Exploring palm–insect interactions across geographical and environmental gradients

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INTRODUCTION

Mutualistic relationships, such as plant-pollinator interactions, have been suggested to drive ecological divergence (Ehrlich & Raven, 1964; Thompson & Cunningham, 2002; Weiblen & Bush, 2002; Thompson, 2005; Whittall & Hodges, 2007). Therefore, it is likely that plant reproductive isolation is closely related to pollination biology and differences in pollinator distribution and plant-pollinator specificity may contribute to population differentiation and coevolution and even drive speciation. Insects are the predominant pollinators across flowering plants and therefore plant-insect interaction is expected to be an important evolutionary factor in most clades across the angiosperm phylogeny (Farrell, Mitter & Futuyma, 1992). Classic examples of plant-insect coevolution are found throughout the plant tree of life and include yucca moths and Yucca L. (e.g. Pellmyr, 2003), fig wasps and Ficus L. (e.g. Wiebes, 1979) and ants and Acacia Mill. (Janzen, 1966). These studies have shown that high pollinator specificity results in high species turnover between host plants (here defined as visited or pollinated plant...
individuals), although plant–insect coevolution is diffuse when pollinator species use several host plants. Further, insect species turnover between plant hosts can be driven by various factors, including environmental and spatial distance, without the influence of host identity (Carstensen et al., 2014). Therefore, the roles of extrinsic and intrinsic mechanisms shaping plant–insect relationship are unclear.

Analysis of species turnover across space (i.e. beta diversity) has been important to disentangling the role of different mechanisms in local species assemblages such as species interactions, environmental heterogeneity and dispersal limitation (Novotny et al., 2007; Bell et al., 2013; Carstensen et al., 2014). A primary hypothesis in plant–insect systems is that species interactions are the strongest driver of insect turnover. This hypothesis suggests that mutualism is strong and species co-vary across their geographical ranges (Thompson, 1999). It also predicts that insect species turnover is low throughout the host plant species range, even across broad environmental or geographical gradients (Godsoe et al., 2009). In contrast, a second hypothesis posits that environmental heterogeneity may increase species turnover within the host plant range. Species distributions are limited by environment (e.g. physiological tolerances), particularly for insects as they are highly affected by climatic conditions (Archibald, Greenwood & Mathewes, 2013). This hypothesis predicts high species turnover across climatic gradients (e.g. along mountain sides). Thirdly, the dispersal limitation hypothesis suggests that dispersal capacity influences insect species turnover, resulting in spatially autocorrelated species composition across landscapes (Novotny et al., 2007). If environmental heterogeneity or dispersal limitation explains insect species variation, the hypothesis of coevolution would be rejected based on plant–insect networks. However, appropriate data to evaluate these hypotheses are limited.

Palms are a well studied plant group that display a wide variety of pollination syndromes and, although they were long thought to be wind pollinated (Faegri & van der Pijl, 1979; Anderson, Overall & Henderson, 1988), they have been shown to maintain strong interactions with insects (Porter-Morgan, 2007; Barfod, Hagen & Borchsenius, 2011). For example, beetles are reported to pollinate 29% of all palms (Barfod et al., 2011). Sap beetles in the genus Mystrops are particularly associated with palm flowers, are highly abundant, have been reported to pollinate a large proportion of palm genera and potentially generate coevolutionary relationships with some of these species (e.g. Henderson, 1986; Barfod, Henderson & Balslev, 1987; Anderson et al., 1988; Silberbauer-Gottsberger et al., 2001; Padilha, Couturier & Beserra, 2003; Kirejtshuk & Couturier, 2009, 2010; Barfod et al., 2011; Balhara et al., 2013; Nuñez, 2014). Visitor insects on flowers have been studied in some palm species (reviewed in Barfod et al., 2011), providing an excellent system to analyse insect species turnover at large geographical scales.

In this study, we sampled Mystrops assemblages from eight species of Wettinia Poepp. ex. Endl. to determine the degree of plant–insect interaction. Specifically, we aimed to explore the factors that drive plant–insect interaction in the Wettinia–Mystrops system. Here, we used three working hypotheses: (1) Specificity of plant–insect interaction is strong and it is maintained across the host plant range, predicting insect turnover to be low within and high between host species. In addition, host species filter pollinators as a mechanism of reproductive isolation, resulting in large pollinator assemblage differences between closely related plant species. Thus, insect species turnover increases with phylogenetic distance between host plants (Kay & Sargent, 2009). (2) Environmental heterogeneity drives insect species turnover. Specifically, climatic tolerance limits species distribution and composition (e.g. Archibald et al., 2013). Thus, species dissimilarity is predicted to increase with climatic differences, irrespective of host identity. (3) Dispersal limitation is an important driver of insect species turnover, assuming low dispersal abilities based on their small body size and short flight distances (Kirejtshuk & Couturier, 2010). Insect species dissimilarity is predicted to increase with spatial distance. To test among these hypotheses, we correlated insect species dissimilarity between host species and across spatial and climatic distance. We also examined phylogenetic distance between host species to explore the effect of relatedness in plant–insect networks.

**METHODS**

**STUDY SYSTEM:** WETTINIA SPECIES AND MYSTROPS SPECIES

Wettinia comprises 21 species distributed from central Panama and Venezuela south to Bolivia (Henderson, Galeano & Bernal, 1995). Most of the species are found in Colombia and Ecuador and the Choco region is especially diverse with ten endemic species. Wettinia spp. are widely represented in the Andes, in contrast with lowland Amazonia, where only three species are found (Henderson et al., 1995; Galeano & Bernal, 2010). We sampled eight species representing sister lineages from each of the major clades of Wettinia from a robust molecular phylogenetic tree of tribe Iriarteeae (Fig. 1; Bacon et al., 2016, this issue; for voucher information, see that paper). Selected sister taxa were chosen based on accessibility of field sites: three endemics from the Choco...
region, *Wettinia aequalis* (O.F.Cook & Doyle) R.Bernal, *W. quinaria* (O.F.Cook & Doyle) Burret and *W. radiata* (O.F.Cook & Doyle) R.Bernal; *W. fascicularis* (Burret) H.E.Moore & J.Dransf. and *W. kalbreyeri* (Burret) R.Bernal from the Central Cordillera of Colombia and Ecuador; *W. microcarpa* (Burret) R.Bernal and *W. praemorsa* (Willd.) Wess.Boer from the Eastern Cordillera of Colombia; and the Amazonian species *W. drudei* (O.F.Cook & Doyle) A.J.Hend. (Supporting Information, Table S1).

We collected insect visitors from 31 staminate inflorescences from at least three individuals of each of the eight palm species. Whole inflorescence were collected and placed in a plastic bag containing 70% ethanol to preserve insects, which were subsequently sorted and counted and morphological and anatomical characters were compared between all samples. We used dorsal pubescence, body coloration and pronotum shape diagnostic characters to define morphospecies, as widely used in this insect group (Kirejtshuk & Couturier, 2010).

**DETERMINANTS OF INSECT ASSEMBLAGES**

We assessed insect species turnover in relation to spatial, climatic and phylogenetic distance between palm species. We applied three metrics of species turnover that measure different assemblage properties. The Bray-Curtis metric is focused on shared species and includes the variation present in insect abundance. We also used Manhattan and Euclidean distances (log-transformed), which incorporate species absence and therefore facilitate comparison with results from Bray-Curtis. We used the ‘cophenetic’ function to estimate divergence between palm species based on the most recent *Wettinia* molecular phylogenetic tree (Bacon et al., 2016) and the ‘pointDistance’ function to measure the spatial distance between points on a sphere based on geographical coordinates, both in the R package raster (Hijmans & Etten, 2012). Climatic data were obtained for each locality from the Worldclim database (Hijmans et al., 2005). We found high correlation between bioclimatic variables (Pearson $R > 0.7$), so we reduced the dataset to include only minimum annual temperature and precipitation of the driest quarter, considered the major limiting factors of insect distribution (Deutsch et al., 2008). Finally, we estimated the Euclidean distance of each these variables between localities.

We regressed insect turnover as a response variable with climate, spatial distance and phylogenetic
distance as independent variables using a multiple regression matrix (Lichstein, 2007). We ran this analysis for each combination of variables to estimate the individual effect on the total explained variance. These analyses were run using the ‘MRM’ function implemented in R package ecodist (Goslee & Urban, 2007). We chose this method to analyse beta diversity over others because our sampling is geographically biased and the behaviour of other methods under these conditions is unclear (Legendre, Borcard & Peres-Neto, 2005). Finally, we assessed the degree of pollinator specificity using an analysis of similarity (ANOSIM; Clarke, 1993) to compare insect species assemblages within and between palm species. ANOSIM estimates the proportion of differences within and between categories, in our case host palm species. We used the ‘plotweb’ function implemented in the R package bipartite (Dormann, Gruber & Fründ, 2008) to visualize the degree of specificity between insects and palms.

Palm species distribution modeling

Insect species dissimilarity may be related to climatic differences because each Wettinia sp. occupies a different climatic profile. Thus, the effect of climate and host identity could be mixed. To disentangle these effects, we obtained occurrence data for the eight Wettinia spp. derived from the Missouri Botanical Garden (Tropicos), Instituto Sinchi (Colombian Amazon herbarium), Universidad de Antioquia herbarium, Universidad Nacional de Colombia herbarium and the Flora de Antioquia databases. All georeferenced biases were carefully reviewed to avoid geographical biases. We used the MaxEnt algorithm (Phillips, Dudik & Schapire, 2004) in the R package dismo version 0.9–3 (Hijmans et al., 2011) to model palm species distributions. Because our purpose was compare amongst palm species, rather than understand their relationship with bioclimatic variables, we included all 19 climatic layers from the WorldClim database (Hijmans et al., 2005). We used 10 000 background points from which 90% were randomly selected to characterize the surrounding environment of each palm species and 75% of the randomly sampled points from the presence data used as input for MaxEnt. The remaining background and presence data points were used to evaluate the fit of the models.

RESULTS

We found 21 insect species belonging to the genus Mystrops associated with the eight Wettinia spp. (N = 31 inflorescences). Of all insects collected in each palm inflorescence, 40–68% belong to Mystrops, the most abundant visitor. Lowland palm species tend to host lower Mystrops abundance and species richness than highland palms (Table 1). The sister species W. fascicularis and W. kalbreyeri share their principal visitor species. Choco Wettinia spp. share one Mystrops sp., although the morphospecies was present in low abundances. Despite being sampled in different cordilleras, W. kalbreyeri and W. microcarpa share their principal visitors. In addition, W. microcarpa shares one pollinator species with W. praemorsa, although it was rarely collected and had low abundance. The Amazonian species, W. drudei, does not share any pollinator with other of the host species analysed (Fig. 2). We found a general pattern of high differentiation in the pollinator species assemblage between species based on ANOSIM results (R: 0.98, P < 0.001; Fig. 2).

Beta diversity metrics affect the interpretation of our results. Bray–Curtis and Euclidean log-transformed based analyses show similar results and large proportions of explained variance. In contrast, Manhattan distance is poorly related to predictor variables (Table 2). We interpret our results based on log-transformed Euclidian metric because

<table>
<thead>
<tr>
<th>Host species</th>
<th>Elevation min</th>
<th>Elevation max</th>
<th>Morphospecies richness (number of shared species)</th>
<th>Total abundance</th>
<th>Abundance (%)</th>
<th>Mystrops abundance</th>
<th>Main visitor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wettinia microcarpa</td>
<td>1600</td>
<td>2300</td>
<td>5 (3)</td>
<td>2819 ± 1427</td>
<td>39.95</td>
<td>1126 ± 464</td>
<td>29.49</td>
</tr>
<tr>
<td>Wettinia fascicularis</td>
<td>1200</td>
<td>2000</td>
<td>5 (3)</td>
<td>2426 ± 1122</td>
<td>56.55</td>
<td>1372 ± 570</td>
<td>40.12</td>
</tr>
<tr>
<td>Wettinia kalbreyeri</td>
<td>400</td>
<td>2200</td>
<td>4 (2)</td>
<td>5426 ± 3752</td>
<td>41.67</td>
<td>2259 ± 727</td>
<td>77.74</td>
</tr>
<tr>
<td>Wettinia radiata</td>
<td>0</td>
<td>1000</td>
<td>3 (2)</td>
<td>2120 ± 340</td>
<td>67.08</td>
<td>1422 ± 231</td>
<td>73.89</td>
</tr>
<tr>
<td>Wettinia praemorsa</td>
<td>400</td>
<td>2400</td>
<td>3 (2)</td>
<td>1320 ± 534</td>
<td>67.73</td>
<td>894 ± 500</td>
<td>62.34</td>
</tr>
<tr>
<td>Wettinia aequalis</td>
<td>0</td>
<td>800</td>
<td>3 (1)</td>
<td>1800 ± 650</td>
<td>52.78</td>
<td>950 ± 274</td>
<td>85.97</td>
</tr>
<tr>
<td>Wettinia quinaria</td>
<td>0</td>
<td>1100</td>
<td>2 (1)</td>
<td>4123 ± 2011</td>
<td>57.36</td>
<td>2365 ± 1148</td>
<td>96.29</td>
</tr>
<tr>
<td>Wettinia drudei</td>
<td>75</td>
<td>700</td>
<td>1 (1)</td>
<td>515 ± 211</td>
<td>41.94</td>
<td>216 ± 87</td>
<td>100</td>
</tr>
</tbody>
</table>

Pollination interactions are influenced by pollinator abundance. We found that phylogenetic distance between palms is positively related with turnover of Mystrops spp. and explains the largest proportion of variance (Table 2), implying that closely related species share a larger proportion of pollinators (Fig. 2). In addition, climatic divergence between sites in conjunction with phylogenetic relatedness between hosts explains 57% of the variance. Finally, our results show that spatial distance has a minor role driving species turnover (Table 2; Fig. 2).

The environmental niche models for Wettinia highlight the variation in species distributions, which could be the result of adaptations to different climatic regimes, producing different distributional patterns. The spatial distribution models inferred using MaxEnt show some species are widespread (W. dru dei and W. praemorsa), some are restricted to high elevations in the Andes (1500–2500 m; W. fascicularis and W. kalbreyeri) and others that are restricted to the lowland regions of Choco (W. aequalis, W. quinaria and W. radiata; Fig. 3). Some palm species have overlapping ranges, and thus they could share pollinator species where they occur in sympatry, as is the case for the species in the lowlands in Choco (sharing one Mystrops sp.) and for two palm species distributed along the Andes (sharing two Mystrops spp.).

**DISCUSSION**

Taken together, our results indicate that Mystrops spp. are common visitors to Wettinia spp. and probably play a strong role in pollination biology. Furthermore, we found low compositional similarities between host palm insect communities and that insect turnover is affected by climate rather than spatial distance. Similar patterns have been reported in other pollination studies (Henderson, 1986; Barfod et al., 1987; Anderson et al., 1988; Silberbauer-Gottsberger et al., 2003; Padilha, et al., 2003; Nuñez, Bernal & Knudsen, 2005). For example, the oil palm Elaeis guineensis Jacq. is highly dependent on a single insect species and the pollinator assemblage of

**Table 2.** Multiple regression matrix using three methods and their respective statistical support values

<table>
<thead>
<tr>
<th>MODEL A</th>
<th>Method = 'euclidean'</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geog</td>
<td>−0.0008</td>
<td>0.0534</td>
<td>0.01</td>
</tr>
<tr>
<td>Climate</td>
<td>1.2788</td>
<td>0.5271</td>
<td>0.01</td>
</tr>
<tr>
<td>Phy</td>
<td>0.1393</td>
<td>0.4732</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0.6749</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MODEL B</th>
<th>Method = 'manhattan'</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geog</td>
<td>−0.9619</td>
<td>0.0223</td>
<td>0.07</td>
</tr>
<tr>
<td>Climate</td>
<td>255.2996</td>
<td>0.1872</td>
<td>0.01</td>
</tr>
<tr>
<td>Phy</td>
<td>37.7329</td>
<td>0.1674</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0.3737</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MODEL C</th>
<th>Method = 'bray'</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geog</td>
<td>0.0001</td>
<td>0.2571</td>
<td>0.01</td>
</tr>
<tr>
<td>Climate</td>
<td>0.0606</td>
<td>0.4652</td>
<td>0.01</td>
</tr>
<tr>
<td>Phy2</td>
<td>0.0078</td>
<td>0.5344</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>0.7062</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
Attalea phalerata Mart. ex Spreng. is dominated by Mystrops mexicana, even in individuals separated by 600 km (Barfod et al., 1987).

Our data show that climatic differences and host identity together influence insect species turnover. Also, we found that spatial distance is a weak predictor of species turnover, rejecting the hypothesis of dispersal limitation as a possible driver of turnover of Mystrops spp. (Fig. 2, Table 2; e.g. Novotny et al., 2007). A lack of spatial signal may be due to the scale of measurement, although Mystrops spp. are shared between individuals located in different mountain ranges from Colombia, suggesting that strong geographical barriers have a weak effect on Mystrops dispersion. Therefore, insect presence is limited by palm ranges, which suggests that insect composition is strongly affected by host palm presence (Godsoe et al., 2009). Moreover, we found that climate has an important role in shaping insect species turnover, even after controlling for spatial distance. In this way, species turnover is probably related to the climatic affinities of host species (Archibald et al., 2013), but it is unclear whether this influence is over insect species, palm species or both. Wettinia spp. are distributed across different climatic conditions, as shown in the distribution models (Fig. 4). Further, palm flowers control the temperature and microclimate conditions that provide refuge for insect species (e.g. Nuñez, 2014). Thus, the effects of climate on insect species turnover could be indirect and may be related to palm distribution across climatic space. Taken together, we suggest that host identity and species interaction are the strongest drivers of insect turnover although explicit, fine-scale hypotheses and further experiments are needed.

Despite the role of climate, insect assemblages are largely divergent between palm species. However, closely related species share more species, contrary to expectations related to pollinator divergence increasing reproductive isolation (Kay & Sargent, 2009). In this way, major Wettinia clades demonstrate divergence in pollinators, implying clear reproductive isolation between them and possibilities for hybridization between closely related species. Accordingly, phylogenetic reconstruction of Wettinia relationships showed evidence of hybridization between closely related species, specifically in the two distinct clades of W. kalbreyeri resolved in Bacon et al. (2016: Fig. 1). This leads to the question: can pollinators

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![Graphs showing beta diversity of Mystrops palm-visitor insect as a function of spatial, climate, and phylogenetic distance using three different statistical metrics.](image)

**Figure 3.** Plot of beta diversity of Mystrops palm-visitor insect as a function of spatial, climate and phylogenetic distance using three different statistical metrics, of which the Bray-Curtis was the most supported from our data.

influence population isolation and potential diversification in palms? If species are co-distributed and maintain long-term interactions, co-evolutionary relationship may emerge (Thompson, 2005). Population reproductive isolation at large scales can also be an important mechanism of species diversification, but explicit tests of this hypothesis require well-resolved phylogenetic trees for both taxa (e.g. Ramírez et al., 2011).

It is important to bear in mind that insect speciation rates must be higher than speciation rates in palms based on differences in generation time (i.e. affecting mutational rate; Kay & Sargent, 2009). With this, palm population isolation may have produced rapid insect speciation rates, resulting in species-specific relationships, in accordance with our observations (Fig. 2). Insect adaptability to specific phenology or phytochemical signals to each palm species could be determined by the effectiveness of coevolutionary relationships in this system. However, the physical signals, resource gains and physiological advantages for insects in Wettinia flowers are unknown and need further study (Bawa, 1990; Armbruster, 2014). We suggest future studies include broad geographical sampling and ecological, morphological and physiological traits to understand the mutualistic relationship in this system across geographical ranges as possible mechanism of palm

Figure 4. Ecological niche models for the studied Wettinia spp. All niche models recovered a betamultiplier of 1 and all AUC scores were > 0.94. Probability of occurrence increases with the heat of the colour; cold blue and hot red hues represent low and high probability, respectively.

speciation. Also, these analyses provide evidence that plant–insect networks maybe affected indirectly by climate change (Hegland et al., 2009).

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Localities of collection points in this study in which staminate inflorescences were collected from at least three individuals of each host palm species.