & Baum, 2006). Also, we studied two recently named taxa, *I. ayabacense* S. Leiva and *I. stenanthum* S. Leiva, Quipuscoa & N. W. Sawyer, which appear to be of hybrid origin (Smith & Baum, 2006). Phylogenetic analyses have suggested that *I. ayabacense* is a hybrid between *I. lehmannii* Bitter and *I. cyaneum* (Lindl.) M. L. Green, and *I. stenanthum* is probably a hybrid between *I. cornifolium* (Kunth) Miers and *A. arborescens* (Smith & Baum, 2006). Vouchers were collected for all studied populations; these are given in Appendix 1.

Study sites

Iochroma species are mainly distributed in the Andes of Colombia south to Peru, where they occur as sparsely distributed large shrubs or treelets in scrub or cloud forest between 2200 and 2900 m. The greatest species richness occurs in the Amotape–Huanacamba zone at the border of Ecuador and Peru (Smith & Baum, 2006; Fig. 1). Whereas outside of this zone *Iochroma* species are typically found in allopatry, geographic ranges frequently overlap within the Amotape–Huanacamba zone, and up to four species can co-occur within a single 1-km^2 area. A few species pairs (e.g., *I. cyaneum* and *I. cornifolium*) hybridize in areas of contact, but many do not (e.g., *I. umbellatum* and *I. edule* S. Leiva) (Smith, 2006). There are some differences among taxa in microhabitat preferences (e.g., soil, light, and moisture conditions), but generally, *Iochroma* species are found in areas of moderate disturbance, such as forest gaps, trails, dry stream beds, or field edges. The 11 study sites were typically located on the outskirts of rural towns or villages, in areas of mixed secondary vegetation and small-scale agriculture; the locations are shown in Figure 1 and listed in Appendix 1.

Iochroma species flower throughout the year, but peak flowering occurs during the rainy season, roughly from December to April in the Andean regions of Ecuador and Peru where these studies took place. Flowering peaks slightly earlier in northern Ecuador (December) than in southern Ecuador and Peru (January to February). During these months, the weather is typically sunny in the morning and cloudy or rainy in the afternoon and evening. Pollinator observations for each species were made over a three- to four-day period during the rainy season; a few studies were extended for a fifth day when extremely rainy conditions persisted for several days or when the overall rate of pollinator visitation was low. The dates of each study are given in Appendix 1. To the extent possible, the pollination studies were conducted in sites that were well within the geographic and altitudinal range of the species and contained many large, flowering individuals (20 to 200). Up to 14 individuals in each population were incorporated into each study (Appendix 1), although the scarcity and/or poor accessibility of some taxa limited the number of available individuals (e.g., four plants for *I. peruvianum* (Dunal) J. F. Macbr.).

Floral biology

Stigma receptivity was judged using the hydrogen peroxide test (Kearns & Inouye, 1993). Styles were collected from flowers throughout anthesis (from bud to a wilted flower) and dipped in hydrogen peroxide. The youngest stigma to yield a positive result (production of bubbles) was taken to indicate the onset of stigma receptivity.

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Nectar volume was measured with calibrated glass micropipettes, and the percentage of sugar was estimated using a temperature-compensated hand refractometer (QA Supplies, Norfolk, Virginia, U.S.A.) accurate to 0.2% between 0% and 32% sugar by volume. Nectar was extracted by pressing the micropipette into the spaces between the filaments where the liquid accumulates in *Iochroma*. Samples were taken from first- or second-day flowers (covered with 1-mm mesh bags before anthesis) at 3-hr. intervals from 0700 to 1900 hr. Each flower was only sampled once. Flowers were sampled from five to 10 individuals per study population.

VISITATION

Pollinator visits were recorded for two to three days during three observation periods: morning (0600 to 0900 hr.), midday (1100 to 1400 hr.), and evening (1700 to 2000 hr.). Two hours of observations were recorded during each period, but with the time required to move between study individuals, each period spanned 3 hr. The time periods were chosen based on previous observations that suggested that these are periods of high pollinator activity. During the studies, the sun rose at ca. 0600 hr. and set at 1900 hr. Observations were made while sitting 2.5 to 3.5 m from a subject plant to minimize the distraction of visitors. Both the number of visits to the subject plant and the number of legitimate flower visits (sensu Jones & Reithel, 2001) were noted. Bird visitors were identified to the lowest possible taxonomic level using field guides and consultation with experts. Visiting insects were collected in ethanol (70%) and later identified by comparing them to reference collections at the University of Wisconsin–Madison, the University of Illinois–Urbana, and the Charles Darwin Research Station. All insect specimens were deposited in the University of Wisconsin–Madison Insect Research collection.

Plant and flower visitation rates were calculated from raw observation data by dividing the total number of visits by a given pollinator by the time observed (in flower hours to correct for differences in display size among individuals, sensu Dafni, 1992). Flower hours were calculated from a series of observations (1 to n) as follows: $FH = (D_1 \times T_1) + (D_2 \times T_2) + \ldots (D_n \times T_n)$, where FH equals the number of flower hours, D is the display size (number of flowers on the plant), and T equals the number of hours the plant was observed. Visitation rates were calculated both for individual species and for four major groups of pollinators (hummingbirds, as well as hymenopteran, lepidopteran, and dipteran insects). Flower visitation rates were used to compute the proportional similarity (PS) of pollinator assemblages between all pairs of *Iochroma* species (Schemske & Brokaw, 1981; Kay & Schemske, 2003). This measure takes into account both the number of pollinator species shared and their visitation frequency; PS for a pair of plant species is $1 - \frac{1}{2} \Sigma |P_{ai} - P_{bi}|$, where P_{ai} and P_{bi} are the proportions of the total visitation rate made up by pollinator species i for plant species a and b, and differences in P_{ai} and P_{bi} are summed across all pollinator species, $1 \ldots i$. PS values range from 0 to 1, with higher values indicating greater overlap in the pollinator visitation between two species.

INTERSPECIFIC POLLINATOR MOVEMENTS

We assessed the potential for interspecific pollen flow in sympatry by observing pollinator visitation to individual plants from different species growing side by side. The closest pairs of plants, typically 1 to 5 m apart, were selected for observation, and movement of the pollinators within the plants and between the plants was recorded. We examined the visitation patterns for bias toward particular *Iochroma* species by a given pollinator by comparing the expected to the observed number of visits with χ^2 analysis (as in Schemske, 1981). Expected values are the number of visits expected if visits are directly proportional to display size. Also, using the observations of the sympatric plant pairs, we compared the number of plant visits that involved movement between species to those that were restricted to one species as an additional measure of pollinator fidelity.

POLLEN DEPOSITION AND POLLINATOR IMPORTANCE

To measure pollen deposition by different pollinator species, virgin flowers (covered with green or black 1-mm mesh bags before anthesis) were presented to pollinators and then re-bagged after a single visit by a single pollinator. The pollen loads on visited stigmas were compared to stigmas from flowers that were bagged for the duration of the experiment to determine if these species require biotic pollination. Also, we collected and examined stigmas from unbagged flowers to assess the typical pollen deposition for flowers exposed to unlimited visits.

To test for nocturnal pollination, stigmas were collected from flowers that had been bagged during the day but left unbagged at night (1900–0600 hr.). Additionally, for 13 study species (excluding *Acnistus arborescens*, *Iochroma cyaneum*, and *I. gesnerioides*), stigmas were collected from flowers that were bagged at night but unbagged during the day (0600–1900 hr.). Where there were sufficient flowers, all four treat-

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ments (always bagged, never bagged, bagged during night only, bagged during day only) were completed for each individual. Styles were removed from flowers that were already open at the beginning of the experiment to ensure that only virgin flowers were included.

Styles from visited or treated flowers were collected 6 to 12 hr. after replacement of the bag, fixed in formalin, acetic acid, and alcohol (FAA) for 12 to 24 hr. and transferred to ethanol (70%) for storage. The fixed stigmas were examined for the presence of germinating pollen grains using a modified version of Martin's (1959) protocol. The styles were soaked in 4M NaOH to soften (10 min.), washed three times in 50 mM KPO_4 buffer (5 min. per wash), stained with 0.05% decolorized aniline blue in 50 mM KPO_4 buffer for 5 min., squashed on a microscope slide, and viewed under ultraviolet fluorescence microscopy. When the style contained fewer than ca. 100 grains, all were counted. When there were greater than 100 grains, the style was divided into sections (e.g., halves, quarters), and a count from a representative section was used to estimate the total load. Non-fluorescing grains (not germinating and/or not Solanaceae) were excluded from counts. *Iochroma* species do not differ markedly in pollen morphology, so it was not possible to identify pollen to species. To relate pollen load to ovule availability, the average number of ovules per flower in each species was estimated by counting ovules from enough flowers to bring the standard error to less than 10% of the mean, when sufficient flowers were available.

Pollen deposition (quality) was combined with visitation rate (quantity) to give an overall estimate of pollinator importance (Waser & Price, 1983; Schemske & Horvitz, 1984; Herrera, 1987; Mayfield et al., 2001). Here, the importance of each group of pollinators was calculated as the product of the relative visitation rate and the proportion of available ovules potentially pollinated by a single visit (using the previously described estimates of ovules per flower). If the average number of pollen grains deposited by a single visit exceeded the estimated number of ovules, the proportion was set to 1.0. We used this scaled deposition for two reasons. First, it allowed us to take into account the differences in ovule number and, thus, in potential per-visit effectiveness across study species. Second, this approach accommodates the fact that seed set typically levels off quickly with increasing pollen load (Silander & Primack, 1978; Kohn & Waser, 1985), so pollen deposition that greatly exceeds the number of ovules (as observed here for many hymenopterans) is unlikely to result in a proportional increase in fitness.

Results

Floral Biology

Measurements of stigma receptivity showed that *Acnistus* and all species of *Iochroma*, except *I. umbellatum*, are protogynous. Stigmas are fully receptive when the flowers open, and the anthers dehisce 1 to 3 hr. later except in *I. umbellatum*, in which the anthers are already dehisced when the flower opens. *Iochroma* flowers open asynchronously throughout the day, but with some tendency toward opening in the morning, and they do not close at night. The stigmas remained receptive until the flowers wilted, two to three days after opening.

Nectar-standing crop varied widely across taxa, but did not show strong diurnal patterns, in large part because of the staggering of flower maturation during the day. For comparison across taxa, we pooled measurements from all sampling times and computed averages for nectar volume and concentration (Table 1). The small-flowered *Acnistus arborescens* produced the lowest volume of nectar (0.5 ± 0.1 μl) and presented the smallest reward, both on a per-flower and a per-plant basis. The large-flowered *Iochroma calycinum* Benth. produced the most nectar (38.4 ± 3.0 μl), although it did not offer the highest per-flower reward because of its low sugar concentration (14.5 ± 0.3%). The most rewarding species was *I. loxense* (Kunth) Miers due to its high nectar volume (37.0 ± 2.7 μl), high sugar concentration (24.0 ± 0.5%), and large display size (120.3 ± 38.1 flowers per plant).

Visitation

Over the course of ca. 264 hr. of observation, 47 pollinators were observed legitimately visiting the 16 study species (Appendix 2). Bees and species of flower-piercing birds (*Diglossa* Chapman [Thraupidae]) were frequent illegitimate visitors, robbing nectar by making holes in the sides of the corollas. These illegitimate visits were not included in visitation rates. Legitimate visitors included hummingbirds (Apodiformes, Trochilidae) and a wide variety of insects from the Hymenoptera, Diptera, and Lepidoptera (Appendix 2).

Hummingbird visits were evenly spread across all observation periods, hymenopteran visits were most common in the midday period (1100–1400 hr.), dipteran visits occurred primarily in the morning (0600–0900 hr.), and lepidopteran visits (mainly moths) were most frequent in the evening period (1700–2000 hr.). Hymenoptera and Diptera visited 2.6 and 2.1 flowers per plant visit, respectively, whereas Lepidoptera visited 4.5 flowers on average and hummingbirds 15.8 flowers.

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Table 1. Nectar rewards across study taxa.[1]

Species	Flowers sampled	Average nectar volume (μl) ± SE	Average percent sugar ± SE	Average reward per flower[2] ± SE	Average reward per plant[3] ± SE
Acnistus arborescens	16	0.5 ± 0.1	14.4 ± 2.5	0.1 ± 0.02	2.3 ± 0.6
Iochroma ayabacense	25	11.8 ± 1.0	22.5 ± 0.3	2.7 ± 0.2	459.9 ± 128.1
I. calycinum	26	38.4 ± 3.0	14.5 ± 0.3	5.7 ± 0.5	169.3 ± 63.9
I. confertiflorum	37	17.5 ± 1.6	20.9 ± 0.6	3.8 ± 0.4	424.4 ± 148.6
I. cornifolium	20	37.3 ± 3.2	18.4 ± 0.3	6.9 ± 0.6	603.0 ± 121.8
I. cyaneum	29	17.7 ± 1.9	23.1 ± 0.7	4.1 ± 0.4	177.8 ± 42.6
I. edule	28	10.9 ± 1.1	20.7 ± 0.4	2.3 ± 0.2	704.7 ± 167.6
I. ellipticum	29	1.8 ± 0.3	7.6 ± 1.4	0.2 ± 0.05	10.22[4]
I. fuchsioides	32	20.2 ± 1.8	26.4 ± 0.7	5.2 ± 0.4	414.8 ± 79.7
I. gesnerioides	11	10.0 ± 2.4	16.3 ± 0.66	1.7 ± 0.4	841.7 ± 482.2
I. lehmannii	26	7.0 ± 0.6	27.2 ± 0.84	1.8 ± 0.2	515.3 ± 229.6
I. loxense	30	37.0 ± 2.7	24.0 ± 0.47	8.7 ± 0.5	1048.1 ± 338.6
I. parvifolium	17	17.5 ± 1.1	20.0 ± 0.7	3.5 ± 0.3	342.6 ± 133.7
I. peruvianum	20	6.9 ± 0.7	21.3 ± 0.6	1.4 ± 0.2	122.6 ± 29.0
I. stenanthum	23	21.4 ± 2.5	19.5 ± 0.4	4.2 ± 0.5	880.5 ± 243.7
I. umbellatum	32	2.2 ± 0.4	16.4 ± 0.6	0.4 ± 0.08	31.1 ± 17.1

[1] All values are shown with standard error (SE).
[2] Reward per flower is the product of volume per flower and percent sugar.
[3] Reward per plant is the product of average reward per flower and average display size (Appendix 1).
[4] Variation in display size across the population was not measured in *Iochroma ellipticum*, thus no standard error was calculated.

Based on relative flower visitation, we classified the species into three broad classes: principally hummingbird pollinated, mixed hummingbird/insect pollinated, and exclusively insect pollinated (Table 2). Taxa for which greater than 75% of the total visitation was concentrated on a single group of pollinators were considered to be specialized for that group (Fenster et al., 2004). With this criterion, 11 of the study species were considered to be principally hummingbird pollinated (Table 2). *Iochroma calycinum* had only 65% hummingbird visitation, but was also classified as principally hummingbird pollinated because most insect visitors were found to be poor pollen vectors (described below; Table 3). Two species, *I. peruvianum* and *I. umbellatum*, were visited almost equally by hummingbirds and hymenopterans and were considered to have a mixed bird and insect pollination system. *Acnistus arborescens* and *I. ellipticum* were visited frequently by all three groups of insects but no hummingbirds were observed (Table 2), so these two taxa were considered insect pollinated. Thus, we did not observe a diverse array of specialized systems, at least at the level of major pollinator groups.

Iochroma species appeared more divergent in pollination system at the pollinator species level, but these differences may be driven more by geography than by specialization for certain pollinators. As described previously, we calculated the pairwise PS metric from the pollinator visitation rates (Appendix 2) and used these values to assess the overlap in pollinator assemblage among species (Fig. 2). We observed that species with different pollination systems (Table 2) did not have significantly lower proportional similarity than species with the same pollination systems (mean PS = 0.13 ± 0.02 vs. 0.18 ± 0.03, unpaired *t*-test: $P = 0.16$). That is, two principally hummingbird-pollinated species did not necessarily show greater overlap in pollinator assemblage than a principally hummingbird-pollinated and an insect-pollinated species. However, species studied in allopatry had lower PS than those in sympatry (mean PS = 0.13 ± 0.02 vs. 0.51 ± 0.10; $P < 0.00001$) and species living in different regions had significantly lower proportional similarity than those living in the same region (mean PS = 0.08 ± 0.01 vs. 0.36 ± 0.04; $P < 0.00001$).

INTERSPECIFIC POLLINATOR MOVEMENTS

To better understand pollinator activity in areas of sympatry, we examined patterns of pollinator visitation to pairs of plants from different sympatric species. Our observations revealed biased patterns for most pollinator species. We observed that the larger hummingbirds (e.g., *Coeligena iris* Gould and *Colibri coruscans* Gould) visited the species that provided the greater nectar reward significantly more frequently, in every case with a sufficient sample size (Table 4). The smaller hummingbirds *Adelomyia melanogenys* Fraser, *Myrtis fanny* Lesson, and *Polyonymus caroli* Bourcier often showed

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Table 2. Plant and flower visitation rates across study taxa.[1,2]

	All visitors		Trochilidae		Hymenoptera		Lepidoptera		Diptera		Principal pollinators
	Plant	Flower	Plant	Flower	Plant	Flower	Plant	Flower	Plant	Flower	
Acnistus arborescens	0.130	0.428	0	0	0.024	0.082	0.010	0.091	0.096	0.255	insects
Iochroma ayabacense	0.010	0.186	0.010	0.186	0	0	0	0	0	0	birds
I. calycinum	0.050	0.140	0.008	0.090	0.017	0.024	0	0	0.025	0.025	birds
I. confertiflorum	0.037	0.221	0.011	0.173	0.024	0.042	0.002	0.006	0	0	birds
I. cornifolium	0.016	0.185	0.012	0.177	0.004	0.008	0	0	0	0	birds
I. cyaneum	0.075	0.299	0.041	0.238	0.027	0.040	0.008	0.021	0	0	birds
I. edule	0.012	0.177	0.011	0.175	4×10^{-4}	0.001	2×10^{-4}	0.001	0	0	birds
I. ellipticum	0.018	0.022	0	0	0.007	0.008	0.007	0.008	0.005	0.005	insects
I. fuchsioides	0.032	0.304	0.008	0.235	0.020	0.049	0.003	0.020	0	0	birds
I. gesnerioides	0.008	0.165	0.006	0.148	0.001	0.010	0.001	0.006	0	0	birds
I. lehmannii	0.007	0.341	0.006	0.338	0.001	0.003	0	0	0	0	birds
I. loxense	0.040	0.283	0.017	0.251	0.023	0.032	0	0	0	0	birds
I. parvifolium	0.023	0.336	0.016	0.309	0.007	0.027	0	0	0	0	birds
I. perwianum	0.033	0.107	0.008	0.047	0.025	0.060	0	0	0	0	birds/insects
I. stenanthum	0.012	0.263	0.010	0.259	0.002	0.004	0	0	0	0	birds
I. umbellatum	0.034	0.298	0.008	0.154	0.022	0.140	0.001	0.001	0.002	0.003	birds/insects
All species	0.032	0.232	0.011	0.174	0.012	0.032	0.002	0.009	0.008	0.017	

[1] Rates are presented as the numbers of visits per plant or flower per flower hour (see Methods, Visitation).
[2] Pollinator groups are hummingbirds (Trochilidae) and the three orders of insects observed.

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Table 3. Pollen deposition by pollinators and during treatments.[1,2]

	Trochilidae		Hymenoptera		Lepidoptera		Diptera		Day control[3]		Night control[3]		Bagged control		Unbagged control	
	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size	Average grains deposited ± SE	Sample size
Acnistus arborescens			898 ± 128	11	266 ± 118	5	149 ± 59	22	*		718 ± 241	4	0 ± 0	4	811 ± 204	3
Iochroma ayabacense	264 ± 56.0	21							285 ± 149	5	17 ± 12	7	1 ± 1	8	414 ± 89	13
I. calycinum	579 ± 428	3	2248 ± 727	4			139 ± 11	2	2058 ± 322	11	60 ± 38	8	134 ± 83	5	1208 ± 170	14
I. confertiflorum	971 ± 217	7	389 ± 75	4	212 ± 106	3			1262 ± 178	17	111 ± 48	19	70 ± 45	7	1462 ± 178	12
I. cornifolium	1436 ± 215	3	1538 ± 603	7					1396 ± 331	27	910 ± 193	5	152 ± 33	6	2209 ± 242	27
I. cyaneum	1076 ± 332	7	732 ± 720	2	88	1			*		115 ± 77	14	24 ± 11	14	775 ± 110	26
I. edule	1290 ± 240	22	2355 ± 137	2	2144 ± 944	2			2422 ± 404	16	878 ± 312	11	1 ± 1	6	1650 ± 140	28
I. ellipticum			97 ± 51	3	1326 ± 387	5	257 ± 78	2	860 ± 151	16	534 ± 174	11	29 ± 13	10	1769 ± 464	6
I. fuchsioides	279 ± 194	7	1151 ± 343	4	317 ± 199	4			1447 ± 265	13	0 ± 0	10	2 ± 2	8	1699 ± 177	19
I. gesnerioides	1107 ± 791	3	2780 ± 301	11	†				*		1 ± 1	5	8 ± 4	19	1423 ± 114	33
I. lehmannii	3083 ± 577	11	594 ± 594	2					4155 ± 853	10	122 ± 119	4	23 ± 13	4	4367 ± 487	14
I. loxense	407.1 ± 259	5	447 ± 205	6					804 ± 142	21	238 ± 91	12	2 ± 2	16	790 ± 106	28
I. parvifolium	14 ± 7	5	720	1					109 ± 52	9	17 ± 11	8	0 ± 0	13	438 ± 86	27
I. peruvianum	1545	1	3328 ± 334	4					2614 ± 608	9	173 ± 151	5	142 ± 65	6	1956 ± 473	12
I. stenanthum	166 ± 59	9	1014	1					761 ± 237	14	263 ± 181	6	1 ± 1	5	686 ± 114	26
I. umbellatum	58	1	727 ± 493	2	†		0	1	438 ± 255	5	365 ± 177	6	17 ± 17	13	937 ± 132	22
All taxa	940 ± 121	105	1472 ± 157	64	712 ± 194	20	151 ± 49	27	1453 ± 122	154	262 ± 42	135	28 ± 6	144	1323 ± 67	310

SE = standard error.

[1] For each plant species, the average number of pollen grains deposited by each group of visitors is presented; blank cells indicate that the pollinator group was not observed visiting that plant species.

[2] The sample size is the number of visited stigmas counted for each pollinator group or treatment.

[3] Day control refers to flowers unbagged only during the day, and night control to those unbagged only during the night.

* Treatment not completed for this species.

† Although this class of visitor was recorded for that species, no pollen counts were obtained.

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	Species (site)	*Acnistus arborescens* (ALL)	*I. calycinum* (GUA)	*I. gesnerioides* (PUL)	*I. confertiflorum* (MAL)	*I. fuchsioides* (COJ)	*I. cyaneum* (CIS)	*I. loxense* (LOJ)	*I. edule* (AGA)	*I. parvifolium* (AGA)	*I. umbellatum* (AGA)	*I. ayabacense* (AYA)	*I. lehmannii* (AYA)	*I. peruvianum* (GUZ)	*I. cornifolium* (GUZ)	*I. stenanthum* (GUZ)
	Iochroma ellipticum (GAL)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Acnistus arborescens (ALL)		0	0.06	0.02	0.09	0	0.01	0.01	0.08	0.11	0	0.01	0.11	0.03	0.01
N. ECU	*I. calycinum* (GUA)			0.02	0	0.07	0	0	0	0	0	0	0	0	0	0
	I. gesnerioides (PUL)				0.26	0.09	0	0.01	0.19	0.23	0.31	0.24	0.25	0.31	0.28	0.26
C. ECU	*I. confertiflorum* (MAL)					0.39	0	0.01	0.19	0.19	0.43	0.41	0.42	0.43	0.30	0.42
	I. fuchsioides (COJ)						0	0.01	0.01	0.08	0.09	0	0.01	0.09	0.03	0.01
S. ECU	*I. cyaneum* (CIS)							0.72	0	0	0	0.17	0.17	0	0.17	0.17
	I. loxense (LOJ)								0.01	0.01	0.01	0	0.01	0.01	0.01	0.01
N. PER	*I. edule* (AGA)									0.76	0.20	0.19	0.22	0.22	0.19	0.22
	I. parvifolium (AGA)										0.25	0.17	0.18	0.27	0.20	0.18
	I. umbellatum (AGA)											0.48	0.44	0.88	0.31	0.53
	I. ayabacense (AYA)												0.96	0.44	0.28	0.48
	I. lehmannii (AYA)													0.44	0.31	0.70
	I. peruvianum (GUZ)														0.31	0.46
	I. cornifolium (GUZ)															0.55
	I. stenanthum (GUZ)															

Figure 2. Pairwise similiarity in pollinator assemblage across *Iochroma* species. The names of the principally hummingbird-pollinated taxa are in boldface. Study site names are abbreviated as follows: AGA = Agallpampa, AYA = Ayabaca, CIS = Cisne, COJ = Cojitambo, GUZ = Guzmango, LOJ = Loja, MAL = Malpote, and PUL = Pululahua. Regions are abbreviated as follows: N. ECU = northern Ecuador, C. ECU = central Ecuador, S. ECU = southern Ecuador, and N. PER = northern Peru. Values for taxa in the same region are boxed in thin black lines, and taxa in sympatry are boxed in thick black lines. Shading of cells denotes degree of similarity.

significant preference for a particular species, although, in some cases, for the less rewarding species. For instance, over the course of three bouts, *A. melanogenys* visited 49 flowers on the low-reward *Iochroma umbellatum* and no flowers on *I. edule* ($P < 0.001$). Insect visits were typically too infrequent to show any significant pattern, but often they tended toward the less rewarding species (Table 4).

We also considered the movement of individual pollinators between these sympatric plant pairs. Although pollinator species could be observed visiting multiple sympatric plant species, we rarely saw movement between species by individual pollinators (Fig. 3). For instance, when observing an individual of *Iochroma cyaneum* and *I. lehmannii* side by side, only one of 60 visits involved movement from *I. lehmannii* to *I. cyaneum* and five involved movement in the reverse direction. The smaller hummingbirds (mentioned above) were largely responsible for these occasional interspecific movements. However, the overall rarity of interspecific movements between these individual plant pairs points to some individual-level pollinator fidelity. Due to the sparse distribution of *Iochroma* plants, we could not explore the frequency with which individual pollinators continued to be constant to a particular species after moving beyond the observed pairs.

POLLEN DEPOSITION AND POLLINATOR IMPORTANCE

Results from the bagged and unbagged controls suggested that the study species require biotic pollination. Styles from bagged flowers typically had very few pollen grains, but in three cases (*Iochroma calycinum*, *I. cornifolium*, and *I. peruvianum*) they had larger loads, with means of 134, 152, and 142 grains, respectively (Table 3). These loads, which are small relative to those typically on visited flowers, could be due to very small insects penetrating the mesh, but were more likely due to self-pollen deposited during anthesis. If the observed loads in bagged flowers of *I. cornifolium*, *I. peruvianum*, and *I. calycinum* are indeed self-pollen, they might not result in fertilization as crossing studies indicate that most *Iochroma* species are self-incompatible (Smith & Baum, 2007).

Comparison of the night- and day-bagged flowers indicated that, on average, 5.5 times more pollen was deposited during the day (0600–1900 hr.) than during the night (1900–0600 hr.; Table 3). In three species, *Iochroma ellipticum*, *I. umbellatum*, and *I. cornifolium*, pollen tube counts for the night-exposed flowers were greater than 50% that of day-exposed flowers. *Iochroma ellipticum* received the most visits during the

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Table 4. Visitation patterns to sympatric species in Guzmango, Ayabaca, and Agallpampa, Peru. Pairs of plants of sympatric taxa were observed in the study localities (see Appendix 1), with interplant distances (d) in meters and the time observed in hours (hr.). For pairs of plants greater than 3 m apart, only hummingbird movements could be recorded. Species names are abbreviated to the first letters of the specific epithet. Although the larger study of *Iochroma cyaneum* involved an allopatric population, it was found in sympatry with other taxa in Ayabaca, Peru, and so was included in these observations. Observed plant and flower visits were compared to expected visits using a χ^2 analysis with Yates' correction. Expected values (in parentheses) are the number of visits expected if visits were directly proportional to display size. For instance, if one of the two plants being observed has twice as many flowers as the other, it would be expected to receive twice as many visits. Significant χ^2 values are noted with asterisks where * is $P < 0.05$ and ** is $P < 0.01$. Boldface values are significant after Bonferroni correction.

Site: Guzmango, Peru		Hummingbird visitors						Insect visitors					
		Coeligena iris			*Adelomyia melanogenys*			*Apis mellifera*			Vespid wasp		
Plant pair observed; time observed, interplant distance		*per*	*cor*	χ^2	*per*	*cor*	χ^2	*per*	*cor*	χ^2	*per*	*cor*	χ^2
I. peruvianum with 120 flowers and *I. cornifolium* with 50 flowers; 3.6 hr., 1.5 m	Plant visits	0 (3)	4 (1)	6.5*	3 (4)	3 (2)	0.4	7 (5)	0 (2)	1.7	2 (1)	0 (1)	0.02
	Flower visits	0 (19)	27 (8)	**61.4****	18 (36)	33 (15)	**28.9****	14 (10)	0 (4)	4.5*	2 (1)	0 (1)	0.02
I. peruvianum with 75 flowers and *I. cornifolium* with 25 flowers; 4 hr., 1 m	Plant visits				1 (1)	0 (0)		0 (1)	1 (0)	0.3	3 (2)	0 (1)	0.1
	Flower visits				2 (2)	0 (0)	0	0 (2)	2 (0)	2.7	5 (4)	0 (1)	0.6

Site: Ayabaca, Peru		Hummingbird visitors						Insect visitors								
		Coeligena iris			*Adelomyia melanogenys*			*Bombus*			cf. *Plebeia*			Vespid wasp		
Plant pair observed; time observed, interplant distance		*leh*	*cya*	χ^2	*leh*	*cya*	χ^2	*leh*	*cya*	χ^2	*leh*	*cya*	χ^2	*leh*	*cya*	χ^2
I. lehmannii with 60 flowers and *I. cyaneum* with 125 flowers; 3 hr., 1 m	Plant visits	1 (6)	16 (11)	4.3*	2 (2)	5 (5)										
	Flower visits	2 (66)	203 (139)	**91.0****	45 (30)	46 (61)	**11.0****									
I. lehmannii with 144 flowers and *I. cyaneum* with 35 flowers; 3 hr., 2 m	Plant visits	0 (7)	9 (2)	**32.1****	20 (18)	2 (4)	0.9	1 (1)	0 (0)		2 (2)	0 (0)		3 (2)	0 (1)	0.02
	Flower visits	0 (29)	36 (7)	**143.0****	224 (184)	5 (45)	**42.8****	10 (8)	0 (2)	1.3	4 (3)	0 (1)	0.1	5 (4)	0 (1)	0.3

Site: Agallpampa, Peru		Hummingbird visitors												Insect visitors								
		Colibri coruscans			*Adelomyia melanogenys*			*Myrtis fanny*			*Polyonymus caroli*			*Bombus*			*Apis mellifera*				
Plant pair observed; time observed, interplant distance		*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2	*par*	*edu*	χ^2
I. parvifolium with 220 flowers and *I. edule* with 300 flowers; 2 hr., 2.5 m	Plant visits	0 (1)	2 (1)	0.3	2 (2)	2 (2)											2 (1)	0 (1)	0.9
	Flower visits	0 (7)	16 (9)	**19.5****	15 (21)	34 (28)	**13.6****										7 (3)	0 (4)	7.3**
I. parvifolium with 50 flowers and *I. edule* with 60 flowers; 4.75 hr., 5 m	Plant visits							2 (5)	10 (7)	2.9	7 (4)	2 (5)	2.6						
	Flower visits							9 (66)	136 (79)	**88.5****	125 (80)	50 (95)	**46.6****						
I. parvifolium with 175 flowers and *I. edule* with 300 flowers; 5 hr., 2.5 m	Plant visits	1 (6)	16 (11)	5.7**										1 (0)	0 (1)	0.1	6 (2)	0 (4)	7.8**
	Flower visits	34 (108)	258 (184)	**78.6****										2 (1)	0 (1)	1.3	26 (10)	0 (16)	**41.9****
		umb	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2	*umb*	*edu*	χ^2
I. umbellatum with 160 flowers and *I. edule* with 350 flowers; 2.3 hr., 9 m	Plant visits	0 (6)	18 (12)	6.8**	3 (1)	0 (2)	3.8												
	Flower visits	0 (39)	125 (86)	**55.7****	49 (15)	0 (34)	**104****												

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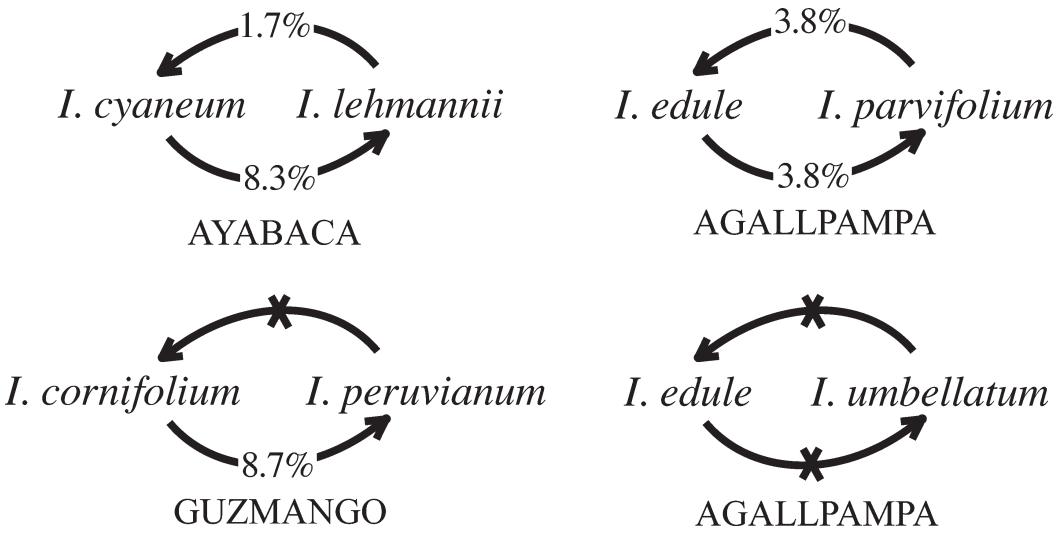

Figure 3. Observed interspecific pollinator movements among sympatric species in mixed populations. Arrows indicate percentage of interspecific movement observed between pairs of individual plants from different species, and crossed arrows indicate no observed interspecific pollinator movement. Sites where pairs were observed are given below pairs in capital letters. The percentage between *Iochroma peruvianum* and *I. cornifolium* is based on data from 23 visits to two pairs of plants, between *I. lehmannii* and *I. cyaneum* on 60 visits to two pairs of plants, *I. edule* and *I. parvifolium* on 52 visits to three pairs of plants, and *I. edule* and *I. umbellatum* on 21 visits on one pair of plants. Display sizes for these pairs are given in Table 4.

evening period (data not shown); thus, it was not surprising to find substantial night pollination in this species. Although no evening visits were observed for *I. umbellatum*, it could be visited by the same night-flying moths that visited the sympatric *I. edule* in the evening. It is unclear what could account for the night pollination in *I. cornifolium*, but the pollen loads (large relative to bagged flowers and comparable to visited flowers) implicate unidentified, nocturnally active animal visitors. Overall, however, the relatively small amounts of nocturnal pollen deposition in most taxa provide assurance that our largely diurnal pollinator observations covered most of the pollinator activity.

Thirty-four of the 47 pollinator species observed during the study visited virgin (previously bagged) flowers, allowing for estimation of pollen deposition. Visits by all species but one (an unidentified syrphid fly) resulted in pollen deposition (data not shown). Overall, hymenopterans deposited more pollen per visit on average than other classes of pollinators (Table 3). Dipteran visits resulted in the smallest deposition, on average (Table 3). Considering that the flowers have 50 to 500 ovules per flower (depending on species; Table 5), our deposition estimates suggest that single visits by most pollinator classes, except for dipterans, would result in enough pollen deposition to potentially fertilize all of the ovules. For instance, the average hummingbird visit deposited 264 viable pollen grains on a stigma of *Iochroma ayabacense* and, since this species has on average 124 ovules per flower, a single visit could potentially fertilize all the ovules.

Combining pollen deposition (quality) with visitation rate (quantity), we estimated the importance of each class of pollinators. For *Iochroma gesnerioides* and *I. umbellatum*, pollen counts were not available for the lepidopteran visitors. In the case of *I. gesnerioides*, we used the counts from its closest relative, *I. fuchsioides*, to estimate lepidopteran importance. For *I. umbellatum*, we used the average lepidopteran pollen deposition from *I. confertiflorum*, because for these two species, a hesperid butterfly species was the sole lepidopteran visitor. In this pollinator importance estimation, we scaled pollen deposition to equal the proportion of ovules potentially fertilized by a single visit. Since this proportion was 1.0 in most cases, pollinator importance values were similar to relative visitation (Table 5) as in Olsen (1997). Thus, hummingbirds appeared to be the most important pollinators for most *Iochroma* species, with only a few having either mixed bird-insect pollination or exclusively insect pollination (Table 5).

Discussion

Spatio-temporal considerations

Interactions between plants and their pollinators are subject to temporal variation within a day, within a season, and across years (Herrera, 1988; Schemske &

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Table 5. Relative visitation rates and pollinator importance.

Species	Average ovules per flower ± SE	Scaled pollen deposition[1]				Relative flower visitation rates[2]				Relative pollinator importance[3]			
		Tro	Hym	Lep	Dip	Tro	Hym	Lep	Dip	Tro	Hym	Lep	Dip
Acnistus arborescens	52.0 ± 3.5	—	1	1	1	0	0.19	0.21	0.60	0	0.19	0.21	0.60
Iochroma ayabacense	123.5 ± 13.1	1	—	—	—	1.00	0	0	0	1.00	0	0	0
I. calycinum	488.0 ± 29.3	1	1	—	0.28	0.65	0.17	0	0.18	0.74	0.20	0.00	0.06
I. confertiflorum	152.3 ± 15.1	1	1	1	—	0.78	0.19	0.03	0	0.78	0.19	0.03	0
I. cornifolium	471.0 ± 22.6	1	1	—	—	0.96	0.04	0	0	0.96	0.04	0	0
I. cyaneum	368.7 ± 20.1	1	1	0.24	—	0.80	0.13	0.07	0	0.84	0.14	0.02	0
I. edule	414.7 ± 15.6	1	1	1	—	0.98	0.01	0.01	0	0.99	0.01	0.01	0
I. ellipticum	53.4 ± 13.0	—	1	1	1	0	0.38	0.38	0.23	0	0.38	0.38	0.23
I. fuchsioides	462.7 ± 7.6	0.60	1	0.69	0	0.77	0.16	0.06	0	0.70	0.24	0.07	0
I. gesnerioides	264.7 ± 19.8	1	1	1	—	0.90	0.06	0.04	0	0.90	0.06	0.04	0
I. lehmannii	69.3 ± 5.6	1	1	—	—	0.99	0.01	0	0	0.99	0.01	0	0
I. loxense	254.7 ± 21.7	1	1	—	—	0.89	0.11	0	0	0.89	0.11	0	0
I. parvifolium	69.1 ± 6.9	0.21	1	—	—	0.92	0.08	0	0	0.70	0.30	0	0
I. peruvianum	230.3 ± 11.6	1	1	0	0	0.44	0.56	0	0	0.44	0.56	0	0
I. stenanthum	146.0 ± 12.5	1	1	—	—	0.99	0.01	0	0	0.99	0.01	0	0
I. umbellatum	134.3 ± 7.2	1	1	1	0	0.52	0.47	4×10^{-3}	0.01	0.32	0.67	0.01	0

Tro = Trochilidae, Hym = Hymenoptera, Lep = Lepidoptera, and Dip = Diptera.
[1] Pollen deposition was scaled to equal the proportion of ovules potentially fertilized by a single visit.
[2] Relative visitation rates are the numbers of flowers visited per flower hour, rescaled to sum to 1.0.
[3] Relative pollinator importance is the product of the relative visitation and scaled deposition, rescaled to sum to 1.0.

Horvitz, 1989; Ivey et al., 2003; Price et al., 2005). Here, we sampled across the times of the day, but we did not sample in multiple years or times of the year and each study took place during a three- to five-day period. The issue of temporal variation is mitigated by the fact all studies took place in the same season (the rainy season), during which weather conditions did not vary substantially from day to day. Also, the pollinator fauna in the study areas are resident, as opposed to migratory (Greenewalt, 1960), and, thus, might be expected to shift less from year to year. Casual observations in the same locality across years (S. D. Smith, unpublished data) showed some variation in the animal species visiting a particular *Iochroma* species, but not in the broad class of pollinator species (e.g., bird or insect). Associations of plants with classes of pollinators are probably more robust across time than the specific composition of the pollinator assemblage.

Plant-pollinator associations may also vary across spatial scales, e.g., across sites (Boyd, 2004; Price et al., 2005) or along environmental gradients (Scobell & Scott, 2002; Herrera, 2005). Here, we have conducted studies of each species at a single site. Observations of several study taxa in other sites suggested that relative visitation of different classes of pollinators is similar across the species range despite variation in pollinator fauna (S. D. Smith, pers. obs.). For instance, in El Cisne, Ecuador, *Iochroma cyaneum* is mostly visited by the hummingbird *Amazilia amazilia* Lesson, whereas in Ayabaca, Peru, it is mainly visited by the hummingbirds *Coeligena iris* and *Adelomyia melanogenys*. Thus, while pollinator composition may vary across sites, *I. cyaneum* appears to be principally pollinated by hummingbirds across its range.

FLORAL DIVERSITY AND POLLINATOR RELATIONSHIPS

One goal in undertaking this study was to determine if the floral diversity in *Iochroma* corresponds to a diverse set of pollinator systems. At the broadest level, we observed three basic modes of pollination (bird, mixed bird/insect, and insect pollinated). Unlike other Andean taxa for which comparative pollination studies have been undertaken (Kay & Schemske, 2003; Pérez et al., 2006), pollination systems in *Iochroma* appeared only weakly related to floral differences (see also Smith et al., 2008). The insect-pollinated taxa *Acnistus arborescens* and *I. ellipticum* were both white, scented, and offered a low reward, but varied in shape and size (Fig. 1). The mixed bird-/insect-pollinated species *I. peruvianum* and *I. umbellatum* differed in flower color (one green, one orange), but shared two traits, small flowers and intermediate rewards (on a per-plant basis) (Table 1). The greatest floral variation was observed among the 12 bird-pollinated taxa, whose flower colors included red, white, yellow, blue, and purple and whose corolla size varied nearly three-fold (Fig. 1). One common feature among bird-pollinated species, however, was a large

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nectar reward; all hummingbird-pollinated taxa had higher rewards than the mixed or exclusively insect-pollinated taxa (see also Smith et al., 2008). This observation is in accord with other studies showing that the amount of reward is more important than visual cues (e.g., flower color) in determining hummingbird visitation (Collias & Collias, 1968; Stiles, 1976; Melendez-Ackerman et al., 1997).

We also considered the possibility that the diversity of flower form among species sharing the same pollination system could reflect lower-level specialization, e.g., for particular pollinator species. However, we found no evidence to support such an explanation. A single hummingbird or insect species (e.g., *Adelomyia melanogenys* and *Apis mellifera* L.) was observed visiting multiple species of *Iochroma*, and, conversely, a single plant species was visited by multiple pollinator species (Appendix 2). For example, an average bird-pollinated *Iochroma* species was visited by 2.4 hummingbird species, and an average insect-pollinated species by 8.0 insect species. Furthermore, measurements of pollen deposition suggested that the vast majority of these visitors were effective pollinators. Thus, it appears that *Iochroma* species do not have tightly coevolved, specialized pollination systems.

Despite visits by many pollinator species, it is possible that a given *Iochroma* species could be specialized on a guild or functional group of pollinators, which collectively explain the particular floral traits seen. If this were the case, one might expect a lack of pollinator sharing among geographically proximate but florally distinct *Iochroma* species. However, our analysis of pollinator assemblage similarity showed that diverse taxa from the same geographic region and from the same study site shared pollinator species significantly more often than those from different regions or sites (Fig. 2). This is consistent with the idea that *Iochroma* species are generalists within a broad class (such as hummingbirds) and that they tend to be visited by whichever pollinator species are locally abundant.

POLLINATOR BEHAVIOR AND REPRODUCTIVE ISOLATION

The overlap in pollinator assemblage among sympatric taxa has significant implications for the maintenance of species boundaries. Considering that nearly all pollinators were capable of transferring loads of pollen in excess of the number of ovules on any given visit, any foraging bout that included visits to multiple species would almost certainly result in interspecific pollen flow. All sympatric species studied here shared at least two pollinator species, and some as many as four (Appendix 2), making interspecific pollen flow in sympatry potentially common. On the other hand, subtle differences in visitation rates and patterns appear to restrict interspecific pollen flow. First, PS values for sympatric taxa, even those with the same broad pollination system, were typically much lower than 1 (range, 0.20–0.96; mean, 0.51), reflecting differential visitation by pollinator species. Second, we observed that individual pollinators do not tend to move between sympatric species even when the plants are growing side by side (Fig. 3). This could be explained by individual preferences (Jones & Reithel, 2001) or optimal foraging (Heinrich, 1976; Waser, 1986), but is perhaps better explained by territoriality. Areas of *Iochroma* sympatry contained several hummingbird species, including small birds (e.g., *Adelomyia melanogenys* and *Polyonymus caroli*) and larger birds (e.g., *Colibri coruscans* and *Coeligena iris*). As mentioned previously, smaller hummingbirds tended to visit less nectar-rewarding species in mixed populations, even though there is no mechanical barrier preventing them from retrieving nectar from the more rewarding species. Larger, more aggressive birds dominate the more rewarding species and defend individuals of the rewarding species from the smaller birds (Feinsinger & Colwell, 1978; Stiles, 1981). One can envision that territorial behavior might prevent or reduce gene flow among plant species in mixed patches, and that this effect would be enhanced by differences in reward (Table 1). The combination of local spatial separation of populations, perhaps due to microhabitat specialization, and hummingbird territoriality might then reduce the potentially frequent interspecific pollen flow in sympatry. The low incidence of hybridization among sympatric *Iochroma* might reflect individual pollinator fidelity driven by interactions among hummingbirds, additional pre-fertilization mechanisms (e.g., pollen competition), and/or post-fertilization mechanisms. Although artificial interspecific crosses suggest wide crossability among *Iochroma* species (Smith & Baum, 2007; S. D. Smith, unpublished data), none of the species pairs that grow in sympatry without observed hybrids have yet been examined. Thus, additional crossing studies and tests of hybrid fitness will be required to understand how *Iochroma* species coexist in northern Andean communities.

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Appendix 1. Description of plant populations used in pollination studies in Ecuador and Peru.

Species	Voucher	Study dates	Plants studied	Avg. display per plant ± SE	Hours observed	Flower hours observed[1]
Acnistus arborescens (L.) Schltdl.	Ecuador, Alluriquin, 0.32°S 78.99°W, *S. D. Smith 209* (MO, QCNE, WIS)	26–28 Dec. 2002	8	21 ± 4	12.1	208.0
Iochroma ayabacense S. Leiva	Peru, Ayabaca, 4.63°S 79.71°W, *S. D. Smith & S. Leiva G. 337* (F, HAO, MO, NY, USM, WIS)	17–19 Jan. 2004	6	186 ± 45	14.9	3156.3
I. calycinum Benth.	Ecuador, Guajalito, 0.25°S 78.81°W, *S. D. Smith 471* (F, QCNE, WIS)	28–31 Dec. 2004	6	30 ± 11	18.7	784.6
I. confertiflorum (Miers) Hunz.	Ecuador, Malpote, 1.91°S 78.97°W, *S. D. Smith & L. Lopez 482* (MO, NY, QCNE, WIS)	16–18 Jan. 2005	6	112 ± 37	14.1	1770.8
I. cornifolium (Kunth) Miers	Peru, Guzmango, 7.39°S 78.90°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 337* (F, HAO, MO, NY, USM, WIS)	27–31 Jan. 2004	9	87 ± 16	23.9	2215.2
I. cyaneum (Lindl.) M. L. Green	Ecuador, Cisne, 3.86°S 79.43°W, *S. D. Smith 227* (MO, QCNE, WIS)	7–11 Jan. 2003	14	42 ± 6	36.6	1354.0
I. edule S. Leiva	Peru, Agallpampa, 7.95°S 78.56°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 359* (F, HAO, MO, NY, USM, WIS)	6–8 Feb. 2004	6	311 ± 66	15.1	4773.2
I. ellipticum (Hook. f.) Hunz.	Ecuador, Los Gemelos, 0.63°S 90.38°W, *P. R. Izquierdo 15022* (CDRS)	8–10 May 2004	10	50[2]	12.0	200.0
I. fuchsioides (Humb. & Bonpl.) Miers	Ecuador, Cojitambo, 2.75°S 78.89°W, *S. D. Smith & L. Lopez 488* (F, NY, QCNE, WIS)	21–23 Jan. 2005	6	79 ± 14	21.1	1792.6
I. gesnerioides (Kunth) Miers	Ecuador, Pululahua, 0.039°N 78.50°W, *S. D. Smith 200* (MO, QCNE, WIS)	19–21 Dec. 2002	5	505 ± 253	12.0	7640.0
I. lehmannii Bitter[3]	Peru, Ayabaca, 4.63°S 79.71°W, *S. D. Smith & S. Leiva G. 330* (F, HAO, MO, NY, USM, WIS)	21–23 Jan. 2004	5	275 ± 119	13.2	3916.7
I. loxense (Kunth) Miers	Ecuador, Loja, 3.98°S 79.23°W, *S. D. Smith 499* (QCNE, WIS)	26–28 Jan. 2005	8	120 ± 38	13.4	1822.1
I. parvifolium (Roem. & Schult.) D'Arcy	Peru, Agallpampa, 7.95°S 78.56°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 303* (F, HAO, MO, NY, USM, WIS)	4–8 Feb. 2004	6	98 ± 38	15.1	1806.1
I. peruvianum (Dunal) J. F. Macbr.	Peru, Guzmango, 7.39°S 78.90°W, *S. D. Smith & S. J. Hall 353* (F, HAO, F, MO, NY, USM, WIS)	29–31 Jan. 2004	4	85 ± 18	11.7	830.0
I. stenanthum S. Leiva, Quipuscoa & N. W. Sawyer	Peru, Guzmango, 7.39°S 78.90°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 313* (F, HAO, MO, NY, USM, WIS)	29–31 Jan. 2004	5	209 ± 52	12.0	2511.0
I. umbellatum (Ruiz & Pav.) Hunz. ex D'Arcy	Peru, Agallpampa, 7.95°S 78.56°W, *S. D. Smith, S. Leiva G., S. J. Hall & A. Rodriguez 360* (F, HAO, MO, NY, USM, WIS)	6–8 Feb. 2004	4	81 ± 40	17.8	1556.8
Total					263.58	36337.24

[1] Flower hours were calculated from a series of observations (1 to n) as follows: FH = $(D_1 \times T_1) + (D_2 \times T_2) + \ldots (D_n \times T_n)$, where FH equals the number of flower hours, D is the display size (number of flowers on the plant), and T equals the number of hours the plant was observed.

[2] Variation in display size across the population was not recorded in *Iochroma ellipticum*.

[3] We treat *I. lehmannii* and the recently described *I. squamosum* S. Leiva & Quipuscoa as synonyms.

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Appendix 2. Pollinator taxa and visitation rates. Animals are listed by order and identified to species when possible, and only legitimate pollinators (which made contact with the reproductive organs) are included. Each unidentified visitor is given a unique number within its taxonomic group, and these identifiers are shared across plant species. For example, both *Iochroma cornifolium* and *I. peruvianum* were visited by Vespidae, sp. indet. 1. Only pollinators observed during the individual species studies are included; additional visitors observed during subsequent experiments (e.g., *Myrtis fanny*; Table 5) are not listed here. For each pollinator, plant and flower visitation rates (per flower hour) are listed (separated by a slash).

Acnistus arborescens	Hymenoptera	Apidae, *Apis mellifera* L., 0.014/0.048
		sp. indet. 1, 0.010/0.034
	Lepidoptera	sp. indet. 2, 0.010/0.091
	Diptera	Syrphidae, sp. indet. 1, 0.024/0.058
		Tipulidae, sp. indet. 1, 0.043/0.115
		sp. indet. 1, 0.019/0.048
		sp. indet. 2, 0.005/0.019
		sp. indet. 3, 0.005/0.014
Iochroma ayabacense	Apodiformes	Trochilidae, *Adelomyia melanogenys* Fraser, 0.005/0.089
		Trochilidae, *Coeligena iris* Gould, 0.005/0.097
I. calycinum	Apodiformes	Trochilidae, *Coeligena torquata* Boissoneau, 0.001/0.013
		Trochilidae, *Phaethornis* Swainson, 0.001/0.045
		Trochilidae, sp. indet. 1, 0.005/0.033
	Hymenoptera	Apidae, *Parapartamona vittigera* Moure, 0.001/0.001
		Apidae, *Plebia* sp. 2, 0.017/0.023
	Diptera	Drosophilidae, sp. indet. 1, 0.025/0.025
I. confertiflorum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.007/0.090
		Trochilidae, *Heliangelus viola* Gould, 0.004/0.082
	Hymenoptera	Apidae, *Apis mellifera*, 0.002/0.005
		Apidae, *Parapartamona vittigera*, 0.014/0.028
		Halictidae, *Caenohalictus* sp. indet. 1, 0.008/0.010
	Lepidoptera	Hesperidae, sp. indet. 1, 0.002/0.006
I. cornifolium	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.004/0.051
		Trochilidae, *Coeligena iris*, 0.008/0.125
	Hymenoptera	Apidae, *Apis mellifera*, 0.003/0.006
		Vespidae, sp. indet. 1, 0.001/0.001
I. cyaneum	Apodiformes	Trochilidae, *Amazilia amazilia* Lesson, 0.032/0.188
		Trochilidae, *Coeligena iris*, 0.008/0.049
	Hymenoptera	Vespidae, *Polybia* sp. indet., 0.027/0.040
	Lepidoptera	Sphingidae, sp. indet. 1, 0.001/0.007
		sp. indet. 1, 0.007/0.014
I. edule	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.002/0.033
		Trochilidae, *Colibri coruscans* Gould, 0.003/0.031
		Trochilidae, *Polyonymus caroli* Bourcier, 0.005/0.089
	Hymenoptera	Apidae, *Apis mellifera*, 0.0004/0.001
	Lepidoptera	Noctuidae, sp. indet. 1, 0.0003/0.001
I. ellipticum	Hymenoptera	Formicidae, *Paratrechina*, 0.002/0.002
		Formicidae, *Wasmannia auropunctata* Roger, 0.002/0.002
		Vespidae, *Pachodynerus galapagoensis* Williams, 0.003/0.005
	Lepidoptera	Geometridae, *Oxydia lignata* Warren, 0.002/0.002
		Noctuidae, *Agrotisia williamsi* Schaus, 0.002/0.002
		Noctuidae, sp. indet. 2, 0.002/0.003
		sp. indet. 3, 0.002/0.002
	Diptera	Syrphidae, *Xanthandrus agonis* Walker, 0.005/0.005
I. fuchsioides	Apodiformes	Trochilidae, *Coeligena torquata*, 0.001/0.021
		Trochilidae, *Heliangelus viola* Gould, 0.008/0.214
	Hymenoptera	Apidae, *Apis mellifera*, 0.004/0.027
		Halictidae, *Caenohalictus* sp. 2, 0.016/0.022
	Lepidoptera	Danaidae, sp. indet. 1, 0.003/0.020

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Appendix 2. Continued.

I. gesnerioides	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.002/0.040
		Trochilidae, *Coeligena torquata*, 0.0001/0.004
		Trochilidae, *Boissonneaua flavescens* Loddiges, 0.004/0.102
		Trochilidae, sp. indet. 3, 0.0001/0.003
	Hymenoptera	Apidae, *Apis mellifera*, 0.001/0.010
	Lepidoptera	Sphingidae, sp. indet. 2, 0.001/0.006
I. lehmannii	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.002/0.148
		Trochilidae, *Coeligena iris*, 0.005/0.190
	Hymenoptera	Apidae, *Apis mellifera*, 0.001/0.003
		Apidae, *Plebeia*, 0.0003/0.001
I. loxense	Apodiformes	Trochilidae, *Amazilia amazilia*, 0.017/0.251
	Hymenoptera	Apidae, *Apis mellifera*, 0.001/0.002
		Colletidae, *Chilicola* cf. *pedunculata*, 0.005/0.005
		Vespidae, *Polybia*, 0.016/0.024
I. parvifolium	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.004/0.057
		Trochilidae, *Colibri coruscans*, 0.001/0.001
		Trochilidae, *Polyonymus caroli*, 0.010/0.230
		Trochilidae, sp. indet. 2, 0.001/0.021
	Hymenoptera	Apidae, *Apis mellifera*, 0.007/0.027
		Vespidae, sp. indet. 2, 0.001/0.001
I. peruvianum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.008/0.047
	Hymenoptera	Apidae, *Apis mellifera*, 0.021/0.055
		Vespidae, sp. indet. 1, 0.004/0.005
I. stenanthum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.006/0.192
		Trochilidae, *Coeligena iris*, 0.004/0.068
	Hymenoptera	Apidae, *Apis mellifera*, 0.002/0.004
I. umbellatum	Apodiformes	Trochilidae, *Adelomyia melanogenys*, 0.007/0.153
		Trochilidae, *Colibri coruscans*, 0.001/0.001
	Hymenoptera	Apidae, *Apis mellifera*, 0.019/0.131
		Anthophoridae, *Melissodes*, 0.003/0.009
	Lepidoptera	Hesperidae, sp. indet. 2, 0.001/0.001
	Diptera	Syrphidae, sp. indet. 2, 0.002/0.003