Pollinator Assemblages and Visitation Rates for 11 Species of Neotropical *Costus* (Costaceae)[1]

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ABSTRACT

Most plant species in tropical forests are pollinated by animals, and yet the diversity and specificity of pollinator assemblages are poorly documented. Here, we investigated pollinator relationships for 11 species of understory herbs in the genus *Costus*, with the goal of documenting visitation rates and pollinator assemblages among a variety of habitats. For a subset of species, we documented pollinator visitation for multiple years and/or multiple sites to examine temporal and spatial variation in pollinator relationships. Furthermore, we examined the extent to which specialization in pollination systems can contribute to reproductive isolation for sympatric species. Each species was primarily pollinated by either euglossine bees or hummingbirds. Total visitation rates were generally low, averaging 3.2 visits per flower per hour for bee-pollinated species and 0.5 visits per flower per hour for hummingbird-pollinated species. All of the higher elevation species studied were hummingbird-pollinated, while low elevation species were pollinated either by euglossine bees or hummingbirds. Spatial and temporal variation in visitation rates and pollinator identities was minimal. Pollinator specificity was found to contribute strongly to reproductive isolation for the 11 pairwise combinations of sympatric species differing in pollination syndrome, in some cases functioning as a complete barrier to potential pollen flow.

RESUMEN

La mayoría de las especies vegetales de los bosques tropicales son polinizadas por animales, pero la diversidad y las especies de los gremios de polinizadores son poco conocidas. En este trabajo investigamos las interacciones de los polinizadores de 11 especies de hierbas del sotobosque pertenecientes al género *Costus*, con el objetivo de documentar la frecuencia de visitas y los gremios de polinizadores en distintos hábitats. Para un subconjunto de las especies, documentamos las visitas de los polinizadores en varios años y/o sitios para examinar la variación espacial y temporal de las interacciones de los polinizadores. Además, examinamos como la especialización de los sistemas de polinización puede contribuir al aislamiento reproductivo de especies simpátricas. Cada especie fue polinizada principalmente ya sea por abejas euglosinas o colibríes. Las frecuencia de visitas fue baja en general, con un promedio de 3.2 visitas/flor/hora en las especies polinizadas por abejas y 0.5 visitas/flor/hora en las especies polinizadas por colibríes. Las especies localizadas a mayor altitud fueron polinizadas por colibríes, mientras que las de bajas elevaciones fueron polinizadas por ambos. La diferencia espacial y temporal en frecuencia de visitas e identidad de polinizadores fue mínima. Se encontró que la especificidad de los polinizadores contribuye al aislamiento reproductivo, en las 11 combinaciones reciprocas de especies simpátricas se observaron síndromes de polinización diferentes, que en algunos caso sirvieron como una barrera efectiva al flujo potencial de polen.

RELATIONSHIPS BETWEEN PLANTS AND THEIR POLLINATORS have been the subject of much interest because of their implications for the evolution of floral characters, patterns of gene flow, and the number, strength, and variance of species interactions in communities. Tropical regions harbor a spectacular diversity of plant species and floral phenotypes, and tropical pollination systems are often assumed to be more highly specialized than temperate systems (Feinsinger 1983, Johnson & Steiner 2000), although recent reviews of specialization across latitudinal gradients have reached conflicting conclusions (Olesen & Jordano 2002, Ollerton & Cranmer 2002). Pollinator specificity can also contribute to reproductive isolation between closely related plant species (Stebbins 1970, 1974; Grant 1981; Fulton & Hodges 1999; Schemske & Bradshaw 1999), and so specialization in pollination systems could promote speciation. Nevertheless, detailed information on pollinator relationships is lacking for most tropical plant taxa. Furthermore, the po-
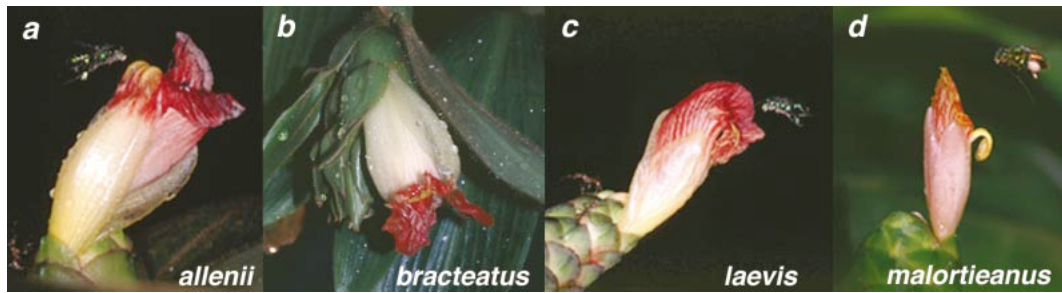
FIGURE 1.   *Costus* species included in the study that were classified by Maas (1977) as bee-pollinated.

tential role of pollinator specificity in reproductive isolation has not been directly quantified for any tropical plant group.

The deficiency of data on pollination in the tropics is understandable in light of the special challenges presented by tropical forests. While plant diversity is high, the density of individual plant species is often extremely low (Janzen 1970), making large sample sizes difficult to obtain. Trees and epiphytes in the canopy and subcanopy often possess abundant displays of flowers, but are logistically difficult to observe, while understory herbs often produce only one or a few flowers at a time. Interestingly, for plant species growing at low density, specialization may be favored to ensure pollen transfer between highly dispersed conspecifics while minimizing deposition of heterospecific pollen (Feinsinger 1978). To better understand tropical pollination systems, information is needed on pollinator identities, visitation rates, and relative efficiencies of pollen transfer, preferably measured throughout a species' range and across time. Unfortunately for most plant taxa, not even the identity of pollinators is known. Often plants are quickly categorized based on sparse observations and suites of floral characters that correspond to recognized pollination syndromes, but it is unclear how well these syndromes predict actual pollinators (Waser *et al.* 1996, Ollerton 1998, Ollerton & Watts 2000).

Maas (1977) used floral characters and scant data on pollinator visitation to classify Neotropical members of the genus *Costus* into pollination syndromes. In the Neotropics, the genus comprises *ca* 60 species of understory terrestrial herbs (Maas 1972). Although a few pollinator observations have been published (Maas 1977, Stiles 1978, Schemske 1981, Grove 1985, Sytsma 1985), there has been no systematic investigation into pollinator relationships in the genus. Thus, this group provided an

opportunity to identify the pollinator assemblages for a diverse set of tropical species and test the pollination syndromes proposed by Maas (1977).

In undisturbed forest, *Costus* generally grow at extremely low density, sometimes with hundreds of meters between flowering individuals. Most have spiral stems with terminal inflorescences that produce only one flower per day (rarely two) over an extended flowering period. Maas (1977) proposed that Neotropical *Costus* species are pollinated by either euglossine bees or hummingbirds. Flowers of the putatively bee-pollinated *Costus* have a short, broad labellar tube with a distinct white or yellow limb; the lateral lobes of the limb are often striped with red or purple; entry to the flower is blocked by the stamen; and the floral bracts are green (Fig. 1). The putatively hummingbird-pollinated species have flowers with a narrow, tubular labellum without an attached limb, and the labellum and floral bracts are yellow, orange, or red (Fig. 2). Flowers in both pollination categories are odorless and diurnal, and they produce relatively large quantities of nectar.

Evolutionary specialization by plants on their pollinators has been considered important to the diversification of many angiosperm lineages because it may drive adaptive divergence in floral morphology and can contribute to the origin and maintenance of reproductive isolation among close relatives (Stebbins 1970, 1974; Grant 1981). Much attention has been paid to evolutionary shifts in pollination syndromes as isolating mechanisms between sympatric congeners (Grant 1994, Fulton & Hodges 1999, Schemske & Bradshaw 1999). This attention is justified, however, only if shifts in syndrome accurately indicate specialization on different pollinators and other strong isolating mechanisms are absent. Other potentially important prezygotic mechanisms of reproductive isolation in plants include habitat segregation, differences in
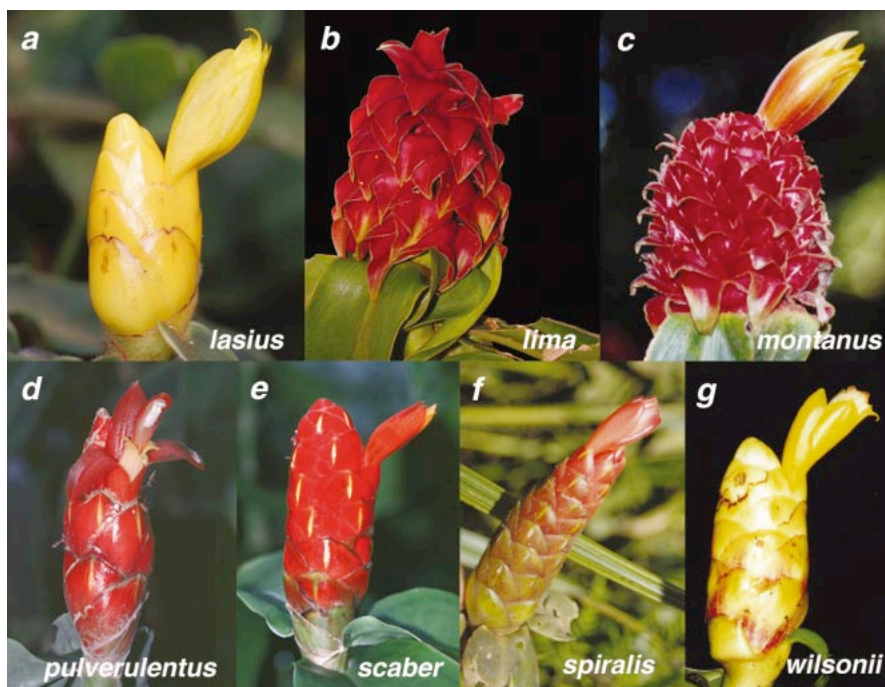
FIGURE 2.   *Costus* species included in the study that were classified by Maas (1977) as hummingbird-pollinated.

flowering phenology, floral constancy of shared pollinator species, mechanical inefficiency of pollen transfer, and incompatible pollen–pistil interactions, while post-zygotic mechanisms may include hybrid inviability, infertility, or reduced fitness. *Costus* presented an excellent system to investigate the role of plant–pollinator interactions in reproductive isolation because it has undergone a species radiation in the Neotropics involving multiple shifts in pollination syndrome (Specht *et al.* 2001). Furthermore, it is common to find multiple *Costus* species, representing both bee and hummingbird syndromes, in sympatry at any given Neotropical lowland rain forest site.

We documented pollinator assemblages and visitation rates for 11 species of *Costus*. For a subset of these species, observations were made for multiple years and/or at multiple sites, and intensive observations were made of sympatric species at three sites. We explored the following questions: (1) How well do the suites of floral characters proposed by Maas (1977) for bee- and hummingbird-pollinated *Costus* predict their actual pollinators?; (2) How much variation in pollinator visitation is there across time and space?; and (3) Does pollinator specificity contribute to the reproductive isolation of sympatric *Costus*?

MATERIALS AND METHODS

STUDY SPECIES AND SITES.—Observations were made at five lowland sites and three mid-elevation sites. Lowland sites included Barro Colorado Island Nature Monument (BCI) in Panama, La Selva Biological Station (La Selva) and Sirena Biological Station (Sirena) in Costa Rica, and along the Rio Moile (Moile) and near the town of Asunción de Guarayos (Guarayos) in Bolivia. BCI (9°09′N, 79°51′W) is a 16 km² island located in Gatun Lake in the Panama Canal that consists of mature, tropical moist forest (Croat 1978). La Selva (10°25′N, 84°00′W) is a 1536 ha reserve in the Atlantic lowlands of Costa Rica that shares a boundary with the extensive Braulio Carillo National Park. It consists of mature tropical wet forest as well as regenerating agricultural lands (McDade & Hartshorn 1994). Sirena (8°29′, 83°35′W) is located along the Pacific Coast of Costa Rica in the expansive Area Conservación de Osa, and the surroundings consist of mature, tropical wet forest and regenerating agricultural lands. The Moile site (17°01′S, 64°02′W) was located on the border of the 430,000 ha Parque Nacional y Área de Uso Múltiple Amboró in Bolivia, in fragments of seasonal moist forest. The Guarayos site (15°23′S,

TABLE 1. *Pollination syndromes and observation periods of* Costus *species.*

Species	Syndrome[a]	Site[b]	Peak flowering[c]	Year observed	Month(s) observed	No. plants	No. hours
C. allenii	bee	BCI	June–Aug.	1998	July	2	24
C. bracteatus	bee	LS	Apr.–June	2000	May–Aug.	4	113
C. laevis	bee	BCI	May–Aug.	1998	July	2	26
		Sirena	May–Aug.	2002	July	6	41
C. lima	hummingbird	Sirena	May–July	2002	July	10	69
C. malortieanus	bee	LS	Aug.–Dec.	1997	Aug.	3	20
				1999	Feb.	2	12
				2000	July–Aug.	4	50
				2002	Oct.	6	47
C. lasius	hummingbird	EV	NA	1999	July	3	13
C. montanus	hummingbird	MV	May–July	1999	July	3	11
C. pulverulentus	hummingbird	BCI	June–Aug.	1998	July	4	65
				1999	July–Aug.	7	36
		LS	May–Aug.	1998	July	3	43
				1999	July	5	24
				2000	May–Aug.	19	282
		Sirena	May–July	2002	July	10	61
C. scaber	hummingbird	BCI	May–Aug.	1999	July–Aug.	4	28
		LS	May–Aug.	1999	July	5	62
				2000	May–Aug.	21	351
		Moile	NA	2001	Mar.	8	31
		Sirena	May–July	2002	July	14	78
C. spiralis	hummingbird	Guarayos	NA	2001	Apr.	8	8
C. wilsonii	hummingbird	LA	NA	2000	Sept.	3	9

[a] Based on Maas (1977).
[b] Sites: BCI: Barro Colorado Island, Panama; EV: El Valle de Antón, Panama; Guarayos: Asunción de Guarayos, Bolivia; LA: Las Alturas, Costa Rica; LS: La Selva Biological Station, Costa Rica; Moile: Rio Moile, Bolivia; MV: Monteverde, Costa Rica; Sirena; Sirena Biological Station, Costa Rica.
[c] Flowering phenology of all species is based on unpublished data and personal observations, except for *Costus malortieanus* and *C. pulverulentus* at La Selva (Stiles 1978), and *C. allenii* and *C. laevis* at BCI (Schemske 1981). NA: information not available.

63°00′W) was outside of the town of Asunción de Guarayos in forest patches and along small creeks, in a patchwork matrix of semievergreen moist forest and cleared rangeland. Our mid-elevation sites included (1) El Valle, Cocle Province, Panama, (800–1000 m; 8°38′N, 80°07′W), (2) Monteverde, Puntarenas Province, Costa Rica (1200–1400 m; 10°17′N, 84°47′W), and (3) Parque Nacional La Amistad near the Las Alturas Field Station, Puntarenas Province, Costa Rica (1500–1600 m; 8°57′N, 82°50′W). These sites represent lower montane wet forest, premontane wet forest, and montane wet forest, respectively.

Our study included four species classified by Maas (1977) as bee-pollinated (Fig. 1a–d) and seven classified as hummingbird-pollinated (Fig. 2a–g). At La Selva, we studied *C. bracteatus* (Fig. 1b), *C. malortieanus* (Fig. 1d), *C. pulverulentus* (Fig. 2d), and *C. scaber* (Fig. 2e), and at BCI, we studied *C. allenii* (Fig. 1a), *C. laevis* (Fig. 1c), *C. pulverulentus*, and *C. scaber*. At Sirena, we studied all species, including *C. laevis*, *C. lima* (Fig. 2b), *C. pul-*

verulentus, and *C. scaber*. *Costus scaber* was studied at Moile, and *C. spiralis* (Fig. 2f) at Guarayos. All species studied at mid-elevation sites exhibited hummingbird pollination syndromes and included *C. lasius* (Fig. 2a) at El Valle, *C. montanus* (Fig. 2c) at Monteverde, and *C. wilsonii* (Fig. 2g) at Las Alturas.

FLOWER VISITATION.—We made 1504 total hours of observations from 1997 to 2002, primarily during May through August when *Costus* was at its peak flowering in the Central American rainy season. Dates and sites of observations and sample sizes are summarized for each species in Table 1. Peak months of flowering, when known, are also noted for each species, although it is typically possible to find a few individuals flowering throughout the year. Because of low plant density and sporadic flower production, we were unable to sample plants for a standardized amount of time. The number of individuals observed typically represents what we could find during the time spent at each site, and

TABLE 2. *Pollinator visitation rates*[a] *for bee-pollinated* Costus.

Plant species	Site	Year	Pollinator species[b]					
			Euglossa	*Eulaema*	*Exaerete*	*Trigona*	Other bees	*P. s.*
C. allenii	BCI	1998	**9.66** (216)	—	—	—	—	—
C. bracteatus	LS	2000	**0.18** (42)	—	—	—	—	—
C. laevis	BCI	1998	**7.61** (176)	—	—	—	—	—
	Sirena	2002	**0.55** (25)	—	—	**0.06** (2)	**0.02** (1)	**0.08** (2)
C. malortieanus	LS	1997	**0.51** (52)	—	**0.01** (1)	—	—	—
		1999	**0.55** (9)	**0.19** (3)	—	—	—	—
		2000	**0.56** (20)	**0.10** (6)	**0.03** (2)	—	—	—
		2002	**0.27** (13)	**0.06** (3)	—	**0.04** (2)	—	**0.33** (26)

[a] Rates presented in bold are population means per flower per hour. Parentheses under each rate represent total number of legitimate visits.
[b] Pollinators: *P. s.*: *Phaethornis superciliosus.*

every effort was made to spread the hours of observation evenly among them. Because *Costus* has the ability to spread clonally, plants within 5 m of each other possibly represent the same genet; therefore, only one was chosen for observation. Distances between conspecific individuals observed at a site typically ranged from tens of meters to a few kilometers. The majority of our observations was made by video, using cameras set on tripods and covered with camouflage plastic covers and/or vegetation. Cameras ran for four hours between tape changes, which allowed us to observe multiple isolated plants for a major portion of the day. Direct observations were made with binoculars from a distance sufficient to avoid disturbing pollinators. The flowers of each *Costus* species open at dawn and last for only a single day, and observations were made from dawn until mid-afternoon, at which point the flowers either fell off or wilted and were no longer visited. Visitors were considered legitimate pollinators if they appeared to contact the anthers and stigma. We did not quantify the pollination success of the legitimate visitors. Hummingbirds were identified to species and bees were identified to genus. Identification of bees to species would have required collecting, which was impractical for video observations.

DATA ANALYSIS.—Visitation rates are first presented separately by plant species, site, and year. For each type of visitor, a population mean visitation rate (per flower per hour) was calculated from the individual rates of each observed plant. To estimate the potential contribution of pollinator specificity to reproductive isolation, we determined the proportional similarity (PS) in pollinator assembly for pairs of sympatric congeners, calculated as $PS = 1 - 1/2 \sum_{i=1}^{n} |P_{ai} - P_{bi}|$, where P_{ai} and P_{bi} are the proportion of the total visitation rate made up by taxon *i* for plant species *a* and *b*, respectively (modified from Schemske & Brokaw 1981). This index, which ranges from 0 to 1, takes into account both the identity of pollinators and their relative visitation rates. Data were combined across years for populations sampled more than once. Lower values indicate less overlap in pollinator use, and therefore, a larger potential contribution of pollination system to reproductive isolation. Although the *Costus* species studied appeared to have subtly distinct habitat preferences, all species growing at a site were considered sympatric, as distances between conspecifics were similar to distances between heterospecifics.

RESULTS

Bees (Apoidea; Table 2) and hummingbirds (Trochilidae; Tables 3 and 4) were the sole pollinators of the *Costus* in our study, and each *Costus* species was specialized on one of these two groups of pollinators. The pollination syndromes proposed by

TABLE 3. *Pollinator visitation rates[a] for low elevation, hummingbird-pollinated* Costus.

| Plant species | Site | Year | Hermits[b] | | | | | Non-hermits[b] | | | | Bees[b] |
			G. h.	P. p.	P. r.	P. sub.	P. sup.	A. t.	A. n.	T. c.	T. f.	Eug.
C. lima	Sirena	2002	—	—	—	—	**0.29** (19)	—	—	—	—	—
C. pulverulentus	BCI	1998	—	—	—	—	**0.13** (8)	—	—	—	—	—
		1999	—	—	—	—	**0.09** (4)	—	—	—	—	—
	LS	1998	—	—	—	—	**0.16** (11)	—	—	—	—	—
		1999	—	—	—	—	**0.23** (5)	—	—	—	—	—
		2000	—	—	—	—	**0.16** (30)	—	—	—	—	—
	Sirena	2002	—	—	—	—	**0.25** (15)	—	—	—	—	—
C. scaber	BCI	1999	—	—	—	—	**1.13** (51)	—	—	**0.08** (6)	—	**0.06** (2)
	LS	1999	—	—	—	—	**0.06** (6)	**0.03** (11)	—	**0.22** (81)	—	—
		2000	—	—	—	—	**0.25** (111)	—	—	**0.05** (23)	—	—
	Moile	2001	—	**0.03** (1)	**0.75** (32)	**0.21** (9)	—	—	—	—	**0.01** (1)	—
	Sirena	2002	—	—	—	—	**0.22** (14)	—	—	**0.10** (10)	—	—
C. spiralis	Guarayos	2001	**1.34** (18)	—	—	—	—	—	**0.25** (15)	—	—	—

[a] Rates presented in bold are population means per flower per hour. Parentheses under each rate represent total number of visits.
[b] Pollinators: *G. h.*: *Glaucis hirsuta*; *P. p.*: *Phaethornis pretrei*; *P. r.*: *Phaethornis ruber*; *P. sub.*: *Phaethornis subochraceous*; *P. sup.*: *Phaethornis superciliosus*; *A. t.*: *Amazilia tzacatl*; *A. n.*: *Anthracothorax nigricollis*; *T. c.*: *Thalurania columbica*; *T. f.*: *Thalurania furcata*; *Eug.*: *Euglossa*.

TABLE 4.   *Pollinator visitation rates*[a] *for high elevation, hummingbird-pollinated* Costus.

Plant species	Site	Year	Pollinator species[b]				
			C. h.	*E. e.*	*E. c.*	*P. l.*	*P. g.*
C. lasius	EV	1999	—	—	**0.21** (5)	**0.14** (4)	—
C. montanus	MV	1999	**0.40** (4)	**0.08** (34)	—	—	—
C. wilsonii	LA	2000	—	—	—	—	**0.16** (2)

[a] Rates presented in bold are population means per flower per hour. Parentheses under each rate represent total number of legitimate visits.
[b] Pollinators: *C. h.*: *Campylopterus hemileucurus*; *E. e.*: *Eupherusa eximia*; *E. c.*: *Elvira chionura*; *P. l.*: *Phaethornis longuemareus*; *P. g.*: *Phaethornis guy.*

Maas (1977) were effective at predicting the pollinators for all 11 species. Of the 601 legitimate visits to the 4 species predicted to be bee-pollinated, 573 were by bees and 568 were specifically by euglossine bees (Euglossini). Of 532 legitimate visits to the 7 species predicted to be hummingbird-pollinated, 530 were by hummingbirds. Of the 12 site/species combinations for the 7 putatively hummingbird-pollinated species, the mean percentage of visits by hummingbirds was 99.6 (range = 96.6–100). Of the 5 site/species combinations representing the 4 putatively bee-pollinated species, the mean percentage of visits by bees was 94.9 (range = 81.0–100). Only *C. malortieanus* received a substantial number of visits by both bees and hummingbirds, and this was only observed during the fall of 2002. In all other years, bees were the only legitimate visitors to *C. malortieanus*. Illegitimate visits, primarily by shorter-billed hummingbirds that pierced the base of the corolla, were common for both bee- and hummingbird-pollinated species (KMK, pers. obs.).

Within the hummingbirds, there was a variety of pollinating species, including both Phaethornine (hermit) and Trochiline (non-hermit) hummingbirds. Hermits included *Glaucis hirsuta*, *Phaethornis guy*, *P. longuemareus*, *P. pretrei*, *P. ruber*, *P. subochraceous*, and *P. superciliosus*. Non-hermits included *Amazilia tzacatl*, *Anthracothorax nigricollis*, *Campylopterus hemileucurus*, *Elvira chionura*, *Eupherusa eximia*, *Thalurania columbica*, and *T. furcata*. Most bees belonged to three genera in the tribe Euglossini (Apidae), *Euglossa*, *Eulaema*, and *Exaerete*, with *Euglossa* by far the most common. Overall, there was a mean of 2.5, a median of 2, and a mode of 1 visiting taxa per plant species, and for every species/site combination sampled, the most frequent visitor made up at least 60 percent of the total

visitation rate. The visitation rates to the bee-pollinated species at BCI were considerably higher than those at La Selva or Sirena. In general, the hummingbird-pollinated species, especially those with only one visiting taxon, had extremely low visitation rates. Across plant species, visitation rates were generally slightly higher at mid-morning and declined after noon, but no patterns in the identity of visitors across the day were detected (KMK, pers. obs.).

There were only minor fluctuations in total visitation rate among years and sites, except in the case of *C. scaber*, for which BCI and Moile had much higher visitation rates than either Costa Rican site, and *C. laevis*, for which the bee visitation rate at BCI was 12 times higher than at Sirena. At the level of visiting taxa, there was little temporal and spatial variation in the composition of the pollinator assemblage and the relative visitation rates for most *Costus* species. One notable exception was the difference between Central and South American populations of *C. scaber*. The observed hummingbird species do not occur both in Central America and at Moile in Bolivia; however, while the species of hummingbird visitors at Moile were different than in Central America, the genera were the same. Also, the pollination system of *C. malortieanus* exhibited some temporal variation at La Selva, with *P. superciliosus* only observed visiting during the October 2002 sampling.

Proportional similarity in pollinator assemblage was consistently low ($\bar{x} = 0.08$) for sympatric congeners differing in pollination syndrome, while it was consistently high ($\bar{x} = 0.81$) for pairs sharing a syndrome (Table 5). These values indicate that there was little overlap in pollinator use for species living at the same site but exhibiting different pollination syndromes. In contrast, specialization by

TABLE 5. *Mean proportional similarity[a] $\pm$ 1SD in pollinator assemblage for sympatric* Costus *species.*

Site	Same syndrome	Different syndrome
BCI	**0.95** $\pm$ 0.078 (2)	**0.03** $\pm$ 0.029 (4)
LS	**0.70** $\pm$ 0.071 (2)	**0.10** $\pm$ 0.114 (4)
Sirena	**0.79** $\pm$ 0.180 (3)	**0.11** $\pm$ 0 (3)
All sites	**0.81** $\pm$ 0.152	**0.08** $\pm$ 0.076

[a] Proportional similarity (PS) in pollinator assemblage is calculated as $1 - \frac{1}{2} \sum_{i=1}^{n} |Pai - Pbi|$, where P_{ai} and P_{bi} are the proportion of the total visitation rate made up by taxon *i* for species *a* and *b,* respectively. PS was calculated separately for each pairwise combination of species at a site using composite visitation rates calculated across all individual plants regardless of sampling year, and then averaged across all pairs in each category. Sample sizes of pairwise combinations in each category follow PS values in parentheses. Some pairwise combinations of species occurred at more than one site.

Costus species within functional groups of pollinators did not greatly reduce overlap in pollinator use.

DISCUSSION

Specialization and pollination syndromes in *Costus*.—Of the scores of potential pollinators in the tropical forest, each *Costus* species is pollinated by a limited number of taxa, indicating a high level of specialization by these plants to their pollinators. Although sample sizes for some *Costus* species were low, we probably have not missed many important pollinators for a given species at the site and time period sampled. Species accumulation curves constructed for well-sampled site/year combinations saturate quickly. Curves were computed using EstimateS (Colwell 1997) to randomize across sample order, and they give Michaelis–Menten estimates of the asymptote equal to the total observed number of pollinating taxa with just one to three individual plants observed. For most species, we found that sampling at multiple sites or across multiple years uncovered no or only very rare new visitors. For *C. scaber*, however, adding a geographically distant site in South America doubled the number of hummingbird species but did not increase the number of genera. Further, we found that sampling *C. malortieanus* at the end of the wet season at La Selva uncovered previously unobserved hummingbird visits. In general, we do not know the relative success of each pollinator at effecting seed set, which may or may not correspond to visitation rates (Schemske & Horvitz 1984); thus, it

is possible that we are underestimating specialization by including ineffective visitors. Finally, specialization in these systems was greater for plants than for pollinators because most of the visiting taxa are known to visit other plant species (Dressler 1968, Snow & Snow 1972, Feinsinger 1976, Stiles and Wolf 1979).

Our results support the idea that suites of characters associated with bee and hummingbird pollination syndromes indicate specialization by plants to these pollinators. Although the *Costus* species exhibit traits generally associated with bee and hummingbird pollination syndromes, more detailed knowledge of their natural history and floral morphology may have predicted that many would be further specialized on euglossine bees or hermit hummingbirds. Plants growing at low density and displaying only one or a few flowers at a time are unlikely to be pollinated by animals defending foraging territories. Both euglossine bees and hermit hummingbirds have been noted for their long foraging routes among isolated flowers in tropical forests (Skutch 1964, Janzen 1971, Stiles & Wolf 1979). *Costus* species growing in denser patches, such as *C. montanus*, *C. spiralis*, and some individuals of *C. scaber*, were visited by more territorial non-hermit hummingbirds. The long, curved corolla tubes of the hummingbird-pollinated species also suggests some specialization on hermits, which generally have long, decurved bills, and the large size of the bee-pollinated flowers fits with the large body sizes and long tongues of euglossine bees. Nevertheless, pollination syndromes did not predict all visits for all species. For example, the putatively bee-pollinated *C. malortieanus* at La Selva displayed substantial temporal variation in pollinator assemblage, with bees composing 100 percent of the visitation rates from 1997 to 2000 when it was observed during the dry season or at the peak of the wet season, but only 53 percent of the visitation rate in the late wet season of 2002 when the hummingbird *P. superciliosus* was a common visitor. *Phaethornis superciliosus* had previously been observed visiting *C. malortieanus* at that time of year (Stiles 1978), and this has been interpreted as a response to a seasonal nectar shortage in the forest (Grove 1985). The uncommon visits of *P. superciliosus* to bee-pollinated species (Table 2) and *Euglossa* sp. to a hummingbird-pollinated species (Table 3) show that specialization is not absolute and suggest potential mechanisms by which evolutionary shifts in pollination syndrome could occur. Phylogenetic evidence suggests that there have

been multiple pollinator shifts in the genus (Specht *et al.* 2001).

PLANT–POLLINATOR INTERACTIONS AND REPRODUCTIVE ISOLATION.—Our studies of sympatric *Costus* differing in pollination syndrome allowed us to explicitly quantify the potential contribution of pollination syndromes to reproductive isolation. The sympatric pairs of species differing in syndrome (Table 5) included in this analysis all showed very little or no overlap in pollinator use. Comparatively, sympatric pairs sharing a syndrome all had high overlap in pollinator use. Since there was extensive overlap in flowering phenology and spatial distribution for sympatric pairs (Table 1; KMK & DWS, pers. obs.), specialization in pollination syndrome is an important mechanism for preventing pollen flow between sympatric congeners. Furthermore, we have conducted artificial interspecific crosses in the greenhouse for 5 of the 11 sympatric pairs differing in syndrome and found that all 5 readily set seed and that hybrids show high fertility (KMK & DWS, pers. obs.). Therefore, specialization in pollination system is an important mechanism for preventing actual gene flow between these sympatric *Costus*. Although it is unknown whether there were other important isolating mechanisms in place at an earlier stage of speciation, our studies show that specialization in pollination system is currently a primary mechanism of reproductive isolation for these species pairs.

In our study, five sympatric species pairs shared the same pollination syndrome and major pollinators, and therefore provided an excellent opportunity to investigate reproductive isolation not caused by specialization in pollination systems. What other mechanisms might contribute to preventing gene flow between these species pairs? For *C. bracteatus* and *C. malortieanus* at La Selva, flowering phenology may be important, since typically most *C. bracteatus* finish flowering before *C. ma-lortieanus* starts. For *C. pulverulentus* and *C. scaber*, differences in floral morphology greatly reduce pollen transfer by their shared pollinator, *P. superciliosus* (KMK, pers. obs.). At BCI, *C. allenii* and *C. laevis* flower concurrently, share pollinators, and experience substantial interspecific pollen flow but do not successfully hybridize (Schemske 1981) because of post-pollination interactions between the pollen and pistil (KMK, pers. obs.). All of these mechanisms are currently under investigation to better understand speciation processes throughout the genus.

Our results suggest a limited set of strong and consistent ecological connections between *Costus* species and their pollinators. Further, divergence in floral phenotypes associated with syndromes can contribute to reproductive isolation among close relatives living in sympatry. Although it is not clear how generally our results apply to other groups of plants, geographic regions, or syndromes, they are consistent with emerging evidence for specialized plant–pollinator relationships in other systems (McDade 1984, Armbruster 1993, Hodges & Arnold 1994, Johnson *et al.* 1998, Schemske & Bradshaw 1999), and they greatly contribute to the empirical data available for tropical pollination systems.

ACKNOWLEDGMENTS

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