RESEARCH PAPER

Relative effectiveness of insects *versus* hummingbirds as pollinators of Rubiaceae plants across elevation in Dominica, Caribbean

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ABSTRACT

- Most angiosperms rely on animal pollination for reproduction, but the dependence on specific pollinator groups varies greatly between species and localities. Notably, such dependence may be influenced by both floral traits and environmental conditions. Despite its importance, their joint contribution has rarely been studied at the assemblage level.

- At two elevations on the Caribbean island of Dominica, we measured the floral traits and the relative contributions of insects *versus* hummingbirds as pollinators of plants in the *Rubiaceae* family. Pollinator importance was measured as visitation rate (VR) and single visit pollen deposition (SVD), which were combined to assess overall pollinator effectiveness (PE).

- In the wet and cool Dominican highland, we found that hummingbirds were relatively more frequent and effective pollinators than insects, whereas insects and hummingbirds were equally frequent and effective pollinators at the warmer and less rainy midelevation. Furthermore, floral traits correlated independently of environment with the relative importance of pollinators, hummingbirds being more important in plant species having flowers with long and wide corollas producing higher volumes of dilute nectar.

- Our findings show that both environmental conditions and floral traits influence whether insects or hummingbirds are the most important pollinators of plants in the *Rubiaceae* family, highlighting the complexity of plant–pollinator systems.

INTRODUCTION

The majority of angiosperms rely on animals for pollination (Ollerton *et al.* 2011; Rech *et al.* 2016), thus plant–pollinator relationships are crucial for the functioning of most terrestrial ecosystems (Kearns *et al.* 1998). However, not all flower visitors are equally important as pollinators (Ollerton *et al.* 2007; King *et al.* 2013). At one extreme, some plants have ecologically and functionally generalised pollination systems (Ollerton *et al.* 2007), being pollinated by numerous species and groups of animal pollinators (Waser *et al.* 1996; Fumero-Cabán & Meléndez-Ackerman 2007; Dalsgaard *et al.* 2009), whereas other plants are phenotypically, ecologically and functionally specialised, the extreme being plants adapted to pollination by one or a few functionally similar pollinators (Temeles *et al.* 2000; Lindberg & Olesen 2001; Ollerton *et al.* 2007; Geerts & Pauw

2009; Schäffler *et al.* 2015). Furthermore, the role of a specific pollinator or functional group of pollinators may vary spatially along environmental gradients (Herrera 2005; Dalsgaard *et al.* 2009; Martín González *et al.* 2009). In fact, pollinator assemblages can vary considerably across an environmental gradient, even limiting plant reproduction (Chalcoff *et al.* 2012).

When quantifying the importance of a pollinator or group of functionally similar pollinators for a given plant species, a key issue is to distinguish flower visitors from pollinators (King *et al.* 2013). Traditionally, visitation frequency, or visitation rate (VR), has been used as a measure of pollinator importance and dependency (Dalsgaard *et al.* 2013; Souza *et al.* 2018). However, VR might not correlate with pollinator importance, as a frequent flower visitor does not necessarily act as an effective pollinator (Mayfield 2001; Watts *et al.* 2012). Thus, to accurately quantify the importance of a given pollinator, a

measure of pollinator effectiveness (PE) – such as pollen deposition per visit – should be incorporated along with VRs (King *et al.* 2013; Ballantyne *et al.* 2015). Although it is very time consuming to collect this kind of data, the inclusion of effectiveness measures has led to some support of the 'pollination syndrome hypothesis', which may predict the most effective pollinator for a given plant based on floral trait combinations (Fenster *et al.* 2004; King *et al.* 2013; Rosas-Guerrero *et al.* 2014; Ashworth *et al.* 2015; Fernández de Castro *et al.* 2017; Wester & Johnson 2017). However, such studies rarely consider the variation in the relative importance of pollinators across localities with contrasting environmental conditions.

Here, we used plants in the coffee family (*Rubiaceae*) to test how the relative importance – including per-visit effectiveness – of insects *versus* hummingbirds as effective pollinators relates to both floral traits and environmental conditions. Specifically, we tested: (i) whether the relative importance of insects and hummingbirds as pollinators changes between elevations using VR, single visit pollen deposition (SVD) and a combination of the two expressing overall PE, and (ii) whether floral traits in addition to elevation influence VR, SVD and PE. We tested this on the Caribbean island of Dominica where many plants from the *Rubiaceae* have previously been shown to be visited legitimately by both hummingbirds and an array of insect groups (Dalsgaard *et al.* 2009; Martín González *et al.* 2009). We worked in two distinct elevation bands: mid-elevation rain forest habitat and high-elevation cloud forest and elfin habitat. Due to the environmental differences between these bands, we expected a shift in both plant species composition and in the importance of different groups of pollinators. Notably, as bees and most other insect pollinators, except flies, thrive better in warmer and drier habitats, we expected the floral traits to be more specialised towards hummingbirds in the wetter and cooler elfin/cloud forest in the highland, and the importance of insects, especially bees, to be higher in the warmer and drier rain forest at midelevation (Cruden 1972; Dalsgaard *et al.* 2009; Martín González *et al.* 2009). We also expected floral traits to impact the VR and importance of hummingbirds *versus* insects as pollinators (Dalsgaard *et al.* 2009; Bergamo *et al.* 2016). Notably, although many Caribbean flowers have both insect and hummingbird pollinators (Dalsgaard *et al.* 2009), typical hummingbird-pollinated flowers have long and narrow floral corollas, preventing all or most insects from reaching their nectar. Also, flowers exclusively pollinated by hummingbirds often have more dilute and copious amount of nectar, as well as other characteristics such as unscented flowers, making them less attractive to insect pollinators (Castellanos *et al.* 2004; Dalsgaard *et al.* 2009; Bergamo *et al.* 2016).

MATERIAL AND METHODS

Study site and period

The study was carried out on the southern part of the Lesser Antillean Island of Dominica in the Morne Trois Pitons National Park. The island is small and mountainous (751 km^2; 1447 m a.s.l.), with a steep environmental gradient. The average annual precipitation ranges from about 1200 mm along the west coast to 2500 mm along the east coast, with a gradual increase in rainfall from the coast to the interior, with some exposed mountainsides receiving about 8000 mm of rainfall

(Malhotra & Thorpe 1991). This generates a heterogeneous environment and associated habitats, ranging from dry shrubland along the western coastline to rain forest in wetter areas at midelevation to montane thicket and elfin forest in the cooler and wetter highlands. We focused on two elevations covering different vegetation types: rain forest (500–600 m a.s.l.) and montane thicket and elfin forest (750–975 m a.s.l.). At each elevation, data were collected at two sites separated by a minimum of 1.5 km. Here, environmental variables were recorded during sampling and an ANOVA confirmed the expected differences between sites in humidity ($F_{3,646} = 22.18$, $P < 0.01$) and temperatures ($F_{3,646} = 13.86$, $P < 0.01$). A following *post-hoc* Tukey test showed that the sites were more alike within each elevation than between elevations (Table 1). Thus, we found higher humidity and lower temperatures at high elevation compared to midelevation. The fieldwork was conducted from April 2015 to July 2015, covering the end of the dry season and beginning of the rainy season and thereby including the flowering period for different *Rubiaceae* species. All species in this study have flowers with relatively generalised morphological features and are visited by an array of distinct pollinator groups (Dalsgaard *et al.* 2009; Martín González *et al.* 2009).

Floral traits

All flowering *Rubiaceae* species in the two elevation bands were identified and, whenever possible, a minimum of 20 individuals were sampled for homostylous (no distinct style morphs among individuals) species. For distylous (distinct style morphs among individuals) species, 10 individuals for each morph were sampled and combined in the following analyses. For each flowering *Rubiaceae* species, floral morphological traits were measured with a digital calliper to the nearest 0.10 mm; *corolla length* as the internal distance from the tube opening to the base of the flower; *internal width* at the inside distal opening of the flower; and *outer width* as the longest distance between the flower petals. We measured corolla length and internal width as reflecting the morphological fit and nectar accessibility for distinct floral visitors, with typical hummingbird flowers usually presenting longer and more narrow corolla tubes (Castellanos *et al.* 2004; Dalsgaard *et al.* 2009). The distance between the petals (outer width) may potentially reflect a 'platform' where many insects land while visiting a

Table 1. *Post-hoc* Tukey's test comparisons of temperature and humidity of sites at high and midelevation; High1, High2, Mid1 and Mid2.

site	n	mean	SD	Tukey's HSD comparisons		
				High1	High2	Mid1
Temperature						
High1	290	24.4	3.3			
High2	90	23.6	5.2	0.3		
Mid1	62	26.4	3.5	<0.001	<0.001	
Mid2	211	25.6	3.1	<0.001	<0.001	0.04
Humidity						
High1	290	81.2	12.2			
High2	90	84.7	16.2	0.01		
Mid1	62	72.3	12.5	<0.001	<0.001	
Mid2	211	76.3	10.4	<0.001	<0.001	0.1

flower (Castellanos *et al.* 2004). For nectar sampling, we collected nectar volume (μl) and concentration (%) between 16:00–18:00 h from flowers previously covered with nylon mesh bags in late stage flower buds, totalling ~10–12 h of nectar production. Nectar volume was measured with a microsyringe and nectar concentration with a handheld refractometer. We expected flowers that are mainly visited and pollinated by hummingbirds would have a higher volume of rather dilute nectar, when compared to flowers having insects as their dominant pollinators (Castellanos *et al.* 2004; Dalsgaard *et al.* 2009). Furthermore, we noted colour and odour of flowers in the field, but as all plant species had odourless flowers according to human olfaction, and all except one species had white flowers, we decided to exclude these variables from subsequent analysis. If the same species was present in both elevations, it was sampled separately for each elevation.

Relative role of pollinators: visitation, pollen deposition and effectiveness

Flower visitation by hummingbirds and insects was monitored separately by direct sighting and also with video recordings for hummingbirds. We identified hummingbirds to species level and insects to functional group, *i.e.* butterflies, bees, flies, ants and beetles. Hummingbirds were observed from ca. 10 m and insects from ca. 2 m. Observations were conducted in dry and calm weather from 06:00 to 16:00 h. For each plant species, we conducted at least 10 h of observation for hummingbirds and 10 h for insects, covering the complete time span from 06:00 to 16:00 h (see Table S1 for details on visitation observations). Plant individuals were chosen in a semi-random fashion, where a minimum of five different individuals were observed at different times of the day. Due to changeable weather conditions at both elevations, observations were performed for variable time periods, ranging from 0.25 h to 3.00 h duration (a total of 181 h for insects and 275 h for hummingbirds). There were no differences in time periods spent on observing plant–pollinator interactions between elevations and visitor groups at each elevation ($F_{5,574} = 1.9$, $P > 0.05$). We only included observations when visitors touched the reproductive structures of the flower, *i.e.* potentially mutualistic associations. For each visit we also recorded the time of day, temperature, humidity, number of observed flowers and visitor group, *i.e.* whether a hummingbird, butterfly, bee, fly, ant or a beetle was the visitor. For each visitor group of each plant species, VR was calculated as visits flower^{-1}·h^{-1}.

To quantify the effectiveness of pollinators, we measured pollen deposition on the stigma, which has been shown to provide a first estimate of a floral visitor's effectiveness, allowing discrimination between floral visitors in their role as pollinators (Mayfield 2001; Watts *et al.* 2012; Freitas 2013; King *et al.* 2013). Data on SVD were collected for hummingbirds and insects as two groups. For each plant species, a minimum of 10 samples per visitor group per flower morph were collected and, when possible, 10 SVD samples were also collected for each visiting insect functional group. The day prior to collection of each SVD sample, flower buds were covered with a nylon mesh bag to prevent visitation. When the flowers opened and became receptive, the bags were removed and the flowers were exposed to visitors. After a single visit, the visitor was noted (*i.e.* hummingbird, bee, fly, butterfly, *etc.*) and the flower was collected

to transfer its stigma, including pollen attached to it, onto a glass slide prepared with conserving and colouring glycerine jelly containing fuchsin dye. Later, the number of conspecific pollen grains was counted under a light microscope (Mayfield 2001; King *et al.* 2013). Although pollen grains in distylous and other heteromorphic plants may show differences between morphs, such differences may be slight, making it impractical to count the pollen grains separately (Maruyama *et al.* 2010). Additionally, in distylous plants most pollen deposited would come from a different morph, as expected due to the reciprocal herkogamy found in distylous species, hence pin and thrum morphs were pooled in the analyses below.

The SVD samples were collected from different plant individuals at different times of day. For each plant species and each floral morph, controls (*i.e.* flowers previously covered and kept isolated from visitors) were collected to account for pollen deposition caused by possible sources other than pollinators, such as wind, handling or possible self-deposition. The mean number of pollen grains from controls for each plant species and each floral morph was then subtracted from each SVD sample.

To calculate overall PE of hummingbirds and insects for each plant species, the mean SVD was multiplied by each VR estimate of the same visitor group (Sugden 1986; Freitas 2013). The same procedure was conducted for each insect functional group, *i.e.* butterflies, bees, flies, ants and beetles. In a few cases (1.6%), an insect group was observed visiting a plant, but due to very low VRs it was not possible to measure SVD for this group. In these few cases, the PE value was set to zero, as the rarity of the visits was treated as indicative of a non-significant contribution to the pollination of the given plant.

Data analysis

To account for interspecific differences among plant species that could affect pollinator importance (*e.g.* pollen production) and standardise the measures of relative importance of pollinator groups, for each plant species we calculated Cohen's *d* effect size (Nakagawa & Cuthill 2007; Sullivan & Feinn 2012). This was calculated as the standardised mean difference between hummingbirds and insects for each pollinator importance variable (VR, SVD and PE). More specifically, for each plant species across both visitor groups, we calculated Cohen's *d* as the difference between the means, divided by the standard deviation of VR, SVD and PE, respectively. For a given variable, positive values, with 95% confidence intervals (CI) not overlapping zero, indicate that hummingbirds are significantly more important than insects, whereas negative values with 95% CIs not overlapping zero indicate that insects are more important. To test the relative importance of hummingbirds and insects as pollinators in each elevation band, we then calculated the mean Cohen's *d* effect size and the 95% CIs of VR, SVD and PE for all *Rubiaceae* species within each elevation band. This was also used to examine whether the relative pollinator importance of hummingbirds and insects differs between elevations depending on using VR, SVD or PE for its quantification. Insects were grouped in the major analysis, as we were mostly interested in comparing vertebrate and invertebrate pollinators, which resulted in increased sample size. However, we also examined the differences in pollinator group importance within each elevation band for each plant species between

hummingbirds and insects as one major group and each insect functional group with Kruskall–Wallis test and Dunn's *post-hoc* test (Figures S4–S6).

Finally, to evaluate the relative role of floral traits and environment on different measures of pollinator importance, we used a linear mixed model with the Cohen's *d* effect sizes as response variable using the lme4 R package (Bates *et al.* 2015). Because all floral traits showed moderate to strong correlation (Table 2) and to reduce the number of predictors, we used principal components analysis (PCA) on the five floral traits measured for each plant species in each elevation band. We log-transformed the data prior to analysis and the first two principal components (PCs) explained 88.3% of the variation in the data (PC1: 72.4%, PC2: 15.9%). These two PCs were taken as predictors representing the variation in floral traits of *Rubiaceae* species (Table 2). Importantly, there were no differences in the PC values between the elevation bands (PC1: $F_{1,12} = 1.60$, $P = 0.23$; PC2: $F_{1,12} = 0.44$, $P = 0.52$), thus allowing inclusion of these predictors together with elevation band as fixed effects, with elevation reflecting the variation in environmental conditions. Because some species were common among the elevation bands, species identities were included as a random factor in the analysis. We used the likelihood ratio test to assess the significance of each fixed effect; for significant effects we compared the difference between the marginal R^2 of the full model and the marginal R^2 of the model lacking the fixed variable of interest to estimate its relative importance (Nakagawa & Schielzeth, 2013).

RESULTS

We found 10 flowering Rubiaceae species, eight species at high elevation and six species at midelevation, of which four were found at both elevations (*Palicourea crocea*, *Psychotria urbaniana*, *Gonzalagunia hirsuta* and *Spermacoce assurgens*; Table S2, Figure S3). Blue-headed hummingbird (*Cyanophaia bicolor*) and Antillean-crested hummingbird (*Orthorhyncus cristatus*) were observed as floral visitors at both elevations. All insect functional groups were found at both elevations, although butterflies, beetles and bees were rare at high elevation (*e.g.* one single visit by a bee). *S. assurgens* and *P. uliginosa* were solely visited by insects, whereas the other plant species were visited by insects and hummingbirds to varying

degrees (Figure S4). In total, we recorded 579 visits by hummingbirds and 70 visits by insects (one bee, 56 flies, four butterflies, three beetles and six ants) in the high elevation and 395 visits by hummingbirds and 323 visits by insects (50 bees, 59 flies, 158 butterflies, 46 beetles and 25 ants) at the midelevation. We sampled 356 stigmas for pollen deposition counts, 106 visited by hummingbirds and 77 visited by insects (no bees, 38 flies, 22 butterflies, no beetles and 17 ants) at the high elevation and 63 visited by hummingbirds and 110 visited by insects (34 bees, 30 flies, 22 butterflies, five beetles and 18 ants) at midelevation.

Hummingbirds were the most important pollinators based on VR and PE for plants at the high elevation. There was no significant difference between hummingbirds and insects for SVD in this elevation band (Fig. 1B). At midelevation, insects and hummingbirds did not differ in relative importance, irrespectively of using VR, SVD or PE (Fig. 1B). The effects of elevation and floral PCs on PE are primarily associated with the effects they have on VR of pollinators, as both PC1 and PC2, as well as elevation, are significantly associated to VR and PE (Table 2). Especially PC1, which relates positively to floral trait size (width and length) and nectar volume, while negatively related to nectar concentration, had a strong positive effect on VR and PE, meaning that PC1 is associated with higher importance of hummingbirds in relation to insects (Tables 2 and 3). Moreover, PC1 was significantly associated with SVD, also having a positive effect, indicating higher importance of hummingbirds. PC2, which is primarily negatively associated with outer width (Table 2), indicated that narrower flowers are most favoured by hummingbirds for VR and consequently PE. The elevation results are consistent with the effect size analysis, in which hummingbirds have higher importance on PE at higher elevations due to their higher importance for VR (Fig. 1B, Table 3).

DISCUSSION

The relative importance of hummingbirds and insects as pollinators of *Rubiaceae* assemblages in Dominica differed according to elevation. At high elevation, hummingbirds were relatively more effective pollinators than insects (PE), which was mostly related to differences in VR and less so to differences in SVD. At midelevation, in contrast, we found no

Table 2. Pearson correlation coefficients between floral traits in *Rubiaceae* from Dominica island.

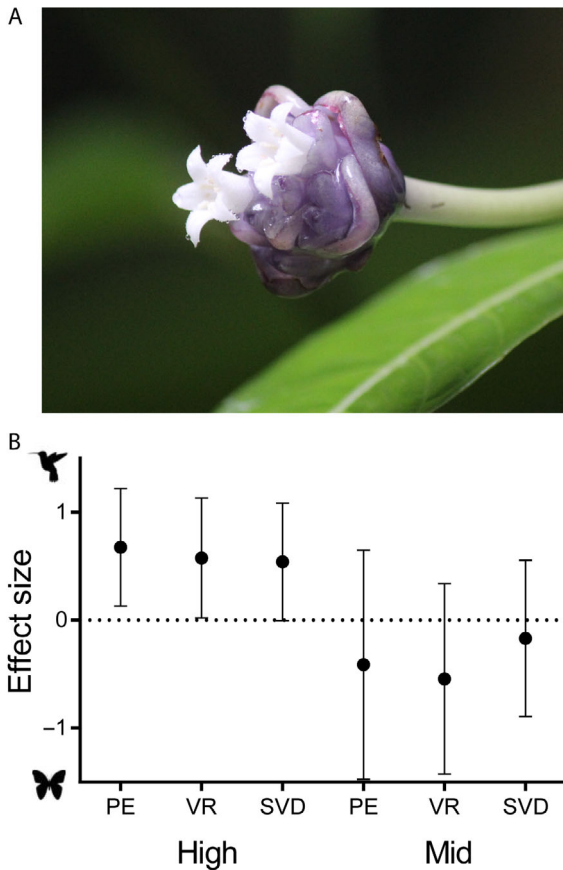| | width (mm) | | | nectar | | |
	outer	internal	length (mm)	volume (µl)	concentration (%)	PC1
Internal width (mm)	0.60*					
Length (mm)	0.53*	0.85***				
Nectar volume (µl)	0.44	0.88***	0.82***			
Nectar concentration (%)	−0.23	−0.70**	−0.63*	−0.70**		
PC1	0.63*	0.96***	0.92***	0.92***	−0.78***	
PC2	−0.74**	−0.03	−0.01	0.15	−0.47	0.00

We also show the correlation between floral traits and principal components scores (PC). PCs were able to explain 88.3% of the variation in the data (PC1: 72.4%, PC2: 15.9%).

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

Fig. 1. A: *Psychotria urbaniana* Steyerm, one of the studied *Rubiaceae* species in Dominica. This species was found in both elevation bands and was visited by both hummingbirds and insects. B: Pollinator importance in relation to elevation in *Rubiaceae* assemblages of Dominica. Mean (±95% CI) Cohen *d*'s effect sizes of pollinator effectiveness (PE), visitation rate (VR) and single visit deposition (SVD) for high (n = 8 plant species) and mid (n = 6 plant species) elevation. Hummingbirds are more important at positive values not overlapping zero and insects at negative values not overlapping zero.

Table 3. Model results from linear mixed effect models with distinct measures of relative pollinator importance, hummingbird *versus* insects, as response variables.

effect size	predictors	estimate	χ^2	*P*-value	variance explained
Visitation rates (VR)	Elevation	0.83 ± 0.27	9.54	<0.01	0.17
	PC1	0.29 ± 0.07	12.3	<0.001	0.44
	PC2	0.39 ± 0.14	7.14	<0.01	0.15
Pollen deposition (SVD)	Elevation	0.13 ± 0.21	0.44	>0.05	–
	PC1	0.24 ± 0.12	3.99	<0.05	0.32
	PC2	0.04 ± 0.19	0.06	>0.05	–
Pollinator effectiveness (PE)	Elevation	0.72 ± 0.28	6.50	<0.05	0.10
	PC1	0.35 ± 0.08	13.12	<0.001	0.55
	PC2	0.39 ± 0.15	6.02	<0.01	0.13

Elevation and principal components scores (PCs, see Table 2) were used as fixed effects and species identity as random effect in the models. We show results from the likelihood ratio test (chi-square) as well as the variance explained by each significant predictor (see Methods for details).

detectable difference between the pollination service of insects and hummingbirds. Therefore, we detected a general pattern of hummingbirds being relatively more important than insects at high elevation, which is characterised by higher humidity and cooler temperatures, but insects and hummingbirds being equally important pollinators at midelevation.

The environmental differences represented by the elevation bands were important through their influence on the composition of the pollinator assemblages. Specifically, the colder and more humid environment at high elevation may limit the activity, density and flight conditions for some insects, especially bees, leading to lower VRs of insects and consequently less insect pollination, while hummingbirds remain active under these conditions (Cruden 1972; Dalsgaard *et al.* 2009; Martín González *et al.* 2009). For example, *P. urbaniana* (Fig. 1A; Figure S3G) with small white flowers, is nearly exclusively visited by hummingbirds at high elevation (only a single ant was observed visiting), but at midelevation it was visited by hummingbirds, ants, bees and butterflies (Figure S4 panel f and j). Although not as clear, the results for the three other species found at both elevations support the overall pattern of insects (especially bees) being less important in the highlands. For *G. hirsuta* there is no significant difference in VR by hummingbirds and insects in either elevation band (Figure S4 panel e and i). However, although visited by a wide variety of insects at both elevations, insects are relatively less effective at high elevation compared to midelevation (*w* = 59.50, *P* = 0.044; Figure S6 panel e and i). *S. assurgens* is only visited by insects at both elevations but not visited by bees and butterflies at high elevation (Figure S4 panel h and l), probably due to lower activity and density of bees and butterflies at high elevation. Lastly, *P. crocea* is exclusively pollinated by hummingbirds (visited but not pollinated by a single ant at high elevation) and it did not differ in VR or effectiveness between elevations, confirming the trend of no or less effect of environment on hummingbird activity (Figures S4 and S6 panel g and k). These results illustrate how elevational/environmental differences in the availability of potentially effective pollinators may influence the apparent specialisation of plants on functional pollinator groups. Notably, our visitation data were only collected during favourable conditions for insects (*i.e.* warmer and without rain), and the results would probably have been even stronger if we had included periods with rain and cooler temperatures, which are common at high elevation in the Antillean mountains (Dalsgaard *et al.* 2018).

Whereas many studies on variation in pollinator assemblage across environmental gradients are based exclusively on VRs (Dalsgaard *et al.* 2013; Souza *et al.* 2018), we incorporated both estimates of the quantity (VR) and quality (SVD) of the pollination process into the overall PE. Because PE is mostly determined by difference in VR at high elevation, visitation frequency is a good proxy for the importance of pollinator groups in the studied *Rubiaceae* assemblages, particularly on the relative importance of hummingbirds *versus* insects. However, it is worth mentioning that the results probably depend on the assemblage, habitat and region in which the study is focused. Here, all the plant species have flowers with a relatively generalised phenotype, compared to more specialised hummingbird flowers (Dalsgaard *et al.* 2009; Ferreira *et al.* 2016), with short floral tubes that are easily accessible, thus

allowing different groups of visitors to touch the anthers and stigmas and deposit pollen. On the other hand, more phenotypically diverse and specialised assemblages of plants would potentially show greater variation in SVD between pollinators, resulting in a clearer division in importance of pollinators when using PE as compared to VR alone (King *et al.* 2013).

Regarding the importance of floral traits, interestingly, the PC representing the major proportion of variation in floral traits (PC1, related positively to flower size and nectar volume, while negatively related to nectar concentration) had a positive effect on all variables (VR, SVD and PE), quantifying the relatively higher importance of hummingbirds in relation to insects. Thus, although environment generated variation in relative pollinator importance, floral traits were also relevant. Floral traits may act in segregating the floral niches between birds and insects (Dalsgaard *et al.* 2009; Bergamo *et al.* 2016), nevertheless, birds and insects often share numerous floral resources but make distinct contributions to pollination, either related to differences in visitation frequency or in their single visit effectiveness (*e.g.* Mayfield 2001; Hargreaves *et al.* 2004; Schmid *et al.* 2011; Watts *et al.* 2012; Sun *et al.* 2017). We contribute to these previous findings by showing that besides floral traits *per se*, which in our study correlate with the relative importance of hummingbirds *versus* insects, differences in PE are also associated with pollinator availability across elevational/environmental gradients. By finding that environment influences the relative importance of hummingbirds and insects, our results suggest that the environment has important consequences for gene flow between plants that is mediated by pollinators, as hummingbirds and insects have markedly different foraging behaviours, with important consequences for how pollen is carried in space (Krauss *et al.* 2017).

In conclusion, for *Rubiaceae* assemblages in Dominica, we found that hummingbirds were relatively more important than insects as pollinators at higher elevation, whereas at mid-elevation sites insects and hummingbirds were equally important. This difference is mediated by differences in the VRs and not by the effectiveness of each single visit, as measured by SVD. Thus, environment-related difference in pollinator assemblages affected the overall PE of hummingbirds *versus* insects across the two elevation bands. Furthermore, we found that floral traits are also important in determining the relative importance of hummingbirds *versus* insects as pollinators. These results add to the recent assessment on the relative importance of vertebrate pollinators (Ratto *et al.* 2018) and the ongoing discussion of predictive ability of floral traits and environmental conditions on pollination services (Waser *et al.* 1996; Chalcoff *et al.* 2012; Rosas-Guerrero *et al.* 2014). Because bird and insect pollination promote distinct pollen flow patterns in space (Krauss *et al.* 2017), our results suggest that floral traits and environmental differences may both lead to differences in mating patterns within and among plant populations. This illustrates the complexity of quantifying the relative importance of distinct pollinator groups and is worthy of additional investigation in the future.

ACKNOWLEDGEMENTS

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Data on visitation observations.

Table S2. Data on floral traits for all 10 *Rubiaceae* species.

Figure S3. Pictures of flowers and pollinators found in mid- and high elevations in Dominica.

Figure S4. Mean visitation rate (VR) by hummingbirds and insects for each plant species at each elevation.

Figure S5. Mean number of pollen grains deposited per visit (SVD) by hummingbirds and insects for each plant species at each elevation.

Figure S6. Mean pollinator effectiveness (PE) of hummingbirds and insects for each plant species at each elevation.

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