Naturwissenschaften (2013) 100:69–79
DOI 10.1007/s00114-012-0995-0

ORIGINAL PAPER

Predominance of self-compatibility in hummingbird-pollinated plants in the Neotropics

Marina Wolowski · Carolina Farias Saad ·
Tia-Lynn Ashman · Leandro Freitas

Received: 13 July 2012 / Revised: 5 November 2012 / Accepted: 8 November 2012 / Published online: 23 November 2012

Abstract Both plant traits and plant–pollinator interactions are thought to influence plant mating systems. For hummingbird-pollinated plants, foraging strategy (territorial or traplining) is also expected to influence plant mating. We hypothesize that the traplining behavior of hermits promotes outcrossing, whereas the behavior of non-hermits favours self-incompatibility. Thus, selection is expected to maintain self-incompatibility in plants pollinated by non-hermits. We explore the incidence of self-incompatibility in Neotropical hummingbird-pollinated plants and its association with hummingbird behavior and plant traits. We conducted a literature review (56 species) and performed hand-pollination experiments in 27 hummingbird-pollinated plants in an Atlantic rainforest. We found that self-incompatibility (measured as <0.3 for the Index of Self-incompatibility [ISI]) occurred in only 33 % of the Neotropical hummingbird-pollinated plants. The interaction of hummingbird and habit type affected ISI, as did phylogenetic relationships. Specifically, herbs pollinated by non-hermits had higher ISI than woody plants pollinated by non-hermits, and herbs pollinated by both hermits and non-hermits. For the Atlantic rainforest plant guild, 30 % of the species were self-incompatible. ISI was higher in herbs than in woody species and increased with plant aggregation but was not dependent on foraging behavior, plant density, or floral display. Although hummingbirds differ in their foraging strategies, these behavioral differences seem to have only a minor influence on the incidence of self-incompatibility. Phylogenetic relatedness seems to be the strongest determinant of mating system in Neotropical hummingbird-pollinated plants.

Keywords Community · Mating system · Ornithophily · Pollination · Self-incompatibility

Introduction

Hummingbirds are among the most important vertebrate pollinators in the Neotropics (Grant and Grant 1968). Hummingbirds differ in their choice of habitat, resources, and foraging behavior (Snow and Snow 1972; Stiles 1981). Thus, floral morphology, nectar production, plant habit, and phenology all dictate which hummingbird species will forage on a given plant species (Stiles 1975; Snow and Snow 1972; Feinsinger 1976; Brown and Bowers 1985; Lara 2006). As a consequence, the faunal composition and visitation dynamics of hummingbird-pollinated plants is expected to vary among species (Feinsinger 1976; Lara 2006).

Two main groups of hummingbirds are recognized based on morphology and foraging strategy: hermits with long curved bills, small feet and larger bodies, and non-hermits with short straight bills, moderate to large feet and smaller bodies (Feinsinger and Colwell 1978). One major behavioral difference between hermits and non-hermits is that the latter frequently hold territories, whereas hermits do so only rarely or inconsistently (Stiles 1975). Hermits also show a consistent foraging behavior, they visit flowers at regular

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-012-0995-0) contains supplementary material, which is available to authorized users.

M. Wolowski (✉)
Programa de Pós-Graduação em Botânica, Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão 2040, Horto,
22460-030, Rio de Janeiro, RJ, Brazil
e-mail: marina.wolowski@gmail.com

C. F. Saad · L. Freitas
Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão 915, Horto,
22460-030, Rio de Janeiro, RJ, Brazil

T.-L. Ashman
Department of Biological Sciences, University of Pittsburgh,
4249 Fifth Avenue,
Pittsburgh, PA 15260, USA

periods, do not stay in a patch after they visit, and are not aggressive with other hummingbirds (Stiles 1975). As a result hermits are expected to disperse pollen over longer distances than non-hermits (Linhart 1973). Thus, we hypothesize that hermits feed by *traplining* (Janzen 1971), a behavior that consists of repeated foraging in a circuit among successive flowers or clumps (Snow and Snow 1972; Linhart 1973; Stiles 1975). Plants chosen by these two types of hummingbirds also differ. A notable example is *Heliconia* species. Species pollinated by hermits bear nectar-rich flowers with long corolla tubes and produce a few flowers each day and thus are sparsely distributed among isolated individuals. In contrast, species pollinated by non-hermits produce nectar-poor flowers with short corollas but flowers occur in clumps (Stiles 1975; Feinsinger and Colwell 1978; Martén-Rodríguez et al. 2011). In addition, at the guild level, hummingbird-pollinated plants form a gradient associated with hummingbird body size (Dalsgaard et al. 2009). Assuming that different foraging behaviors affect pollen dispersal, we predict that non-hermits disperse pollen over short distances and among more related plants whereas hermits promote longer pollen dispersion among less related individuals (Linhart 1973; Stiles 1975).

The main focus of past studies of hummingbird-pollinated plants in the Neotropics has been flowering phenology, resource production, floral morphology, and the composition and foraging behavior of pollinators (Stiles 1975; Snow and Snow 1972; Snow and Teixeira 1982; Arizmendi and Ornelas 1990; Cotton 1998; Vasconcelos and Lombardi 1999; Buzato et al. 2000; Walther and Brieschke 2001; Dziedzioch et al. 2003; Abreu and Vieira 2004; Gutiérrez et al. 2004; Leal et al. 2006; Machado et al. 2007; Dalsgaard et al. 2009; Machado 2009; Araújo et al. 2011; Las-Casas et al. 2012). This work has revealed some common features, e.g., low variation in nectar concentration (ranging from 16 % to 28 %) but high variation in nectar volume (ranging from 8.8 to 72.7 μl) (McDade and Weeks 2004); annual pattern of flowering phenology (cf. Newstrom et al. 1994) and sequential blooming among plant species (Stiles 1975, 1978; Buzato et al. 2000; Abreu and Vieira 2004; Leal et al. 2006; Araújo et al. 2011); presence of a central pollinator (Sazima et al. 1995; Leal et al 2006; Machado 2009); and network specialization at the community level (Dalsgaard et al. 2011). However, information on plant mating system is scarce and dispersed among studies (Kress 1983; McDade 1985; Linhart et al. 1987).

Self-incompatibility is distributed widely throughout all major angiosperm lineages, occurring in 39–60 % of species (Allen and Hiscock 2008; Igic et al. 2008; Raduski et al. 2012). Self-incompatibility is considered the ancestral mating system and suggested as a key factor to the ancient rapid diversification and radiation of flowering plants (Allen and Hiscock 2008). In general, self-incompatibility is associated with woody habit (Arroyo 1981; Bawa 1974) and certain clades, e.g., Magnoliids, Monocots, and Eudicots (Allen and Hiscock 2008; Igic et al. 2008). Detailed studies in Neotropical forests have recorded a high incidence of obligate outcrossing, either by the presence of self-incompatibility systems or dioecy (Bawa 1974; Bawa and Opler 1975; Zapata and Arroyo 1978; Bawa and Beach 1983; Bawa et al. 1985; Bullock 1985). However, self-incompatibility is less common in certain Neotropical plant communities such as in high-elevation montane forests (Sobrevila and Arroyo 1982; Tanner 1982) or among Neotropical herbs and shrubs (Kress 1983; Ramírez and Brito 1990; Kress and Beach 1994). Thus far, there is evidence for the presence of self-incompatibility in the following families that contain hummingbird-pollinated species: Orchidaceae, Amaryllidaceae, Bromeliaceae, Onagraceae, Passifloraceae, Malvaceae, Ericaceae, Rubiaceae, Bignoniaceae, Gesneriaceae, Acanthaceae, and Solanaceae (Igic et al. 2008; Allen and Hiscock 2008).

This study seeks to address the relationship between plant mating system and hummingbird type by answering the following questions: (1) What is the incidence of self-incompatibility in hummingbird-pollinated Neotropical plants? (2) Is the level of self-incompatibility higher in species pollinated by non-hermits? (3) Is mating system related to any plant traits, e.g., habit, density, aggregation, or floral display? We addressed these questions using two approaches: a broad literature review and a detailed study within the hummingbird-pollinated guild of plants occurring in a montane Atlantic rainforest in southeastern Brazil. We expect higher incidence of self-incompatibility in non-hermit pollinated species than hermit-pollinated species in the Neotropics. In addition, we predict that self-incompatibility is positively related to woodiness, floral display size, and aggregation but negatively related to rarity.

Materials and methods

Literature review of hummingbird-pollinated plants

A literature review was conducted using the databases Institute for Scientific Information Web of Science® and Scientific Electronic Library Online — SciELO. The following search terms were used: reproductive system AND hummingbird; breeding system AND hummingbird; mating system AND hummingbird; self-incompatibility AND hummingbird. Our search included papers published through March 2012. Other papers, dissertations, and theses from personal library collections of the authors were added.

Papers were selected based on the following criteria: (1) Studies were conducted on native species; (2) Data from fruit set after hand pollinations (self and outcross pollen) was present; and (3) Information about the hummingbird pollinators at species or genus level was available. Species pollinated by hummingbirds and other vertebrate or invertebrate pollinators were not included. ISI was calculated as the ratio of fruit set after manual self-pollination to that after cross-pollination (Zapata and Arroyo 1978). Following Ramírez and Brito (1990), ISI ≥0.30 indicate self-compatibility (SC), and values <0.30 indicates self-incompatibility (SI). Species studied in more than one site (five species) were included as a single entry for the species, and ISI values were averaged over all sites. When a species was distylous (five species), data from both morphs was averaged, first by calculating fruit set of each pollination treatment for each morph and then using the average per treatment to calculate ISI for the species. For each plant species we characterized pollinator fauna from all reports combined. For all sampled species, we recorded plant family, study site, fruit set following self- and cross-pollination, ISI, mating system category (self-compatible or self-incompatible), plant habit, and hummingbird species. Thus, plant species were classified as pollinated by hermits (H), mixed (M, hermits and non-hermits), and non-hermits (N), based on records of the composition of the hummingbird visitors. The hermit category included *Ensifera ensifera* (Boissonneau, 1840), *Glaucis hirsuta* (Gmelin, 1788), *Ramphodon naevius* (Dumont, 1818) and all species of *Phaethornis*, except *Phaethornis ruber* (Linnaeus, 1758), which usually behaves as a non-hermit (SanMartin-Gajardo and Freitas 1999). All other hummingbirds were considered as non-hermits.

Hummingbird-pollinated plant guild in the Itatiaia National Park

Itatiaia National Park (INP) is located in Serra da Mantiqueira, between Rio de Janeiro and Minas Gerais states in southeastern Brazil. The study site (22°27′S; 44°36′W) is between 900 and 1,200 m a.s.l., and its vegetation is classified as montane tropical rainforest (sensu Veloso et al. 1991). The climate is subtropical humid (i.e., 'Cwa' in Köeppen-Geiger climate classification; Peel et al. 2007) with two distinct seasons: a wet/warm (monthly mean precipitation = 202 mm, monthly mean temperature max = 29 °C, min = 19 °C) period from October to April and a dry/cold (36 mm, 26 °C, 14 °C) period from May to September (data from the climatological normal 1961–1990 at Resende county, 400 m a.s.l.).

Five hummingbird species visited this guild of plants according to Canela (2006): *Phaethornis eurynome* (Lesson, 1832), *P. squalidus* (Temminck, 1822), *Clytolaema rubricauda* (Boddaert, 1783), *Leucochloris albicollis* (Vieillot, 1818) and *Thalurania glaucopis* (Gmelin, 1788). *Phaethornis eurynome* is a resident and pollinated 22 (81 %) plant species (Canela 2006). Both *Phaethornis* species are high-reward trapliners (cf. Stiles 1975; Feinsinger and Colwell 1978) (Fig. 1a). The other hummingbird species were considered territorial, although their behaviors varied from territorial to low-reward trapliners, or territory parasites (cf. Feinsinger and Colwell 1978; Canela 2006) (Fig. 1b).

Twenty-seven hummingbird-pollinated species from nine families were studied. Bromeliaceae dominated this flora (11 species, 41 %), followed by Gesneriaceae (5), Acanthaceae (4) and Rubiaceae (2). Five additional families were represented by only one species. Study species included herbs, shrubs, vines, treelets, and epiphytes. Most studied species in this guild had floral or inflorescence traits typical of hummingbird-pollinated plants, namely tubular shape, reddish display, and imperceptible odor. However, two species, *Abutilon bedfordianum* and *Vriesea longicaulis*, have chiropterophilous characteristics, such as crepuscular anthesis and the production of scent and abundant nectar. Furthermore, butterflies were recorded visiting *Psycothria ruelliflora*. Thus, those three species were not included in the analysis.

We characterized features of the mating system using hand-pollination treatments on bagged flowers and/or inflorescences from September 2009 to August 2011. Mating system of 13 species was studied for 2 years. The number of treated individuals ranged from two to 23, and flowers/treatment from one to 455 among species (see Table 1 for specific values). We subjected flowers to the following four treatments: (1) no pollen (to test for apomixis): pre-anthesis flowers were emasculated, tagged and prevented from deposition of pollen on the stigma (for species where the anthers of one flower can easily touch the stigma of another flower, the stigma was removed prior anthesis); (2) spontaneous self-pollination: flowers in anthesis were tagged and prevented from deposition of outcross pollen on the stigma; (3) manual self-pollination: flowers in anthesis were tagged and pollinated with the self-pollen from one or more anthers per flower; and (4) manual cross-pollination: flowers in anthesis were tagged and pollinated with pollen from one or more anthers of another individual (minimum distance of 50 m, except for *Velloziela dracocephaloides* which crosses were made between branches of the single existing clump separated by at least 50 cm). Flowers and/or inflorescences were kept bagged and fruits were monitored to maturity. Fruit set (number of developed fruits/number of flowers treated) and seed production (number of seeds/fruit) were calculated for each species.

For the species that appeared to be self-incompatible and had a high availability of flowers (*Abutilon bedfordianum*, *Aechmea nudicaulis*, *Billbergia distachia*, *Quesnelia augusto-coburgii*, and *Nidularium itatiaiae*), the growth of self- and cross-pollen tubes was also assessed in five to 15 flowers per treatment per species to confirm self-incompatibility and to identify the type (gametophytic versus sporophytic) using epi-fluorescence microscopy (Martin 1959).

The Index of Autogamy (IA) was calculated as the ratio of fruit set after spontaneous self-pollination to that after

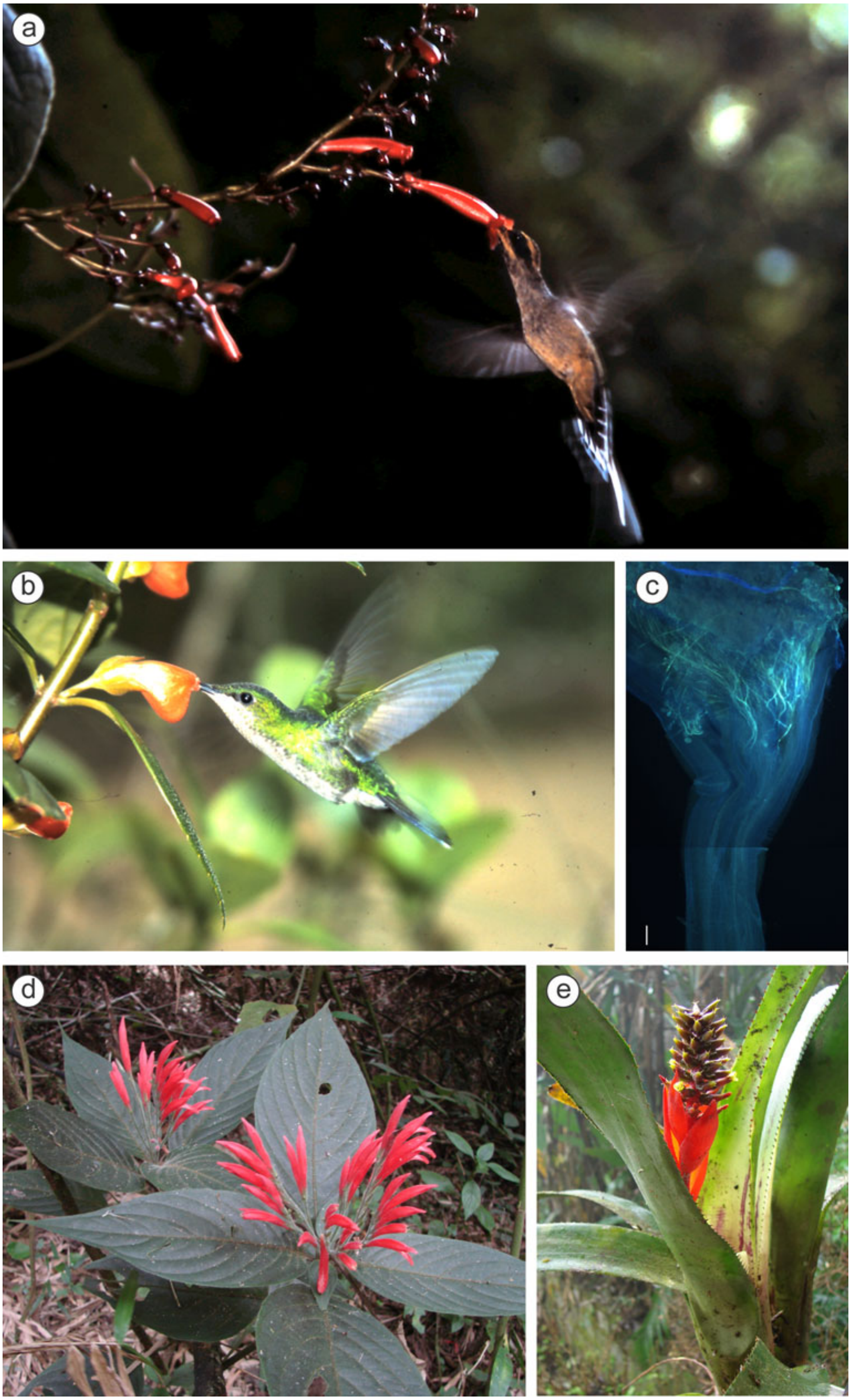

Fig. 1 **a** Pollination of *Sinningia gigantifolia* by the hermit *Phaethornis eurynome*. **b** Pollination of *Nemathantus fornix* by the non-hermit *Thalurania glaucopsis*. **c** Self-pollen tube inhibition at style of *Nidularium itatiaiae* after 36 h, bar = 100 μm. **d** *Justicia sebastianopolitanae* as an example of a species with aggregation among individuals. **e** *Aechmea nudicaulis* as an example of isolation among individuals

cross-pollination (Lloyd and Schoen 1992). Autonomous self-pollination is characterized as IA ≥0.30 (Ramírez and Brito 1990). ISI was calculated based on proportion fruit set and seed production per fruit. When a species' mating system was studied for 2 years, ISI was calculated as the average of years.

Table 1 Mating system and hummingbird pollinators of 27 hummingbird-pollinated species studied in Itatiaia National Park between 2009 and 2011

Species	Year (number of individuals)	Treatments % Fruit set (number of fruits/number of flowers)				Index of Autogamy	Index of Self-incompatibility	Hummingbird pollinator
		NP	SSP	MSP	MCP			
Acanthaceae								
Justicia sebastianopolitanae Profice	2010 (22)	0 (0/4)	7.14 (1/14)	23.08 (9/39)	36.36 (12/33)	0.10^{a}	0.70^{a}	PHEU
	2011 (14)	–	0 (0/2)	23.53 (8/34)	30.95 (13/42)			
Mendoncia vellowiana Mart.	2010 (2)	–	0 (0/22)	14.29 (3/21)	10 (2/20)	0	1.43	LEAL, PHEU, THGL
Odontonema barbelerioides (Nees) Kuntze	2010 (4)	0 (0/1)	0 (0/12)	19.44 (7/36)	24.14 (7/29)	0^{a}	0.41^{a}	PHEU, PHSQ
	2011 (3)	–	0 (0/5)	0 (0/4)	42.86 (3/7)			
Staurogyne itatiaiae (Wawra) Leonard	2010 (10)	0 (0/2)	13.33 (2/15)	58.33 (14/24)	50 (5/10)	0.17^{a}	1.49^{a}	PHEU, PHSQ
	2011 (13)	0 (0/19)	0 (0/24)	40 (18/45)	22.22 (10/45)			
Amaryllidaceae								
Hippeastrum aulicum Herb.	2011 (1)	–	–	100 (2/2)	–	–	–	PHEU, PHSQ, THGL
Bromeliaceae								
Aechmea nudicaulis (L.) Griseb.	2010 (2)	–	0 (0/10)	0 (0/14)	72.73 (8/11)	0.03^{a}	0.03^{a}	CLRU, PHEU, PHSQ, THGL
	2011 (7)	–	3.13 (1/32)	2.94 (1/34)	59.57 (28/47)			
Aechmea vanhoutteana (Van Houtte) Mez	2009 (8)	–	0.44 (1/227)	20.66 (94/455)	91.67 (154/168)	0	0.23	CLRU, PHEU, PHSQ
Billbergia distachia (Vell.) Mez	2009 (7)	0 (0/8)	0 (0/92)	10.34 (3/29)	81.25 (13/16)	0^{a}	0.25^{a}	PHEU, PHSQ, THGL
	2010 (12)	0 (0/4)	0 (0/3)	32.14 (9/28)	90 (27/30)			
Nidularium itatiaiae L.B.Sm.	2009 (23)	–	6.10 (10/164)	22.78 (18/79)	80.49 (66/82)	0. ^{a}	0.16^{a}	PHEU
	2010 (10)	0 (0/1)	0 (0/60)	2.27 (1/44)	85.71 (30/35)			
Pitcairnia flammea Lindl.	2010 (14)	–	57.89 (11/19)	85.37 (35/41)	92.98 (53/57)	0.58^{a}	0.98^{a}	PHEU, PHSQ, THGL
	2011 (5)	0 (0/11)	50 (5/10)	94.74 (18/19)	92 (23/25)			
Quesnelia augusto-coburgii Wawra	2010 (6)	0 (0/19)	3.85 (2/52)	5.71 (2/35)	41.46 (17/41)	0.06^{a}	0.07^{a}	PHEU
	2011 (5)	0 (0/11)	2.5 (1/40)	0 (0/30)	95.45 (21/22)			
Tillandsia geminiflora Brongn.	2010 (5)	–	0 (0/17)	0 (0/23)	95.65 (22/23)	0	0	CLRU, PHEU, PHSQ, THGL
Vriesea carinata Wawra	2011 (8)	–	40 (2/5)	71.43 (5/7)	75 (3/4)	0.53	0.95	PHEU, THGL
Vriesea gradata (Baker) Mez	2010 (20)	–	1.64 (1/66)	27.78 (5/18)	50 (7/14)	0.74^{a}	1.78^{a}	PHSP♦
	2011 (9)	–	18.18 (4/22)	37.5 (3/8)	12.5 (1/8)			
Vriesea longicaulis (Baker) Mez	2010 (17)	0 (0/1)	7.02 (4/57)	66.67 (10/15)	53.85 (7/13)	0.36^{a}	1.4^{a}	PHEU, THGL
	2011 (8)	0 (0/1)	25 (5/20)	66. 67 (4/6)	42.86 (3/7)			
Vriesea penduliflora L.B.Sm.	2011 (1)	–	0 (0/6)	100 (3/3)	–	–	–	CLRU
Gesneriaceae								
Nematanthus crassifolius (Schott) Wiehler	2010 (13)	–	0 (0/36)	33.33 (4/12)	57.89 (11/19)	0	0.58	PHEU
Nematanthus fornix (Vell.) Chautems	2010 (5)	–	0 (0/14)	33.33 (1/3)	75 (3/4)	0	0.44	PHEU, PHSQ, THGL
Nematanthus lanceolatus (Poir.) Chautems	2010 (10)	–	0 (0/22)	50 (7/14)	45.45 (10/22)	0	1.10	PHEU, PHSQ
Sinningia cooperi (Paxton) Wiehler	2011 (6)	0 (0/9)	13.33 (2/15)	90 (27/30)	87.5 (28/32)	0.14	1.03	PHEU, PHSQ, THGL
Sinningia gigantifolia Chautems	2010 (6)	0 (0/26)	14.81 (4/27)	66.67 (22/33)	57.14 (20/35)	0.26	1.17	PHEU, PHSQ
Lamiaceae								
Salvia sellowiana Benth.	2010/11 (8)	–	0 (0/22)	60 (21/35)	51.72 (15/29)	0	1.16	PHEU, PHSQ
Malvaceae								
Abutilon bedfordianum (Hook.) A.St.-Hil. & Naudin	2010 (13)	–	0 (0/43)	14.29 (10/70)	74.6 (47.63)	0^{a}	0.19^{a}	CLRU, PHEU, THGL
	2011 (4)	–	0 (0/60)	12.5 (3/24)	66.67 (16/24)			
Orchidaceae								
Elleanthus brasiliensis (Lindl.) Rchb.f.	2011 (4)	0 (1/7)	0 (0/6)	38.46 (5/13)	53.85 (7/13)	0	0.71	PHSQ♦
Orobanchaceae								
Velloziela dracocephaloides (Vell.) Baill.	2009 (1)	0 (0/2)	–	–	–	0	1.33	PHEU
	2010 (1)	0 (0/1)	0 (0/6)	100 (5/5)	75 (3/4)			

Table 1 (continued)

Species	Year (number of individuals)	Treatments % Fruit set (number of fruits/number of flowers)				Index of Autogamy	Index of Self-incompatibility	Hummingbird pollinator
		NP	SSP	MSP	MCP			
Rubiaceae								
Manettia mitis (Vell.) K.Schum.	2010 (4)	0 (0/7)	16.67 (3/18)	38.10 (8/21)	69.44 (25/36)	0.24	0.51[a]	PHEU, PHSQ, THGL
	2011 (6)			33.33 (12/36)	71.43 (20/28)			
Psychotria ruelliaefolia (Cham. & Schltdl.) Müll.Arg.	2010 (6 thrum)	–	–	0 (0/18)	0 (0/4)	0	0	PHSP♦
	2010 (4 pin)	–	–	0 (0/12)	38.46 (5/13)			

Year (number of individuals per species) of mating system study. Hand-pollination treatments: no pollen (NP), spontaneous self-pollination (SSP), manual self-pollination (MSP), manual cross-pollination (MCP), recorded by % fruit set (number of developed fruits/number of flowers treated). Index of Autogamy = % fruit set after SSP/% fruit set after MCP (Lloyd and Schoen 1992); Index of Self-incompatibility = % fruit set after MSP/% fruit set after MCP (Zapata and Arroyo 1978)

[a] Average values of indices for the 2 years of study of each species. Hummingbird pollinators according to Canela (2006) and this study (black diamond symbol): *Clytolaema rubricauda* (CLRU); *Leucochloris albicollis* (LEAL); *Phaethornis eurynome* (PHEU); *Phaethornis* sp. (PHSP); *Phaethornis squalidus* (PHEU); *Thalurania glaucopis* (THGL)

Composition of the flower-visiting fauna was obtained from Canela (2006) and additional observations following the same methods. Plant species were classified by the hummingbird foraging behavior categories as described above. For each plant species in the INP guild, density, aggregation, and floral display were obtained from flowering phenological data. Flowering phenology was recorded along seven trails. A total of 157 circular plots (5 m radius) were distributed every 25±5 m along the trails. We conducted phenological censuses, each month from September 2009 to February 2012. We counted the number of flowering individuals and open flowers/individual of each species. Plant species density was calculated as the highest number of flowering individuals observed in a month per total sampled area (1.23 ha). Plant species aggregation was estimated as the mean number of flowering individuals of a species in a plot (Fig. 1d and e). Floral display was calculated as the mean number of open flowers per day per individual of a species.

Data analysis

The effect of the predictor variables on mating system was assessed with phylogenetic comparative analysis using phylogenetic generalized linear models (PGLS) from the package caper (Orme 2012) in R 2.15.0 (R Development Core Team 2012). Phylogenetic trees for the species included in the literature review and in the INP guild were obtained using the angiosperm APGIII consensus tree (R20091110) from Phylomatic (Webb and Donoghue 2005). Branch lengths were calibrated from the minimum age of clade divergence (Wikström et al. 2001) using the branch length adjuster function (BLADJ) from Phylocom (Webb et al. 2008). For the literature review (73 species), we assessed the effect of hummingbird type (hermits, mixed, non-hermits), plant habit (herbaceous, woody), and hummingbird by habit interaction on ISI (based on fruit set). For the hummingbird-pollinated plant guild at INP, we assessed the effect of those traits and the following predictor variables: plant density, aggregation, and floral display on ISI (fruit set and seed production, 22 and 19 species, respectively). We conducted analyses on the full model followed by model reduction for the larger data set (literature review). For analyses of the smaller INP guild data set we were restricted to tests of the predictor variables separately. Residuals analysis was conducted in order to ensure model assumptions were met (normal distribution and homoscedasticity).

Results

Literature review of hummingbird-pollinated plants in the Neotropics

Literature review resulted in 56 species exclusively pollinated by hummingbirds with mating system assessment, in addition to the 22 species from INP that met our review criteria (Table S1 and Table 1). Five species existed in both data sets, so the final database included 73 species. Self-incompatibility (ISI <0.3) was found in 24 species (33 %). Self-incompatible species belonged to the following families: Bignoniaceae (1 species, 50 % of the species), Bromeliaceae (14, 39 %), Cactaceae (1, 25 %), Passifloraceae (1, 100 %), Rubiaceae (6, 67 %), and Solanaceae (1, 100 %). Families with all self-compatible species were Acanthaceae (5 species), Ericaceae (1), Gesneriaceae (6), Heliconiaceae (2), Lamiaceae (1), Loranthaceae (2), Orchidaceae (2), and Orobanchaceae (1). For 13 species (18 %) ISI was estimated as >1 and for these we set ISI to 1 for graphic representation (Fig. 2a).

The phylogenetic generalized linear model that best fit the data (lowest Akaike's Information Criterion (AIC) value) was the model with the interaction between hummingbird type and plant habit (AIC=80.95, F=2.99, df=67, p=0.01; Fig. 2b) and a significant phylogenetic signal was detected (λ=0.63 95 % confidence interval [0.22–0.91]). Herbaceous species pollinated by non-hermits were more likely to be self-compatible than woody species pollinated by non-hermits (t=3.33, p=0.001) and herbs pollinated by the mixed hummingbird assemblage (t=3.17, p=0.002) (Fig. 2b).

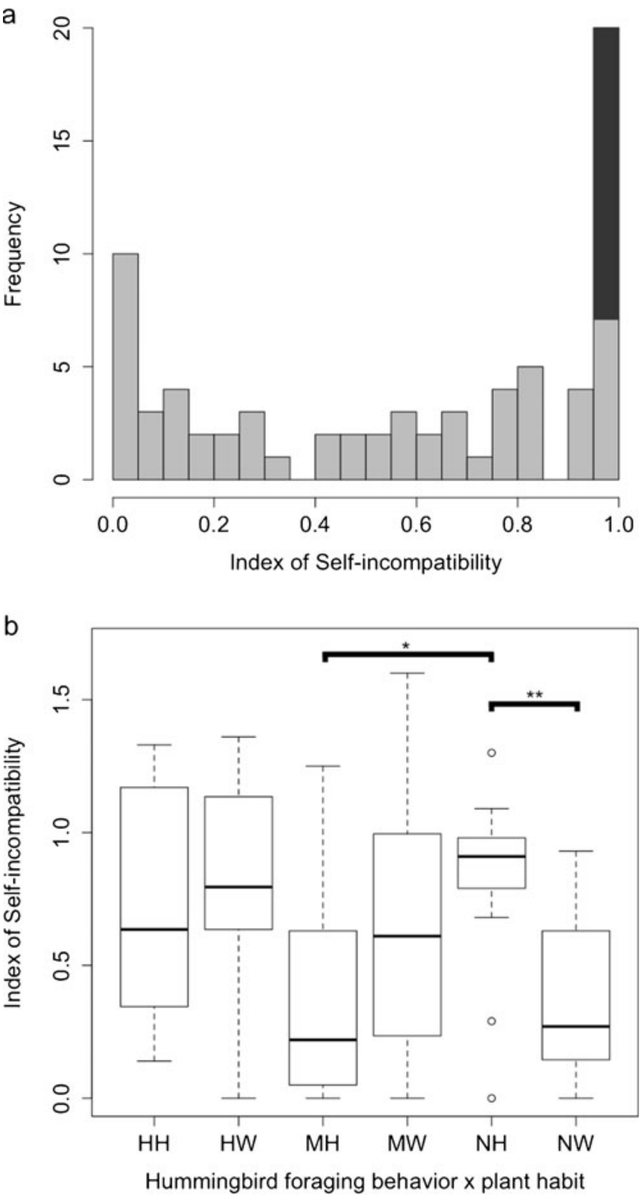

Fig. 2 Mating system of 73 hummingbird-pollinated Neotropical plants measured by Index of Self-incompatibility (Zapata and Arroyo 1978) based on fruit set. **a** Distribution of the Index of Self-incompatibility with values higher than 1 (n=13) maximized to 1 (*black*). **b** Index of Self-incompatibility of plant species grouped by hummingbird foraging behavior and plant habit (F=2.99, df=67, p=0.01, significant comparisons are denoted by *asterisks*: *p=0.002 and **p=0.001). *First upper case letter*: pollination by hermits (H), mixed (M) or non-hermits (N); *second upper case letter*: herbaceous (H) or woody (W) species

Hummingbird-pollinated plant guild in the INP

Mating system of the hummingbird-pollinated plant guild was assessed for 27 species in the INP (Table 1). None of the 13 species tested for apomixis were capable of producing fruits or seeds after bagging. Four species (14.8 %) of Bromeliaceae were able to set seeds via autonomous self-pollination (IA ≥0.30): *Vriesea longicaulis* (IA=0.36), *V. carinata* (0.53), *V. gradata* (0.58), and *Pitcairnia flammea* (0.74). Self-incompatibility was found in eight species (30 %): *Abutilon bedfordianum* (Malvaceae), *Psychotria ruelliaefolia* (Rubiaceae), and six Bromeliaceae species (*Aechmea nudicaulis*, *A. vanhoutteana*, *Billbergia distachia*, *Nidularium itatiaiae*, *Quesnelia augusto-coburgii*, and *Tillandsia geminiflora*). Pollen tube growth was studied in five of these species (*A. bedfornianum*, *A. nudicaulis*, *B. distachia*, *N. itatiaiae*, and *Q. augusto-coburgii*) and in all of them self-pollen grains germinated and grew tubes, but the site of inhibition was different among them. In *A. bedfornianum*, self-pollen tubes grew to the bottom of the style, whereas in *A. nudicaulis* self-pollen tubes were arrested at the top of the style. In *B. distachia*, *N. itatiaiae* (Fig. 1c), and *Q. augusto-coburgii*, inhibition occurred in the top third of the style.

When considering ISI based on seed production, there were significant effects of plant habit (F=10.79, df=17, p<0.001) and plant aggregation (F=5.45, df=17, p=0.01) (Fig. 3), but none for hummingbird foraging behavior (F=0.58, df=17, p=0.57), plant density (F=1.97, df=17, p=0.17), or floral display (F=0.20, df=17, p=0.82). Herbs (mean ± standard deviation 0.38 ± 0.42, median = 0.18, n = 10) had lower ISI than woody species (0.92±0.26, median = 0.96, n=9). ISI increased with plant aggregation (adjusted R^2= 0.20). Considering ISI based on fruit set, there was no significant effect of any of the predictor variables (plant habit: F=2.94, df=20, p=0.08; hummingbird foraging behavior: F=1.2, df=20, p=0.32; plant density: F=0.29, df=20, p=0.75; floral display: F=0.14, df=20, p=0.87; plant aggregation: F=0.19, df=20, p=0.83). No phylogenetic signal was detected for ISI based on seed or fruit set (i.e., 95 % confidence intervals for λ values overlap 0 or 1).

Discussion

Predominance of self-compatibility in hummingbird-pollinated plants in the Neotropics

Our review shows that self-compatibility is predominant in hummingbird-pollinated plants, which contrasts with the

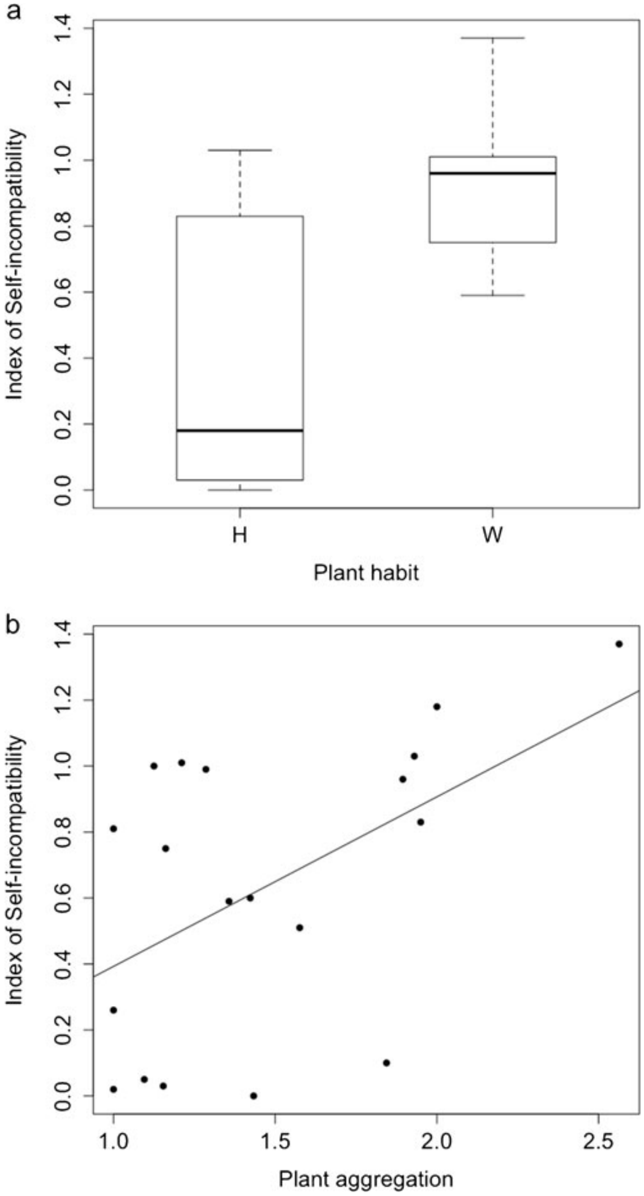

Fig. 3 **a** Mating system of 19 hummingbird-pollinated plant species in Itatiaia National Park measured by Index of Self-incompatibility (Zapata and Arroyo 1978) based on seed production for herbaceous (*H*) (median = 0.18, n=10) and woody species (*W*) (median = 0.96, n=9) (F = 10.79, df = 17, p <0.0001). **b** Index of Self-incompatibility correlation with plant aggregation (adjusted R^2=0.20, F=5.45, df=17, p=0.01)

overall incidence of obligate outcrossing among Neotropical plants (Bawa 1974; Bawa and Opler 1975; Zapata and Arroyo 1978; Bawa and Beach 1983; Bawa et al. 1985; Bullock 1985). However, it is in concordance with the perspective of Kress and Beach (1994) on mating system stratification in forests at La Selva (Costa Rica). Those authors showed that self-incompatibility dominated in the canopy relative to the understory, except for species in the Rubiaceae. Moreover, despite the lack of a physiological barrier to self-fertilization in understory plants in general, it was suggested that outcrossing may be the common mode of mating because their low daily flower production is coupled with reliable long-distance pollinators (e.g., euglossine bees and hummingbirds) (Kress and Beach 1994).

Self-incompatibility was observed in less than half of the species in the studied families (43 % in the review and 34 % in the INP guild), despite the fact that self-incompatibility has been observed in all of these families except for Lamiaceae and Loranthaceae (Igic et al. 2008). Self-compatibility is predominant in Bromeliaceae (Matallana et al. 2010), but self-incompatible species are well represented in our review (39 %) and in the INP guild (55 %). Homomorphic gametophytic self-incompatibility (GSI) is suggested for Bromeliaceae (Allen and Hiscock 2008; Igic et al. 2008), which agrees with the observed inhibition of self-pollen tubes in the styles of four of the self-incompatible species in this family at INP. However, knowledge of the genetic control of SI in the bromeliads is still lacking. In the Malvaceae the genetic control of GSI belongs to the *S-RNase* lineage of the Eudicots (Vieira et al. 2008). Although different self-incompatibility systems are described in the family (Igic and Kohn 2001; Allen and Hiscock 2008; Igic et al. 2008), GSI seems to be the case in *Abutilon bedfordianum* because self-pollen germinated and grew through style. However, the observation of self-pollen tubes near the ovary does not discount the possibility of late-acting ovarian self-incompatibility.

The categorization of mating system as a binary trait (i.e., self-compatible versus self-incompatible) is useful to assess the overall patterns, however, plant fecundity after self-pollination is a continuous trait. We observed a wide range and a bimodal distribution of the expression of self-incompatibility in hummingbird-pollinated plants (26 % [19] with ISI ≤0.2 and 40 % [29] with ISI ≥0.8, categories following Bawa 1974), a pattern similar to that proposed for angiosperms as a whole (Raduski et al. 2012). However, 34 % (25) of hummingbird-pollinated species had ISI values ranging between 0.2 and 0.8, a percentage comparable to that for mixed mating systems in angiosperms in general (Schemske and Lande 1985; Goodwillie et al. 2005; Raduski et al. 2012). Occurrence of intermediate ISI values may be the result of mutations at self-incompatibility loci causing partial compatibility, stabilizing selection acting on the expression of self-incompatibility, and/or by estimation errors (i.e., averaging across distinct morphs) or experimental biases. One must also acknowledge that there is still extensive discussion about the maintenance of mixed mating systems (Goodwillie et al. 2005; Raduski et al. 2012).

Lack of a relationship between mating system and hummingbird foraging behavior

Our results indicate that the difference in foraging behavior between hermits versus non-hermits may only weakly affect mating system. Even if we expect longer pollen dispersal by hermits (Linhart 1973), territorial birds may also disperse sufficient pollen to effect outcrossing because patches usually have more than one genet and occasional movement among patches by birds acting as territory parasites (cf. Feinsinger and Colwell 1978) could counterbalance endogamous crossings. In fact the finding that herbs pollinated by non-hermits were more self-compatible than herbs pollinated by a mixed assemblage of hummingbirds may support the efficient movement of pollen by the non-hermits. In light of the high frequency of self-compatibility and the general lack of foraging type-effects, we surmise that, hummingbirds are efficient in ensuring outcrossing independently of their foraging category.

Plant trait effects on mating system

The results of our review indicate strong phylogenetic conservation of mating system. The restricted occurrence of self-incompatible species in a few families of distinct angiosperm clades (e.g., in Monocots and Eudicots) suggests that there were no frequent changes in mating system following shifts to hummingbird pollination and that the lineages may have lost self-incompatibility before becoming hummingbird pollinated. The lack of significant phylogenetic signal in the INP guild may be due to small sample size with a concentration of self-incompatibility in the heavily represented Bromeliaceae.

Hummingbird pollination has evolved many times, and in many different lineages. In our review data set, hummingbird pollination was present in three families within Monocots and 11 in Eudicots. Hummingbird pollination evolves much more often from bee pollination in New World lineages (Grant 1994; Perret et al. 2003; Kay et al. 2005; Wilson et al. 2007; Cronk and Ojeda 2008), but reverse transitions are also observed (Perret et al. 2003; Tripp and Manos 2008). However, whether shifts to hummingbird pollination within groups have been accompanied by changes in mating system is unknown. Future work should determine whether hummingbird flowers evolve more frequently in lineages that previous lost self-incompatibility or not.

Hermaphroditic flowers can have additional mechanisms that promote outcrossing, such as dichogamy and herkogamy, that when combined with small floral display sizes can increase the likelihood of outcrossing (Webb and Lloyd 1986; Lloyd and Webb 1986; Barrett 2003). For instance, herkogamy and dichogamy can interact to reduce self-interference and selfing (Webb and Lloyd 1986; Lloyd and Webb 1986). In the INP guild, dichogamy (here, protandry) combined with herkogamy was observed in 37 % (10) of the species: all five Gesneriaceae species, plus *E. brasiliensis*, *V. dracocephaloides*, *S. itatiaiae*, *A. bedfordianum*, and *T. geminiflora*, and only the two latter are self-incompatible. Herkogamy is present in two other self-compatible species as *Salvia sellowiana* (Lamiaceae) and *Manettia mitis* (Rubiaceae), as well as *Psychotria ruelliflora*, which displays a special case of reciprocal herkogamy (heterostyly) that is frequently associated with self-incompatibility in Rubiaceae (Bawa and Beach 1983; Barrett 2003).

The higher level of self-incompatibility (i.e., lower value of ISI) for woody species than herbs was only observed in the non-hermits foraging group which agrees with the empirical observations (Arroyo 1981; Kress and Beach 1994). In contrast, the other foraging-types had similar levels of self-incompatibility between the plant habits for Neotropical species, and in the INP, this was in the opposite direction of empirical observations. Neutral or significant associations of plant mating system and plant habit may be due to the lack of self-incompatible woody species in the INP guild and increased representation of other families with self-incompatible woody species (e.g., Bignoniaceae, Rubiaceae, and Solanaceae) in the Neotropics. Moreover, self-incompatibility was pronounced among herbaceous species. Many of species studied here are long-lived clonal plants, and they may be nearly as long-lived as woody species and experience multiple opportunities for outcrossed reproduction. This may be especially prominent in the bromeliads, which propagate by ramets (Benzing 2000). Species in the Bromeliaceae were the greatest contributors to the self-incompatible herbaceous plant group, especially in the INP guild.

In the INP guild, the relationship between mating system and plant aggregation was opposite of our expectations for seed production and no relationship was existed for plant density at either fruit or seed levels. Studies that tested the relationship between outcrossing rates and plant abundance showed distinct responses (Van Treuren et al. 1993; Franceschinelli and Bawa 2000; García et al. 2005). In the case of the self-compatible hummingbird-pollinated shrub *Helicteres brevispira*, outcrossing increased with plant density because hummingbirds visit more flowers on isolated individuals than when individuals are aggregated promoting more selfing in the former. In fact, the positive relationship between selfing and plant aggregation in the INP guild suggests that hummingbirds are promoting outcrossing by moving pollen among near individuals in species with aggregate population and self-incompatibility is maintained to avoid inbreeding in species with sparse population.

Conclusion

Results from our analysis of both data sets support the idea that although hummingbirds differ in their foraging strategies, these behavioral differences do not seem to alter plant mating systems. In fact, phylogenetic relatedness seems to be the strongest determinant of mating system in

Neotropical hummingbird-pollinated species followed by an effect of plant habit that differed among hummingbird foraging strategies and data sets.

Acknowledgments We acknowledge M.B.F. Canela for the use of her Ph.D. data, T.V. Campbell, V. Silvestre, and Itatiaia National Park for field support, National Institute of Meteorology (INMET) for climate data, and G. Arceo-Gómez and R. Spigler for analysis assistance. We would like to thank P. Wilson and three anonymous reviewers for perceptive comments that improved the manuscript. We acknowledge Coordination of Improvement of Higher Education Personnel (CAPES-PROAP) and Research Support Foundation of Rio de Janeiro (FAPERJ) for financial support. MW acknowledges Coordination of Improvement of Higher Education Personnel (CAPES) and National Centre for Flora Conservation (CNCFlora) for Ph.D. scholarships, LF and CFS acknowledge National Council for Scientific and Technological Development (CNPq) for a researcher grant (PQ) and an undergrad scholarship (PIBIC), respectively, and TLA acknowledges logistical support of National Science Foundation (NSF) DEB 1020523. This paper is part of the Ph.D. dissertation of the first author developed at 'Programa de Pós-Graduação em Botânica, Jardim Botânico do Rio de Janeiro'. This paper is a contribution of the 'Rede em Epífitas de Mata Atlântica' supported by Coordination of Improvement of Higher Education Personnel (CAPES-PNADB).

References

Abreu CRM, Vieira MF (2004) Os beija-flores e seus recursos florais em um fragmento florestal de Viçosa, sudeste brasileiro. Lundiana 5(2):129–134

Allen AM, Hiscock SJ (2008) Evolution and phylogeny of self-incompatibility systems in angiosperms. In: Franklin-Tong VE (ed) Self-incompatibility in flowering plants. Springer-Verlag, Berlin, pp 73–101

Araújo FP, Barbosa AAA, Oliveira PE (2011) Floral resources and hummingbirds on an island of flooded forest in Central Brazil. Flora 206:827–835

Arizmendi MA, Ornelas JF (1990) Hummingbirds and their floral resources in a tropical dry forest in Mexico. Biotropica 22(2):172–180

Arroyo MTK (1981) Breeding systems and pollination biology in Leguminosae. In: Polhill RM, Raven PH (eds) Adv Legum Systemat, PT 2. Royal Botanical Garden, Kew, pp 723–769

Barrett SCH (2003) Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. Phil Trans R Soc Lond B 358:991–1004. doi:10.1098/rstb.2003.1301

Bawa KS (1974) Breeding systems of tree species of a lowland tropical community. Evolution 28(1):85–92

Bawa KS, Beach JH (1983) Self-incompatibility systems in the Rubiaceae of a tropical lowland wet forest. Am J Bot 70:1281–1288

Bawa KS, Opler PA (1975) Dioecism in tropical forest trees. Evolution 29:167–179

Bawa KS, Perry DR, Beach JH (1985) Reproductive biology of tropical lowland rain forest trees: I. Sexual systems and incompatibility mechanisms. Am J Bot 72:331–345

Benzing DH (2000) Bromeliaceae: profile of an adaptive radiation. Cambridge University Press, Cambridge

Brown JH, Bowers MA (1985) Community organization in hummingbirds: relationships between morphology and ecology. Auk 102:251–269

Bullock SH (1985) Breeding systems in the flora of a Tropical deciduous forest in Mexico. Biotropica 17(4):287–301

Buzato S, Sazima M, Sazima I (2000) Hummingbird-pollinated floras at three Atlantic Forest sites. Biotropica 32(4b):824–841

Canela MBF (2006) Interações entre plantas e beija-flores em uma comunidade de Floresta Atlântica Montana em Itatiaia, RJ. Dissertation, Universidade Estadual de Campinas

Cotton PE (1998) Coevolution in an Amazonian hummingbird–plant community. Ibis 140(4):639–646

Cronk Q, Ojeda I (2008) Bird-pollinated flowers in an evolutionary and molecular context. J Exp Bot 59(4):715–727. doi:10.1093/jxb/ern009

Dalsgaard B, Martín-González AM, Olesen JM, Ollerton J, Timmermann A, Andersen LH, Tossas AG (2009) Plant–hummingbird interactions in the West Indies: floral specialization gradients associated with environment and hummingbird size. Oecologia 159:757–766. doi:10.1007/s00442-008-1255-z

Dalsgaard B, Magård E, Fjeldså J, Martín González AM, Rahbek C, Olesen JM, Ollerton J, Alarcón R, Araujo AC, Cotton PA, Lara C, Machado CG, Sazima I, Sazima M, Timmermann A, Watts S, Sandel B, Sutherland WJ, Svenning JC (2011) Specialization in plant–hummingbird networks is associated with species richness, contemporary precipitation and Quaternary climate-change velocity. PLoS One 6(10):e25891. doi:10.1371/journal.pone.0025891

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, http://www.R-project.org/ Accessed 22 April 2012

Dziedzioch C, Stevens AD, Gottsberger G (2003) The hummingbird plant community of a tropical montane rain forest in Southern Ecuador. Plant Biol 5:331–337

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

Feinsinger P, Colwell RK (1978) Community organization among Neotropical nectar feeding birds. Am Zool 18:779–795

Franceschinelli EV, Bawa KS (2000) The effect of ecological factors on the mating system of a South American shrub species (*Helicteres brevispira*). Heredity 84:116–123

García C, Arroyo JM, Godoy JA, Jordano P (2005) Mating patterns, pollen dispersal, and the ecological maternal neighbourhood in a *Prunus mahaleb* L. population. Mol Ecol 14:1821–1830. doi:10.1111/j.1365-294X.2005.02542.x

Goodwillie C, Kalisz S, Eckert CG (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu Rev Ecol Evol Syst 36:47–79

Grant V (1994) Historical development of ornithophily in the western North America flora. Proc Natl Acad Sci USA 91:10407–10411

Grant KA, Grant V (1968) Hummingbirds and their flowers. Columbia University Press, New York

Gutiérrez AZ, Rojas-Nossa SV, Stiles FG (2004) Dinámica annual de la interacción colibrí-flor en ecosistemas altoandinos. Ornitol Neotropical 15(Suppl):205–213

Igic B, Kohn JR (2001) Evolutionary relationships among self-incompatibility RNases. Proc Natl Acad Sci USA 98:13167–13171. doi:10.1073/pnas.231386798

Igic B, Lande R, Hohn JR (2008) Loss of self-incompatibility and its evolutionary consequences. Int J Plant Sci 169:93–104. doi:10.1086/523362

Janzen DH (1971) Euglossine bees as long-distance pollinators of tropical plants. Science 171:203–205

Kay KM, Reeves PA, Olmstead RG, Schemske DW (2005) Rapid speciation and the evolution of hummingbird pollination in Neotropical *Costus* subgenus *Costus* (Costaceae): evidence from nrDNA ITS and ETS sequences. Am J Bot 92(11):1899–1910

Kress WJ (1983) Self-incompatibility in Central American *Heliconia*. Evolution 37(4):735–744

Kress WJ, Beach JH (1994) Flowering plant reproductive systems. In: McDade LA et al (eds) La Selva: ecology and natural history of a Neotropical rainforest. University of Chicago Press, Chicago, pp 161–182

Lara C (2006) Temporal dynamics of flower use by hummingbirds in a highland temperate forest in Mexico. Ecoscience 13:23–29

Las-Casas FMG, Azevedo Júnior SM, Dias Filho MM (2012) The community of hummingbirds (Aves: Trochilidae) and the assemblage of flowers in a Caatinga vegetation. Braz J Biol 72(1):51–58

Leal FC, Lopes AV, Machado IC (2006) Polinização por beija-flores em uma área de caatinga no Município de Floresta, Pernambuco, Nordeste do Brasil. Rev Bras Bot 29(3):379–389

Linhart YB (1973) Ecological and behavioral determinants of pollen dispersal in hummingbird-pollinated *Heliconia*. Am Nat 107 (956):511–523

Linhart YB, Busby WH, Beach JH, Feinsinger P (1987) Forager behavior, pollen dispersal, and inbreeding in two species of hummingbird-pollinated. Evolution 41(3):679–682

Lloyd DG, Schoen DJ (1992) Self- and cross-fertilization in plant: I. Functional dimensions. Int J Plant Sci 153(3):358–369

Lloyd DG, Webb CJ (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. N Z J Bot 24:135–162

Machado CG (2009) Beija-flores (Aves: Trochilidae) e seus recursos florais em uma área de caatinga da Chapada Diamantina, Bahia, Brasil. Zoologia 26(2):255–265

Machado CG, Coelho AG, Santana CS, Rodrigues M (2007) Beija-flores e seus recursos florais em uma área de campo rupestre da Chapada Diamantina, Bahia. Rev Bras Ornitol 15(2):267–279

Martén-Rodríguez S, Kress WJ, Temeles EJ, Meléndez-Ackerman E (2011) Plant–pollinator interactions and floral convergence in two species of *Heliconia* from the Caribbean Islands. Oecologia 167:1075–1083

Martin FN (1959) Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol 34:125–128

Matallana G, Godinho MAS, Guilherme FAG, Belisario M, Coser TS, Wendt T (2010) Breeding systems of Bromeliaceae species: evolution of selfing in the context of sympatric occurrence. Plant Syst Evol 289:57–67

McDade LA (1985) Breeding systems of Central American *Aphelandra* (Acanthaceae). Am J Bot 72(10):1515–1521

McDade LA, Weeks JA (2004) Nectar in hummingbird-pollinated Neotropical plants: I. Patterns of production and variability in 12 Species. Biotropica 36(2):196–215

Newstrom LE, Frankie GW, Baker HG (1994) A new classification for plant phenology based on flowering patterns in lowland tropical rain forest trees at La Selva, Costa Rica. Biotropica 26(2):141–159

Orme CDL (2012) Comparative analysis of phylogenetics and evolution in R. http://caper.r-forge.r-project.org Accessed 14 September 2012

Peel MC, Finlayson BL, McMahon TA (2007) Updated world map of the Köppen–Geiger climate classification. Hydrol Earth Syst Sci 11:1633–1644. doi:10.5194/hess-11-1633-2007

Perret M, Chautems A, Spichiger R, Kite G, Savolainen V (2003) Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear NCPGS. Am J Bot 90(3):445–460

Raduski AR, Haney EB, Igic B (2012) The expression of self-incompatibility in angiosperms is bimodal. Evolution 66(4):1275–1283. doi:10.1111/j.1558-5646.2011.01505.x

Ramírez N, Brito Y (1990) Reproductive of a tropical palm swamp community in the Venezuelan llanos. Am J Bot 77:1260–1271

SanMartin-Gajardo I, Freitas L (1999) Hummingbird pollination in *Besleria longimucronata* Hoehne (Gesneriaceae) in southeastern Brazil. Biociências 7:13–24

Sazima I, Buzato S, Sazima M (1995) The saw-billed hermit, *Ramphodon naevius*, and its flowers in southeastern Brazil. J Ornithol 136:195–206

Schemske DW, Lande R (1985) The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. Evolution 39:41–52

Snow BK, Snow DW (1972) Feeding niches of hummingbirds in a Trinidad valley. J Anim Ecol 41:471–485

Snow BK, Teixeira DL (1982) Hummingbirds and their flowers in the coastal mountains of southeastern Brazil. J Ornithol 123(4):446–450

Sobrevila C, Arroyo MTK (1982) Breeding system in a montane tropical cloud forest in Venezuela. Plant Syst Evol 140:19–37

Stiles FG (1975) Ecology, flowering phenology and hummingbird pollination of some Costa Rican *Heliconia* species. Ecology 56:285–310

Stiles FG (1978) Temporal organization of flowering among the hummingbird foodplants of a tropical wet forest. Biotropica 10(3):194–210

Stiles FG (1981) Geographical aspects of bird–flower coevolution, with particular reference to Central America. Ann Mo Bot Gard 68:323–351

Tanner EVJ (1982) Species diversity and reproductive mechanisms in Jamaican trees. Biol J Linn Soc 18:263–278

Tripp EA, Manos PS (2008) Is floral specialization an evolutionary dead-end? Pollination system transitions in *Ruellia* (Acanthaceae). Evolution 62:1712–1737. doi:10.1111/j.1558-5646.2008.00398.x

Van Treuren R, Bijlsma R, Ouborg NJ, van Delden W (1993) The effects of population size and plant density on outcrossing rates in locally endangered *Salvia pratensis*. Evolution 47:1094–1104

Vasconcelos MF, Lombardi JA (1999) Padrão sazonal na ocorrência de seis espécies de beija-flores (Apodiformes: Trochilidae) em uma localidade de campo rupestre na Serra do Curral, Minas Gerais. Ararajuba 7:71–79

Veloso HP, Rangel Filho ALR, Lima JCA (1991) Classificação da vegetação brasileira, adaptada a um sistema universal. Instituto Brasileir de Geografia e Estatística, Rio de Janeiro

Vieira J, Fonseca NA, Vieira CP (2008) An S-RNase-based gametophytic self-incompatibility system evolved only once in Eudicots. J Mol Ecol 67:179–190

Walther BA, Brieschke H (2001) Hummingbird–flower relationships in a mid-elevation rainforest near Mindo, northwestern Ecuador. Int J Ornithol 4:115–135

Webb CO, Donoghue MJ (2005) Phylomatic: tree assembly for applied phylogenetics. Mol Ecol Notes 5(1):181–183. doi:10.1111/j.1471-8286.2004.00829.x

Webb CJ, Lloyd DG (1986) The avoidance of interference between the presentation of pollen and stigma in angiosperms: II. Herkogamy. N Z J Bot 24:163–178

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24:2098–2100

Wikström N, Savolainen V, Chase MW (2001) Evolution of the angiosperms: calibrating the family tree. Proc R Soc B 268 (1482):2211–2220. doi:10.1098/rspb.2001.1782

Wilson P, Wolfe AD, Armbruster WS, Thomson JD (2007) Constrained lability in floral evolution: counting convergent origins of hummingbird pollination in *Penstemon* and *Keckiella*. New Phytol 176:883–890

Zapata TR, Arroyo MTK (1978) Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10 (3):221–230