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Community-wide assessment of pollen limitation in hummingbird-pollinated plants of a tropical montane rain forest

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• *Background and Aims* Although pollen limitation of reproduction (PL) has been widely studied, our understanding of its occurrence in tropical communities, especially for bird-pollinated plants, is underdeveloped. In addition, inclusion of both quantity and quality aspects in studies of PL are generally lacking. Within hummingbird-pollinated plants, a prediction was made for higher PL for the quality than quantity aspects and a minor effect of temporal variation because hummingbirds are constant and efficient pollen vectors but they may transfer low quality pollen.
• *Methods* Field hand and open pollination experiments were conducted on 21 species in a tropical montane rain forest over 2 years. The quantity (fruit set and seeds per fruit) and quality (seed weight and germination) aspects of reproduction were assessed as the response to open pollination relative to outcross hand pollination. The relationships between the effect size of quantity and quality aspects of reproduction and predictive plant features (self-incompatibility, autogamy, density and pollinator specialization level) were assessed with phylogenetic generalized linear models.
• *Key Results* Just over half of all the species expressed PL for one or more response variables. On average, the severity of PL was strong for one quality variable (seed germination; 0·83), but insignificant for another (seed weight; −0·03), and low to moderate for quantity variables (0·31 for seeds per fruit and 0·39 for fruit set). There was only a minor contribution of temporal variation to PL within the studied species. Common predictors of PL, i.e. phylogenetic relatedness, self-incompatibility, autogamy, plant density and pollinator specialization level, did not adequately explain variation in PL within this community.
• *Conclusions* Despite the measurable degree of PL within these hummingbird-pollinated plants, the causes of pollen quality and quantity insufficiency are not clear. Variables other than those tested may contribute to PL or causes of PL may vary among species and cannot adequately be accounted for when assessed from the within-community perspective.

Key words: Atlantic forest, Brazil, Itatiaia National Park, Neotropics, ornithophily, community, pollen limitation, pollination biology, hummingbird.

INTRODUCTION

Pollen limitation (PL) occurs when plant reproductive success is reduced because flowers receive less pollen (quantity limitation) and/or lower quality pollen (quality limitation) than is needed for full seed or fruit set. While quantity limitation has been characterized as the consequence of plants receiving too few pollen grains to fertilize all of their ovules, quality limitation depends on the difference in survival of embryos sired by naturally delivered pollen vs. by pollen of maximal quality (Aizen and Harder, 2007). The causes and consequences of PL have been widely studied (Bierzychudek, 1981; Burd, 1994; Larson and Barrett, 2000; Ashman *et al.*, 2004; Knight *et al.*, 2005). These studies have revealed that the magnitude of PL can vary with response variable chosen, e.g. effect size of 0·15 for seeds per fruit and 0·55 for fruit set in Knight *et al.* (2006) (but see also Larson and Barrett, 2000; Aizen and Harder, 2007; Hegland and Totland, 2008), and thus both measures of quantity and quality of reproduction are necessary to assess levels of pollination sufficiency (Aizen and Harder, 2007; Wesselingh, 2007; Burd *et al.*,

2009; Alonso *et al.*, 2012, and references therein). Estimates of PL have been made for many angiosperm species (e.g. 306 species; Knight *et al.*, 2005), but most studies have measured PL in one or a few species or performed a meta-analysis of data from single-species studies. Far fewer have assessed PL for numerous species within a single community (but see below).

The community perspective may lead to a better interpretation of the occurrence, components and mechanisms of PL. Within a given community, concerns related to habitat differences, weather and pollinator types are kept constant, thus patterns derived from species-specific outcomes after pollination experiments will more closely reflect differences in plant characters. Despite the advantages of this approach (Ashman *et al.*, 2004), only three studies have been performed on all (or most) of the plants within a community (Motten, 1986; Hegland and Totland, 2008; González and Pérez, 2010), and none of these included a single shared group of pollinators. In fact, these studies were conducted in temperate and sub-tropical plant communities pollinated by insects (Motten, 1986; Hegland and Totland, 2008; González and Pérez, 2010), so we still lack a

community-level exploration of PL in a tropical community as well as one among species pollinated by vertebrates. Such a study is necessary if we are to determine whether community-level patterns observed thus far are general [i.e. PL is associated most strongly with mating system (Motten, 1986; González and Pérez, 2010) or varies with response variable (Hegland and Totland, 2008)].

The tropics are a particularly important location for community-based studies of PL because tropical species are hypothesized to be at risk of PL (Larson and Barrett, 2000) and the tropics support high biodiversity and pronounced levels of endemism (Myers *et al.*, 2000). In fact, PL has been seen to increase with species richness (Vamosi *et al.*, 2006) and even more so for endemics (Alonso *et al.*, 2010). Greater PL in high diversity sites is proposed to result from greater interspecific competition for pollinators (Vamosi *et al.*, 2006), or greater heterospecific pollen transfer (Alonso *et al.*, 2010). Endemic species may be particularly at risk of PL because of smaller population sizes, reduced density and/or stronger habitat specificity than widespread species (Karron, 1987; Rymer *et al.*, 2005; Alonso *et al.*, 2010).

Despite these implications, only a small percentage (15 %) of the studies of PL conducted to date have been performed on tropical species, although tropical species represent more than half of animal-pollinated plants (Ollerton *et al.*, 2011), and an equally small percentage (15 %) have been conducted on bird-pollinated species. This has resulted in a void in our understanding of the extent of PL for bird-pollinated species in the tropics [only 5 % of studies in Ashman *et al.*, 2004 – and these were concentrated in one location (Costa Rica; 58 % of sites) and on one genus (*Inga* species; 50 % of species)]. These omissions have precluded a broader understanding of pollen limitation among bird-pollinated species in the tropics.

Several plant and pollinator traits make hummingbird-pollinated plants unique. Not only do bird-pollinated species have floral features associated with bird perception and foraging (e.g. diurnal anthesis, bright colours, lack of perceptible floral scent, nectar as reward, *sensu* Faegri and van der Pijl, 1979) or that restrict visitor access [e.g. tubular narrow corollas in hummingbird-flowers (Lunau *et al.*, 2011)], but they are also significant components of Neotropical forest communities within which they reside, e.g. 15 % of angiosperm species at Monteverde, Costa Rica (Grant and Grant, 1968; Feinsinger, 1983). These plants are mostly represented by species of Acanthaceae, Bromeliaceae, Ericaceae, Gesneriaceae, Heliconiaceae and Rubiaceae (Stiles, 1975; Arizmendi and Ornelas, 1990; Buzato *et al.*, 2000; Dziedzioch *et al.*, 2003; Araújo *et al.*, 2011; Las-Casas *et al.*, 2012). Moreover, hummingbird–plant communities are characterized by sequential blooming among species, which ensures resource reliability over time for hummingbirds (Stiles, 1975, 1978; Buzato *et al.*, 2000; Abreu and Vieira, 2004; Leal *et al.*, 2006; Araújo *et al.*, 2011). Taking these features into account, hummingbirds have been recognized to be especially reliable pollinators of their plant communities (Feinsinger, 1976; Wolf *et al.*, 1976; Stiles, 1985). Thus, hummingbird-pollinated plants are good models to compare species while keeping other community characteristics constant. In fact, due to those peculiarities, hummingbird–plant interactions constitute a well-defined sub-network (Dalsgaard *et al.*, 2008; Danieli-Silva *et al.*, 2012). This is not to say, however, that specialization level does not vary within the network

(Dalsgaard *et al.*, 2008) because hummingbirds differ in morphology and foraging behaviour (cf. Stiles, 1975; Feinsinger and Colwell, 1978), which can result in differences in pollinator composition of hummingbird plants and thus the expected pollen flow among individuals (Stiles, 1975; Snow and Snow, 1972; Linhart, 1973; Feinsinger, 1976; Brown and Bowers, 1985; Lara, 2006).

We sought to answer the following questions with respect to hummingbird-pollinated plants of a tropical montane rain forest. (1) What is the occurrence and magnitude of PL? (2) Does PL vary with the quantity (fruit set and seeds per fruit) and quality (seed weight and seed germination) response variables? (3) Does PL vary over time for species studied in two consecutive years? With respect to these questions, we expected PL to be higher for the quality aspects of reproduction than for the quantity aspects because while hummingbirds are reliable (Feinsinger, 1976; Wolf *et al.*, 1976; Stiles, 1985) and efficient at promoting pollen transfer (Linhart, 1973; Linhart *et al.*, 1987), they may still transfer low quality pollen (e.g. self pollen or pollen from related individuals) as a result of the prevalence of self-compatibility in hummingbird-pollinated plants (Wolowski *et al.*, 2013) coupled with spatial clumping of the understorey plants and a high degree of within-plant movements by hummingbirds (Kress and Beach, 1994). We predicted a minor effect of temporal variation on PL because of hummingbird constancy over time within their communities (Feinsinger, 1976; Wolf *et al.*, 1976; Stiles, 1978, 1985).

We also explored the proximate causes of PL by testing for effects of traits reported from large-scale meta-analyses as being strongly influential, i.e. self-incompatibility, lack of autogamy, pollinator specialization level and low plant density (Burd, 1994; Waser *et al.*, 1996; Larson and Barrett, 2000; Knight *et al.*, 2005). Specifically, for the hummingbird-pollinated plants we asked the following additional questions. (4) Does PL increase with self-incompatibility and specialization level? (5) Does PL decrease with autogamy and plant density?

MATERIALS AND METHODS

Study site and hummingbird-pollinated plant species

The study site is located in the Itatiaia National Park, between the states of Rio de Janeiro and Minas Gerais, in southeastern Brazil. The study site (22°27′S, 44°36′W) is between 900 and 1200 m a.s.l. and its vegetation is classified as tropical montane rain forest (*sensu* Veloso *et al.*, 1991). The climate is sub-tropical humid (i.e. '*Cwa*' in Köeppen–Geiger climate classification; Peel *et al.*, 2007) with two distinct seasons: a wet/warm period (monthly mean precipitation = 202 mm, temperature: 29 °C/ 19 °C max/min) from October to April and a dry/cold period (36 mm, 26 °C/14 °C) from May to September (data from the climatological normal 1961–1990 at Resende county, 400 m a.s.l.). Fieldwork was conducted from September 2009 to August 2011, and data were collected along seven trails through the site that totalled 5 km in length.

We studied 21 species (Table 1), which represent 58 % of the hummingbird-pollinated plants at the study site (Canela, 2006; Wolowski *et al.*, 2013). Plant species were selected according to the availability of reproductive individuals within the study site (i.e. at least four flowering individuals per species).

TABLE 1. *Hummingbird-pollinated plant species studied at Itatiaia National Park and life habit, mating system and pollinators*

Species	Plant habit	Mating system	Pollinators
Acanthaceae			
Justicia sebastianopolitanae	Shrub	SC	Pheu
Odontonema barbelerioides	Shrub	SC	Pheu, Phsq
Staurogyne itatiaiae	Shrub	SC*	Pheu, Phsq
Bromeliaceae			
Aechmea nudicaulis	Herb epiphytic	SI	Clru, Pheu, Phsq, Thgl
Aechmea vanhoutteana	Herb epiphytic	SI	Clru, Pheu, Phsq
Billbergia distachia	Herb epiphytic	SI	Pheu, Phsq, Thgl
Nidularium itatiaiae	Herb	SI	Pheu
Pitcairnia flammea	Herb	SC*	Pheu, Phsq, Thgl
Quesnelia augusto-coburgii	Herb epiphytic	SI	Pheu
Tillandsia geminiflora	Herb epiphytic	SI	Clru, Pheu, Phsq, Thgl
Vriesea carinata	Herb epiphytic	SC*	Pheu, Thgl
Vriesea gradata	Herb epiphytic	SC*	Phsp
Vriesea longicaulis	Herb epiphytic	SC*	Pheu, Thgl, Anca[†]
Gesneriaceae			
Nematanthus crassifolius	Shrub epiphytic	SC	Pheu
Nematanthus lanceolatus	Shrub epiphytic	SC	Pheu, Phsq
Sinningia cooperi	Shrub rupicolous	SC	Pheu, Phsq, Thgl
Sinningia gigantifolia	Shrub rupicolous	SC*	Pheu, Phsq
Lamiaceae			
Salvia sellowiana	Shrub	SC	Pheu, Phsq
Malvaceae			
Abutilon bedfordianum	Treelet	SI	Clru, Pheu, Thgl, Anca, Ange
Orchidaceae			
Elleanthus brasiliensis	Herb epiphytic	SC	Phsq
Rubiaceae			
Manettia mitis	Vine	SC*	Pheu, Phsq, Thgl

For pollinators, hummingbirds comprise *Clytolaema rubricauda* (Clru), *Phaethornis eurynome* (Pheu), *Phaethornis* sp. (Phsp), *P. squalidus* (Phsq) and *Thalurania glaucopis* (Thgl); and bats comprise *Anoura caudifer* (Anca) and *A. geoffroyi* (Ange) (Canela, 2006; Wolowski *et al.*, 2013).

SC, self-compatible; SI, self-incompatible.

* Autogamous species (Wolowski *et al.*, 2013).

[†] The record of bat pollination for this species at other locations according to Sazima *et al.* (1995).

Among these, Bromeliaceae represent half of the species (ten species), and Gesneriaceae and Acanthaceae were also well represented (four and three species, respectively), while four families (Lamiaceae, Malvaceae, Orchidaceae and Rubiaceae) were represented by one species each. Species in the Bromeliaceae represent almost half of the sampled species because they actually represent a disproportionate number of hummingbird-pollinated plants in the montane rain forest (e.g. at least one-third of the species; Buzato *et al.*, 2000), as well in this community (Canela, 2006). The species have traits typical of hummingbird-pollinated plants (e.g. tubular shape, reddish corolla or inflorescences and imperceptible odour), except for *Abutilon bedfordianum* and *Vriesea longicaulis*, which have ornithophilous traits mixed with chiropterophilous traits such as opening at dusk, and producing scent and abundant quantities of nectar (Table 1).

Four hummingbird species visited these plants: *Phaethornis eurynome* (Lesson, 1832), *P. squalidus* (Temminck, 1822), *Clytolaema rubricauda* (Boddaert, 1783) and *Thalurania glaucopis* (Gmelin, 1788), and were present at the study site throughout the year (Canela, 2006). *Phaethornis eurynome* is a resident at the site and is known to pollinate 90 % of the hummingbird-pollinated species (Canela, 2006). Both *Phaethornis* species are long-billed and high-reward trapliners (after Feinsinger and Colwell, 1978), whereas the behaviours of the other species, both short-billed, varied from territorial to low-reward trapliners (Canela, 2006, after Feinsinger and Colwell, 1978). The foraging behaviours of these groups differ: high-reward trapliners forage for dispersed nectar-rich flowers, whereas territorials defend clumped-rich flowers and low-reward trapliners forage for dispersed nectar-moderate flowers when excluded by territorials (Feinsinger and Colwell, 1978).

Procedures

To estimate PL, we collected data following experimental and natural pollination. We conducted manual cross-pollination (Cp) on previously bagged flower buds and/or inflorescences. For each species, flowers in anthesis were tagged, pollinated with the pollen from a flower of another individual (minimum distance of 50 m) and bagged; then, fruits were monitored until maturation. Natural pollination (Np) was estimated for flowers that were tagged at anthesis, exposed to pollinators and bagged once the petals senesced; and then monitored as above. The number of flowers per plant following Cp and Np was dictated by species-specific floral display size (1–200 per plant). The sample size per species ranged from four to 22 individuals (see Table 2). Given the limited number of individuals per species at the study site, both Cp and Np were applied to flowers on the same individual for all species.

For seven species that had multiple branches, Cp and Np were spread across several stalks to eliminate potential resource reallocation (after Zimmerman and Pyke, 1988). For the 14 species with single stalks, the above procedure was not possible, so to test for potential resource reallocation between Np- and

TABLE 2. *Pollen limitation effect size (95 % confidence interval) and sample size for individual species and overall species in hummingbird-pollinated plants at Itatiaia National Park*

Species	Fruit set	Seeds per fruit	Seed weight	Seed germination
*Justicia sebastianopolitanae**	−0·19 (−0·78 to 0·40), 22	−0·96 (−1·90 to −0·03), 10,10	−0·03 (−0·45 to 0·38), 33, 68	ND
*Odontonema barbelerioides**	0·72 (−0·36 to 1·80), 7	ND	**0·67 (0·10 to 1·25), 16, 49**	ND
*Staurogyne itatiaiae**	−0·37 (−1·09 to 0·36), 15	0·33 (−1·30 to 1·95), 3, 3	−3·93 (−5·93 to −1·94), 7, 7	−1·19 (−2·46 to 0·08), 6, 6
*Aechmea nudicaulis**	0·95 (−0·08 to 1·99), 8	0·51 (−0·15 to 1·17), 21, 16	**2·56 (1·32 to 3·81), 10, 10**	**1·98 (0·87 to 3·09), 10, 10**
Aechmea vanhoutteana	−0·12 (−1·17 to 0·93), 7	**0·28 (0·00 to 0·56), 81, 122**	−4·01 (−5·64 to−2·38), 10, 10	0·00 (−1·13 to 1·13), 6, 6
*Billbergia distachia**	0·65 (−0·11 to 1·41), 14	0·35 (−0·22 to 0·92), 25, 23	0·12 (−0·76 to 1·00), 10, 10	−1·67 (−2·72 to −0·62), 10, 10
*Nidularium itatiaiae**	0·26 (−0·38 to 0·90), 19	0·28 (−0·07 to 0·64), 46, 92	0·80 (−0·12 to 1·72), 10, 10	1·19 (−0·08 to 2·46), 6, 6
*Pitcairnia flammea**	0·46 (−0·29 to 1·21), 14	**1·15 (0·25 to 2·04), 11, 12**	−0·69 (−1·60 to 0·21), 10, 10	**1·48 (0·14 to 2·82), 6, 6**
*Quesnelia augusto-coburgii**	0·07 (−0·54 to 0·67), 21	**0·46 (0·03 to 0·90), 34, 55**	−2·13 (−3·27 to −0·99), 10, 10	−2·10 (−3·23 to −0·96), 10, 10
Tillandsia geminiflora	**1·93 (0·25 to 3·61), 4**	0·82 (−0·40 to 2·03), 7, 5	0·92 (−0·02 to 1·85), 10, 10	−0·08 (−1·21 to 1·06), 6, 6
Vriesea carinata	0·90 (−0·56 to 2·35), 4	0·82 (−0·80 to 2·44), 3, 4	−1·38 (−2·37 to−0·38), 10, 10	0·27 (−0·61 to 1·15), 10, 10
*Vriesea gradata**	**0·85 (0·19 to 1·51), 19**	0·89 (−0·76 to 2·53), 3, 4	**1·08 (0·13 to 2·03), 10, 10**	**4·00 (2·37 to 5·63), 10, 10**
*Vriesea longicaulis**	−0·21 (−1·14 to 0·72), 9	0·08 (−1·11 to 1·26), 6, 5	−0·51 (−1·40 to 0·39), 10, 10	0·54 (−0·36 to 1·43), 10, 10
*Nematanthus crassifolius**	0·31 (−0·41 to 1·03), 15	−0·60 (−1·61 to 0·42), 7, 9	**2·54 (1·30 to 3·77), 10, 10**	**2·32 (1·14 to 3·51), 10, 10**
Nematanthus lanceolatus	0·14 (−0·85 to 1·12), 8	0·30 (−0·77 to 1·36), 9, 10	0·26 (−0·62 to 1·14), 10, 10	0·29 (−0·85 to 1·43), 6, 6
Sinningia cooperi	1·04 (−0·44 to 2·51), 4	0·40 (−0·56 to 1·37), 9, 8	**4·54 (1·74 to 7·34), 5, 5**	**5·21 (3·22 to 7·20), 10, 10**
Sinningia gigantifolia	**3·28 (1·55 to 5·02), 6**	1·23 (−1·07 to 3·54), 3, 2	−4·51 (−7·30 to −1·73), 5, 5	0·78 (−0·41 to 1·97), 6, 6
*Salvia sellowiana**	0·23 (−0·61 to 1·07), 11	−0·04 (−0·72 to 0·64), 14,20	0·20 (−0·38 to 0·77), 19,31	ND
*Abutilon bedfordianum**	**1·01 (0·33 to 1·68), 19**	**0·99 (0,33 to 1·65), 29, 15**	**1·12 (0·17 to 2·08), 10, 10**	**1·93 (0·83 to 3·03), 10, 10**
Elleanthus brasiliensis	0·60 (−0·82 to 2·02), 4	ND	ND	ND
*Manettia mitis**	0·17 (−0·71 to 1·05), 10	−0·03 (−0·62 to 0·56), 22, 22	0·85 (−0·07 to 1·77), 10, 10	0·47 (−0·43 to 1·36), 10, 10
Overall	**0·39 (0·17 to 0·61), 21**	**0·31 (0·17 to 0·46), 19**	−0·03 (−0·88 to 0·82), 20	**0·83 (0·01 to 1·66), 17**

The effect size of quantity (fruit set and seeds per fruit) and quality (seed weight and seed germination) aspects of reproduction. Bold indicates significant pollen limitation. Species studied over two years are marked with an asterisk, and values represent the summed individuals of both years. Sample size of fruit set denotes the number of individuals treated; seeds per fruit, the number of developed seeds per fruit counted per treatment [Cp (cross) and Np (natural) pollinations, respectively]; seed weight, the number of seeds or groups of seeds weighed per treatment; and seed germination, the number of Petri dishes per treatment.

ND, not determined.

Cp-treated flowers, we tagged 3–20 flowers on 2–12 individuals per species for four species and only conducted the Np treatment (after Hegland and Totland, 2008). We compared the fruit set of Np flowers on these Np-only individuals with the fruit set of Np flowers on individuals that received both Np and Cp treatments. None of these showed an effect of both treatments on Np fruit set, i.e. there was no evidence of reallocation.

We collected fruits and calculated the proportion of fruit set (number of developed fruits per number of flowers) for each treatment per individual as well as the mean and standard deviation for each species. For 14 species, we repeated the experiment in two years. In the laboratory, we counted the number of developed seeds per fruit (hereafter seeds per fruit) from Cp and Np fruits for 19 species, and for seven of them we also had data for two years. We estimated the weight and germination rate of developed seeds from Cp and Np for 20 and 17 species, respectively. For this, seeds were dried in a chamber for 72 h before being weighed to the nearest 10–200 mg in 5–10 groups of 20–100 seeds per group depending on the seed size; except for *Justicia sebastianopolitanae*, *Salvia sellowiana* and *Odontonema barbelerioides* whose seeds were weighed individually. For each species, 40–50 seeds per treatment per species from Cp and Np treatments were placed in 6–10 Petri dishes per treatment with wet filter paper in a chamber set at 8 h daylength for up to 60 d. The mean percentage of seeds germinating per species was estimated. Appropriate temperature and time for

germination for a given species were determined in previous studies (M. Wolowski, unpubl. res.).

Data analysis

Pollen limitation was estimated as the effect size based on the standardized mean difference between Cp and Np treatments for proportion of fruit set, seeds per fruit, seed weight and percentage germination for each species (Hedges and Olkin, 1985). Overall effect size was calculated by traditional and phylogenetically independent meta-analyses following Adams (2008) and Lajeunesse (2009). Furthermore, we present only the results of the traditional meta-analysis with random effects since this model had the lowest Akaike information criterion (AIC) value (4–14 units lower than traditional fixed effects, as well as the phylogenetically independent meta-analysis) following Lajeunesse (2009). For a given species (and overall), PL was interpreted as significant when the 95 % confidence interval around the effect size does not overlap zero (Hedges and Olkin, 1985). To assess temporal variation in PL for the quantity variables (fruit set and seeds per fruit), we calculated the effect size for 14 and seven species, respectively, with individuals of each species pooled across years (2009–2011).

The relationships between the effect size of quantity and quality aspects of reproduction and predictive plant features (self-incompatibility, autogamy, density and specialization level)

were assessed with phylogenetic generalized linear models separately for each pair of predictor–response variables. This is because the ratio sample size/predictive plant features was inadequate for models with four independent factors. The following plant features were obtained from Wolowski *et al.* (2013): self-incompatibility (measured by the Index of Self-incompatibility; Zapata and Arroyo, 1978), autogamy (measured by the Index of Autogamy; Lloyd and Schoen, 1992) and plant density (measured by the number of flowering individuals ha^{-1}). The specialization level of each plant species was assessed by the *d'*-Index which is a quantitative measure of interaction at the species level based on frequency visitation data and used to assess variation within a pollination network (Blüthgen *et al.*, 2006). The *d'*-Index was calculated from the network obtained with visitation rates from Canela (2006) and M. Wolowski (unpubl. res.). Some variables (effect size of fruit set, autogamy and plant density) were log transformed [log(*x* + 1)] and residuals analysis was conducted in order to ensure that model assumptions (normal distribution and homoscedasticity) were met. After transformation, autogamy was the only variable that did not reach normality; however, residuals were normally distributed and the assumption of linearity was acceptable.

Analyses were conducted in Phylometa 1·3 beta (Lajeunesse, 2009) and R 2.15.0 (R Development Core Team, 2012) using caper (Orme, 2012), bipartite (Dormann *et al.*, 2009) and metafor (Viechtbauer, 2010) packages. The primary phylogenetic hypothesis was obtained using Phylomatic (http://www.phylodiversity.net/phylomatic). Then, the topology was resolved and branch lengths were calibrated in Mesquite 2·75 (Maddison and Maddison, 2011) following phylogenetic studies for angiosperms (Wikström *et al.*, 2001; Bremer *et al.*, 2004; Chaw *et al.*, 2004; Givnish *et al.*, 2011).

RESULTS

Fifty-two per cent (11/21) of species showed significant PL for one or more of the response variables (Table 2). Overall, quantity aspects of PL were low (seeds per fruit: 0·31) to moderate (fruit set: 0·39). Also, while not significant for seed weight (−0·03), the quality aspect of PL, seed germination, was strong (0·83) (Table 2). The overall magnitude of PL varied with the response variable, and there was no clear pattern for quality or quantity aspects (Table 2). *Abutilon bedfordianum* was the only species that showed PL for all response variables.

Temporal variation in PL was observed for two of 14 (fruit set) and three of seven (seeds per fruit) species (Table 3). *Abutilon bedfordianum* was the only species with temporal variation in PL at both levels, while the other species exhibited it at one level: *Vriesea gradata* for fruit set, and *Aechmea nudicaulis*, *Nidularium itatiaiae* and *Quesnelia augusto-coburgii* for seeds per fruit.

The effect sizes of the four response variables were not significantly related to self-incompatibility ($F_{1,15-19} = 0.02-1.54$, $P \geq 0.23$), autogamy ($F_{1,15-19} = 0.11-2.59$, $P \geq 0.13$), density ($F_{1,15-19} = 0.04-2.63$, $P \geq 0.12$) or specialization level ($F_{1,15-19} = 0.01-1.04$, $P \geq 0.32$). No phylogenetic signal was detected in any of the models (i.e. 95 % confidence intervals for *λ* values overlap 0 or 1). Phylogeny of plants and raw data are available in Supplementary Data Fig. S1 and Table S1.

DISCUSSION

Occurrence of PL in hummingbird-pollinated plants of a tropical montane rain forest

Our results show that PL occurs in the hummingbird-pollinated plants at Itatiaia. The proportion (52 %) of species was slightly lower than the 62–73 % recorded for all angiosperms (Burd, 1994; Ashman *et al.*, 2004). The magnitude of PL in the literature, however, may be overestimated due to publication bias, experimental design and the response variable measured (Knight *et al.*, 2006). For instance, higher estimates of PL are recorded when the pollen supplementation experiment is performed at the partial-plant level (flower or inflorescence) compared with the whole-plant level (Knight *et al.*, 2006). These differences are attributed to resource reallocation among flowers and inflorescences (Stephenson, 1981; Haig and Westoby, 1988; Zimmerman and Pike, 1988). However, our test suggested that resource reallocation was not a concern here or in other community-based studies of PL (Hegland and Totland, 2008). In relation to the response variable used, higher PL was recorded when fruit set was measured compared with seeds per fruit (Knight *et al.*, 2006). Within the hummingbird-pollinated plants at Itatiaia, quantity aspects of PL (fruit set and seeds per fruit) did not differ in magnitude. In fact, here the extent of PL seems to be more related to the species-specific response to the quantity and quality aspects measured.

The occurrence of PL at Itatiaia was higher than found in other studies that used a within-community comparison of insect-pollinated species, e.g. PL occurrence ranged from 18 % of species in a temperate grassland community (Hegland and Totland, 2008), to 25 % in a temperate deciduous forest community (Motten, 1986), to 38 % in a sub-tropical community in the Atacama Desert (González and Pérez, 2010). Whether the differences between this hummingbird-pollinated plant community and those other communities are due to pollinator type (hummingbird vs. insects), biome (tropical forest vs. temperate grasslands and forests or sub-tropical desert) or other plant traits is not clear. Species in temperate regions were less likely to express PL than those in the tropics (Larson and Barrett, 2000), which may be due to low plant density in the latter (Baker, 1959; Fedorov, 1966) that may result in low visitation rates. The association of PL and biome could be a valid explanation for the difference between the Itatiaia community and the other communities; however, we would need additional tropical communities to test this hypothesis fully. Pollen limitation of seed quantity is known to be lower in self-compatible species (Burd, 1994; Larson and Barrett, 2000; Knight *et al.*, 2005; Alonso *et al.*, 2010), and even lower in species with autogamy (i.e. species capable of spontaneous self-pollination) (Larson and Barrett, 2000). Thus, if a community has a high representation of self-compatible or autogamous plants, the average incidence of PL is expected to be low. This is the case within the flora of the coastal Atacama Desert where the low occurrence of PL (38 % of species) was attributed to a high incidence of autogamy (63 % of the species) (González and Pérez, 2010). Thirty-three per cent of species were autogamous at the Itatiaia community (Wolowski *et al.*, 2013), thus a lower incidence of autogamy could contribute to higher PL in these hummingbird-pollinated plants; however, most of the species (67 %) are self-compatible (Wolowski *et al.*, 2013). Besides that, the possibility that PL

TABLE 3. *Pollen limitation effect size (95 % confidence interval) and sample size for each species in each year studied in hummingbird-pollinated plants at Itatiaia National Park for fruit set and seeds per fruit*

Species, years studied	Response variable	Year 1	Year 2
Justicia sebastianopolitanae 2010/2011	Fruit set	−0·53 (−1·48 to 0·41), 9	0·12 (−0·65 to 0·89), 13
Odontonema barbelerioides 2010/2011	Fruit set	1·35 (−1·69 to 2·86), 2	0·43 (−0·69 to 1·88), 5
Staurogyne itatiaiae 2010/2011	Fruit set	−0·18 (−1·43 to 1·06), 5	−0·46 (−1·35 to 0·43), 10
Aechmea nudicaulis 2010/2011	Fruit set	1·87 (−2·29 to −6·02), 2	0·58 (−0·58 to 1·75), 6
	Seeds per fruit	**1·95 (0·57 to 3·33) 5, 9**	−0·04 (−0·93 to 0·85), 16, 7
Billbergia distachia 2009/2010	Fruit set	0·0 (−1·60 to 1·60), 3	0·78 (−0·10 to 1·65), 11
	Seeds per fruit	0·17 (−0·77 to 1·1), 8, 10	0·5 (−0·23 to 1·24), 17, 13
Nidularium itatiaiae 2009/2010	Fruit set	0·17 (−0·67 to 1·00), 11	0·53 (−0·47 to 1·53), 8
	Seeds per fruit	**0·58 (0·04 to1·11), 22, 38**	0·09 (−0·39 to 0·57), 24, 54
Pitcairnia flammea 2010/2011	Fruit set	0·56 (−0·38 to 1·51), 9	0·21 (−1·03 to 1·46), 5
Quesnelia augusto-coburgii 2010/2011	Fruit set	−0·56 (−1·46 to 0·34), 10	0·83 (−0·05 to 1·71), 11
	Seeds per fruit	**1·85 (0·91 to 2·79), 13, 13**	0·01 (−0·52 to 0·53), 21, 42
Vriesea gradate 2010/2011	Fruit set	**0·94 (0·09 to 1·78), 12**	0·63 (−0·45 to 1·70), 7
Vriesea longicaulis 2010/2011	Fruit set	−0·14 (−1·27 to 0·99), 6	−0·25 (−1·86 to 1·37), 3
Nematanthus crassifolius 2009/2010	Fruit set	0·62 (−0·46 to 1·71), 7	−0·04 (−1·02 to 0·94), 8
Salvia sellowiana 2010/2011	Fruit set	0·52 (−0·91 to 1·95), 4	0·03 (−1·02 to 1·08), 7
	Seeds per fruit	0·11 (−1·2 to 1·43), 4, 5	−0·12 (−0·92 to 0·68), 10, 15
Abutilon bedfordianum 2010/2011	Fruit set	**1·21 (0·34 to 2·08), 12**	0·58 (−0·49 to 1·65), 7
	Seeds per fruit	**1·35 (0·46 to 2·25) 21,8**	0·45 (−0·58 to 1·48), 8, 7
	Fruit set	0·19 (−1·20 to 1·58), 4	0·13 (−1·00 to 1·26), 6
Manettia mitis 2010/2011	Seeds per fruit	0·94 (−0·12 to 2·0), 11, 6	−0·21 (−0·98 to 0·56), 11, 16

Bold indicates significant pollen limitation.
Sample size of fruit set is the number of treated individuals and for seeds per fruit is the number of fruits counted for Cp and Np, respectively.

differences are due to distinct methodological procedures or variables measured cannot be excluded.

The magnitude of PL varied with response variables

The magnitude of PL varied in relation to the quantity and quality aspects of reproduction, and some of these differed from our expectations. For the quantity variables, the magnitude of PL was similar between fruit set and seeds per fruit in this community. The extent of PL at the fruit set level was comparable with that of the angiosperms as a whole, whereas seeds/fruit was higher at Itatiaia (Knight *et al.*, 2006). For the quality aspects, PL was high for seed germination as expected, perhaps reflecting the transport of low quality pollen by the hummingbirds (Linhart, 1973; Linhart *et al.*, 1987), although the lack of PL for seed weight disagrees with both our expectation and the findings of Hegland and Totland (2008). Even though quality aspects may be biased due to post-pollination events (i.e. resource reallocation), the absence of PL for seed weight here may signify an absence of resource reallocation during seed maturation (e.g. seeds from both Cp and Np treatments may have received sufficient resources for maturation). Nevertheless, other measures (e.g. the relationship of pollen grains on the stigma to pollen tubes on the style) may be more informative for disentangling quantity and quality aspects of PL, especially when assessed across species within communities (Alonso *et al.*, 2012).

Low temporal variation in PL within hummingbird-pollinated plants

In accordance with our predictions, temporal variation in PL was minor in these hummingbird-pollinated plants. Our results suggest that hummingbirds are constant pollinators of plants within their communities, and temporal variation may instead be due to fluctuation in the resource environment. Moreover, since effect size calculation is sensitive to small sample size ($n < 10$; Hedges and Olkin, 1985), it is possible that differences between years may not have been detectable for some species where sample size was restricted in one of the two years (e.g. seeds per fruit of *Aechmea nudicaulis* in 2010 and fruit set of *Abutilon bedfordianum* and *Vriesea gradata* in 2011).

Lack of relationship between PL and plant traits and pollination specialization

Although variation among species in PL was observed, relationships between PL and plant traits or pollination specialization, previously reported or suggested in the literature (Burd, 1994; Larson and Barrett, 2000; Knight *et al.*, 2005; Waser *et al.*, 1996), were not detected in the hummingbird-pollinated plants at Itatiaia. Moreover, PL was consistently dispersed across the phylogeny. This may suggest that observed patterns in global data sets do not exist in this community and/or that in the tropics and at the community level we may need a different explanation for PL. Likewise, we are already dealing with a very specialized pollen transport system with subtle differences among species. At this time, the proximate causes of pollination inefficiency are not clear despite the wide extent of PL within the hummingbird-pollinated plants at Itatiaia.

Conclusions and future directions

Pollen limitation was observed for many species of the hummingbird-pollinated plants and its magnitude was similar

to that of the angiosperms as a whole, but it was higher than found in other community-level studies. Whether the extent of PL in our community is a result of the tropical region or vertebrate pollination is unclear, but comparative studies are strongly encouraged. In particular, in light of the fact that within this community PL could not be attributed to mating system, plant density or pollination specialization, it may be that traits driving patterns of PL within communities are different from those that emerge from global studies; for instance, perhaps environmental variables are more important at the local level or the causes of PL may vary among species within the community.

The extent of PL varied widely with response variable. The quantity variables (fruit set and seeds per fruit) were comparable and exhibited a moderate level of PL, while only one quality aspect (seed germination) had a large effect. Consequently, the choice of the response variable had a major influence on the perception of the magnitude of PL and on our interpretation of its importance for plant reproduction. This result reinforces recent studies that highlight the need to use other measures for PL for single-species or community-wide studies, especially those that that do not confound post-pollination effects and can separate quantity and quality components (Aizen and Harder, 2007; Alonso *et al.*, 2012).

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Figure S1: the phylogeny of plants, response variables and plant traits. Table S1: raw data used to calculate effect size.

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