

INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA – INPA
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**DEPOSIÇÃO DE PÓLEN HETEROESPECÍFICO EM PLANTAS DE SUB-
BOSQUE DE UMA FLORESTA NA AMAZÔNIA CENTRAL**

RODRIGO TAVEIRA SOUZA

Manaus, Amazonas

Maio, 2022

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Dissertação apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de mestre em Biologia (Ecologia).

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Após a exposição, o discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

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Sinopse: Quantificamos a deposição de pólen heteroespecífico em parcelas ripárias na Reserva Florestal Adolpho Ducke; e fizemos um modelo para indicar quais características florais dos indivíduos estão mais relacionadas com a deposição de pólen heteroespecífico.

Palavras-chave: Comunidades que co-florescem; Compartilhamento de polinizadores; Deposição de pólen; Especialização floral; Polinização

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Resumo

Em comunidades de plantas que co-florescem, o movimento dos polinizadores pode levar a transferência de pólen heteroespecífico entre espécies de plantas. Isto pode afetar o sucesso reprodutivo dos indivíduos, seja por impedir que o pólen coespecífico chegue ao estigma ou pela perda de pólen durante o transporte pelos polinizadores. Características ambientais e das plantas podem afetar a proporção de pólen heteroespecífico recebido. Para analisar esse padrão na Amazônia, nós investigamos características bióticas das plantas relacionadas com a capacidade de atração de polinizadores, como tamanho das flores, número de flores, display floral, simetria e distância de um indivíduo florido para outro. O estudo foi realizado nas parcelas ripárias da Reserva Florestal Adolpho Ducke, Amazonia Central durante a estação chuvosa. Estigmas e anteras foram removidos de todos os indivíduos de sub-bosque com flores encontrados nas parcelas. Lâminas foram montadas e todos os grãos de pólen coespecíficos e heteroespecíficos foram identificados e contados. Nós montamos um modelo para determinar como as variáveis estão relacionadas com a deposição de pólen heteroespecífico. Neste estudo, a média de deposição de pólen heteroespecífico nos indivíduos foi de 5,8% e 61% dos indivíduos não apresentaram pólen heteroespecífico e das 15 espécies encontradas, duas não apresentaram pólen heteroespecífico. Tamanho das flores, número de flores e display floral tiveram relação significativa com a deposição de pólen heteroespecífico no modelo. Apesar da grande variação de tipos florais na Amazônia, houve pouca deposição de pólen heteroespecífico. Isto é provavelmente devido à alta especificidade das interações planta-polinizadores que reduzem a deposição inapropriada de pólen nos estigmas.

Abstract

In co-flowering communities, pollinator movement can lead to the transfer of heterospecific pollen between species. This can affect the reproductive success of individuals, either by preventing the conspecific pollen from reaching the stigma or by the pollen lost during transportation. Environmental and plant characteristics can affect heterospecific pollen proportions. To analyse this pattern in Amazon Forest, we investigated biotic characteristics related to capacity of attracting pollinators, such as flower size, number of flowers, floral display, symmetry, and distance from one flowering individual to another. The study was carried out in riparian plots of the Reserva Florestal Adolpho Ducke, Central Amazon during the rainy season. Stigmas and anthers were removed from all flowering understory plants that were encountered in permanent plots. Slides were mounted and all conspecific and heterospecific pollen grains identified and counted. We then fitted a model to determine how each variable is related to heterospecific pollen deposition. In this study, the mean of heterospecific pollen deposition in individuals was 5.8% and 61% of individuals had no heterospecific pollen and from the 15 species found, two had no heterospecific pollen. Flower size, number of flowers and floral display had significant relationships with the heterospecific pollen deposition in the model fitted. Despite the great variation in floral types in the Amazon, there was a low percentage of heterospecific pollen deposition. This is probably due to highly specific plant-pollinator interactions that reduce inappropriate pollen deposition on stigmas.

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1. INTRODUÇÃO

Em comunidades de plantas que co-florescem, o compartilhamento de polinizadores é um processo comum (Mitchell et al., 2009; Fang and Huang, 2013; Ashman and Arceo-Gómez, 2013). Apesar de normal, esse compartilhamento (i.e., polinizadores visitando diferentes espécies de plantas) pode levar à facilitação ou competição por polinizadores. Na facilitação, espécies de plantas se beneficiam pela proximidade a outras plantas que utilizam os mesmos polinizadores (Lopes et al., 2021) e assim, aumentam o sucesso reprodutivo devido à maior disponibilidade de polinizadores (Sargent and Ackerly, 2008; Seifan et al., 2014; Bergamo et al., 2019). Na competição, espécies de plantas podem ter o sucesso reprodutivo reduzido devido ao compartilhamento de polinizadores, levando à limitação de pólen e redução na produção de sementes (Ashman et al., 2004; Knight et al., 2005; Bergamo et al., 2017; Bergamo et al., 2018).

Tanto na competição quanto na facilitação, o pólen transportado por vetores animais podem ser coespecíficos, pertencendo a mesma espécie, e viável para a reprodução, ou pode ser heteroespecífico (Sargent and Ackerly, 2008; Tur et al., 2016). Deposição de pólen heteroespecífico (DPH) ocorre quando o polinizador coleta grãos de pólen de uma ou mais espécies de plantas e deposita em outras (Caruso e Alfaro, 2000; Morales and Traveset, 2008; Arceo-Gómez and Ashman, 2011; Ashman and Arceo-Gómez, 2013). DPH pode causar falha na polinização (Wilcock and Neiland, 2002), e diminuir o sucesso reprodutivo dos componentes tanto masculino quanto feminino.

Para o componente feminino, a DPH pode interferir na adesão do pólen coespecífico no estigma e, conseqüentemente, desenvolvimento de sementes (Morales and Traveset, 2008; Arceo-Gómez and Ashman, 2011). Para o componente masculino, todos os grãos de pólen depositados em outras espécies são considerados uma perda, já que não podem realizar sua função reprodutiva (Morales and Traveset, 2008; Muchhala e Thomson, 2012; Ashman and Arceo-Gómez, 2013).

DPH é comum em ambientes naturais (Rathcke, 1983; Montgomery and Rathcke, 2012; Fang and Huang, 2013; Ashman and Arceo-Gómez, 2013; Tur et al., 2016). Entretanto, a frequência e intensidade variam entre indivíduos de plantas e ambientes, podendo ocorrer em mais de 50% das flores (Ashman and Arceo-Gómez, 2013; Arceo-Gómez and Ashman, 2014; Arceo-Gómez et al., 2019). Entender tais

relações pode ajudar a revelar o processo de organização da diversidade de plantas em ambientes naturais e a diversificação dos tipos florais (Arceo-Gómez et al., 2019).

Características reprodutivas das plantas, como tamanho da flor e display floral são altamente relacionadas a atração de polinizadores (Mitchell et al., 2004; Arceo-Gómez et al., 2016). Assim como simetria floral também é relacionada a atração de polinizadores, com flores radiais atraindo mais polinizadores do que flores bilaterais, que necessitam de mais especialização do polinizador (Arceo-Gómez et al., 2016). Dessa forma, essas características podem afetar a frequência de DPH (Lopes et al., 2021).

Interações com polinizadores podem estar diretamente relacionadas com a distribuição espacial, dependendo da abundância, densidade e distância entre as plantas e polinizadores, manchas com mais flores tendem a atrair mais polinizadores do que indivíduos isolados com poucas flores (Flanagan et al., 2010; Jakobsson et al., 2015; Bruckman and Campbell, 2016). Assim, manchas de plantas floridas da mesma espécie podem receber menos DPH do que plantas mais isoladas. Além das características individuais, o ambiente pode ter um papel decisivo nessas relações (Barônio et al., 2016; Streher et al., 2020; Lopes et al. 2021).

Em ambientes tropicais, plantas evoluíram mecanismos para diminuir o efeito do compartilhamento de polinizadores (Morales and Traveset, 2008; Lagomarsino and Muchhala, 2019), como o aumento de especialização entre plantas e seus polinizadores (Muchhala, 2007; Ollerton et al., 2011; Moreira-Hernández et al., 2019) e diferenças na fenologia (Sakai, 2001), o que limita o número de espécies de plantas visitados pelos polinizadores. Apesar disso, ainda existem modelos controversos sobre os sistemas de polinização tropicais serem mais ou menos especializados, o que poderia causar grande diferença nos níveis de DPH, dependendo da região (Barônio et al., 2016).

Interações através de transferência e deposição de pólen heteroespecífico podem ser importantes na evolução e diversificação dos tipos florais coexistentes na natureza, uma vez que espécies que não conseguem desenvolver mecanismos para evitar muita deposição de DPH podem ter problemas no sucesso reprodutivo e assim, sua persistência no ambiente (Arceo-Gómez et al., 2019).

Entretanto, apesar do seu potencial para ajudar entender o funcionamento das comunidades de plantas em ambientes naturais, em ambientes tropicais pouco é conhecido sobre DPH (Borgella et al., 2001; Muchhala and Jarrín-V, 2002; Muchhala and Thomson, 2012). A maioria dos estudos possuem poucos dados de regiões tropicais

(Arceo-Gómez et al., 2019) ou incluem apenas interações específicas (Muchhala, 2007; Muchhala and Thomson, 2009, 2010; da Fonseca et al., 2016).

Para aumentar o entendimento dos fatores que podem levar a DPH em áreas tropicais, nós avaliamos a percentagem de deposição de pólen coespecífico e heteroespecífico em espécies de plantas floridas numa reserva na Amazônia Central e avaliamos quais atributos florais são associados com DPH.

2. OBJETIVOS

- Avaliar a percentagem de deposição de pólen heteroespecífico em uma floresta na Amazônia Central;
- Avaliar quais atributos florais estão relacionados com a deposição de pólen heteroespecífico.

3. CAPÍTULO 1

Rodrigo Taveira; William E. Magnusson; Juliana Hipólito.

Heterospecific pollen deposition in understory plants of a forest from the Central Amazon

Manuscrito submetido: FLORA

Heterospecific pollen deposition in understory plants of a forest from the Central Amazon

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ABSTRACT

In co-flowering communities, pollinator movement can lead to the transfer of heterospecific pollen between species. This can affect the reproductive success of individuals, either by preventing the conspecific pollen from reaching the stigma or by the pollen lost during transportation. Environmental and plant characteristics can affect heterospecific pollen proportions. To analyse this pattern in Amazon Forest, we investigated biotic characteristics related to capacity of attracting pollinators, such as flower size, number of flowers, floral display, symmetry, and distance from one flowering individual to another. The study was carried out in riparian plots of the Reserva Florestal Adolpho Ducke, Central Amazon during the rainy season. Stigmas and anthers were removed from all flowering understory plants that were encountered in permanent plots. Slides were mounted and all conspecific and heterospecific pollen grains identified and counted. We then fitted a model to determine how each variable is related to heterospecific pollen deposition. In this study, the mean of heterospecific pollen deposition in individuals was 5.8% and 61% of individuals had no heterospecific pollen and from the 15 species found, two had no heterospecific pollen. Flower size, number of flowers and floral display had significant relationships with the heterospecific pollen deposition in the model fitted. Despite the great variation in floral types in the Amazon, there was a low percentage of heterospecific pollen deposition. This is probably due to highly specific plant-pollinator interactions that reduce inappropriate pollen deposition on stigmas.

Keywords: Co-flowering community; Floral specialization; Pollen deposition; Pollination; Pollinator sharing

3.1. Introduction

In co-flowering plant communities, pollinator sharing is a common process (Ashman and Arceo-Gómez, 2013; Fang and Huang, 2013; Mitchell et al., 2009). Although normal, this sharing (i.e., pollinators visiting different plant species) can lead to facilitation or pollinator competition. In facilitation, plant species benefit from being close to other plants that use the same pollinators (Lopes et al., 2021) and thus, increase reproductive success due to greater pollinator availability (Sargent and Ackerly, 2008; Seifan et al., 2014; Bergamo et al., 2019). In competition, plant species have reduced reproductive success due to sharing pollinators, leading to pollen limitation and reduced seed production (Ashman et al., 2004; Knight et al., 2005; Bergamo et al., 2017; Bergamo et al., 2018).

In both competition and facilitation, the pollen grains transported by animal pollinators can be conspecific, belonging to the same species, and viable for reproduction, or can be heterospecific (Sargent and Ackerly, 2008; Tur et al., 2016). Heterospecific pollen deposition (HPD) occurs when the pollinator collects pollen grains from one or more species and deposits them on others (Caruso and Alfaro, 2000; Morales and Traveset, 2008; Arceo-Gómez and Ashman, 2011; Ashman and Arceo-Gómez, 2013). HPD can cause pollination failure (Wilcock and Neiland, 2002), and diminish the reproductive success of both male and female components (Fig. 1).

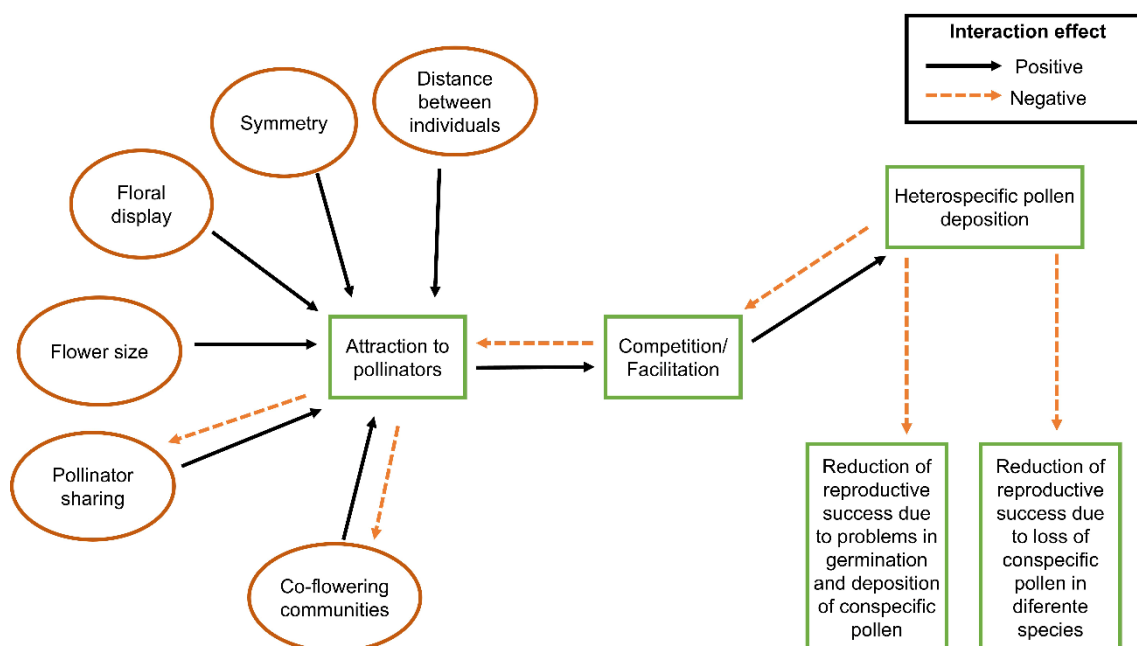


Figure 1. How heterospecific pollen deposition can reduce reproductive success in plants.

For the female component, the HPD can interfere in the adhesion of conspecific pollen to the stigma and, consequently, its germination, as well as preventing pollen tube growth, ovule fertilization and seed set development (Morales and Traveset, 2008; Arceo-Gómez and Ashman, 2011). For the male component, all pollen grains deposited in another species can be considered a loss, since they cannot fulfil their reproductive function (Morales and Traveset, 2008; Muchhala and Thomson, 2012; Ashman and Arceo-Gómez, 2013).

HPD is common in natural environments (Rathcke, 1983; Montgomery and Rathcke, 2012; Fang and Huang, 2013; Ashman and Arceo-Gómez, 2013; Tur et al., 2016). However, the frequency and intensity vary between plant individuals and environments and may occur in more than 50% of flowers (Ashman and Arceo-Gómez 2013; Arceo-Gómez and Ashman 2014; Arceo-Gómez et al. 2019). Understanding such relationships can help unravel the processes that organize the diversity of plants in nature and floral-type diversification (Arceo-Gómez et al. 2019).

Reproductive characteristics of plants, such as flower size and floral display are highly related to pollinator attraction (Mitchell et al., 2004; Arceo-Gómez et al., 2016). Floral symmetry is also related to the attraction of pollinators, with radial flowers attracting more pollinators than bilateral flowers, which need more pollinator specialization (Arceo-Gómez et al., 2016). Thus, these characteristics can affect the frequency of HPD (Lopes et al., 2021).

Pollinator interactions can be directly related to a species spatial distribution, depending on abundance, density and distance between plants that interact with pollinators, as clusters of plants with more flowers tend to attract more pollinators than isolated individuals with few flowers (Flanagan et al., 2010; Jakobsson et al., 2015; Bruckman and Campbell, 2016). In addition to individual characteristics, the environment can play a decisive role in these relationships. (Barônio et al., 2016; Streher et al., 2020; Lopes et al. 2021).

In tropical environments, plants have evolved mechanisms to decrease the effects of pollinator sharing (Morales and Traveset, 2008; Lagomarsino and Muchhala, 2019), such as increased specialization between plants and pollinators (Moreira-Hernández et al., 2019; Muchhala, 2007; Ollerton et al., 2011) and differences in flowering phenologies (Sakai, 2001), which limits the number of plant species visited by pollinators. Despite this, there are still controversial models about tropical pollination

systems being more or less specialized, which could cause a big difference in DPH levels, depending on the region (Barônio et al., 2016).

Interactions through heterospecific pollen transfer and deposition may be important in evolution and diversification of floral types co-existing in nature. However, despite its potential to help understand the functioning of plant communities in natural environments in tropical regions, little is known about HPD (Borgella et al., 2001; Muchhala and Jarrín-V, 2002; Muchhala and Thomson, 2012). Most studies included few data from tropical regions (Arceo-Gómez et al. 2019) or included only specific interactions (Muchhala, 2007; Muchhala and Thomson, 2009, 2010; da Fonseca et al., 2016).

To increase the understanding of the factors which could lead to HPD in tropical areas, we evaluated the percentage of heterospecific and conspecific pollen deposition in understory flowering species from a reserve in central Amazon and evaluated which floral attributes are associated with HPD.

3.2. Material and methods

3.2.1 Study area:

The study was conducted in Reserva Florestal Adolpho Ducke (02°55' e 03°01' S, 59°53' e 59°59' W), which is administered by the Instituto Nacional de Pesquisas da Amazônia – INPA. The reserve is located on the outskirts of Manaus and covers 10.000 ha of terra-firme tropical rainforest (Costa et al., 2005). The mean annual temperature in the reserve is around 26° and mean annual rainfall is 2362 mm with a dry season between July and October, and most rain falling between the months of November and May (Marques-Filho et al., 1981).

Reserva Ducke is part of the Programa de Pesquisa em Biodiversidade – PPBio and has a 64 km² RAPELD trail system (Magnusson et al., 2005, 2013) that gives access to 72 permanent plots in upland areas and 40 permanent plots in riparian zones. The plots are 250 m long and are located along the watercourse. In this study, data was collected in 15 of the riparian plots during the rainy season (Fig. 2).

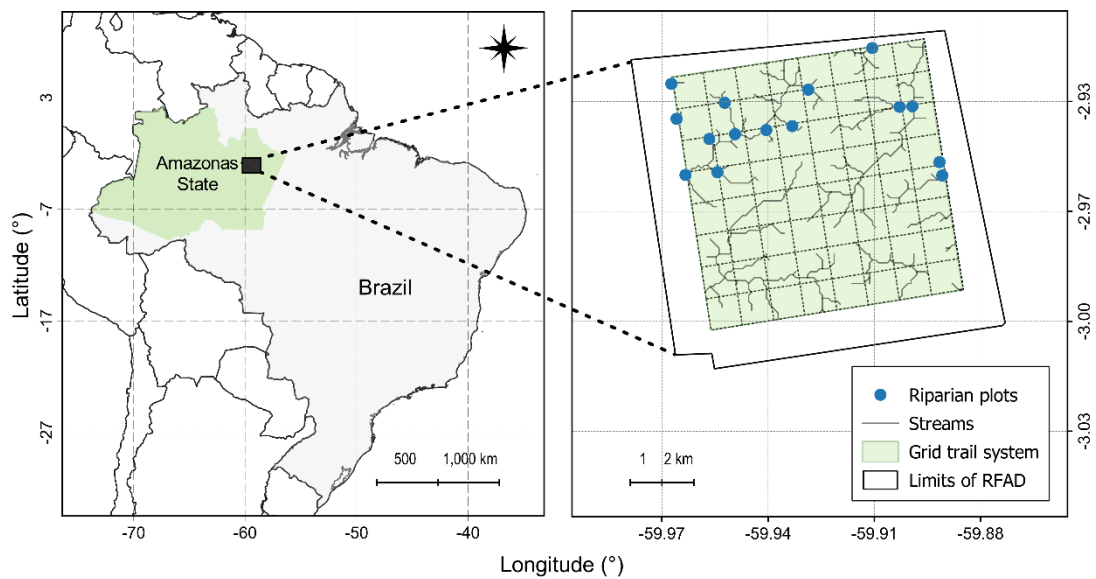


Figure 2. Study area located in Amazonas State and the sampled plots on the grid system in Reserva Florestal Adolpho Ducke.

3.2.2 Data collection

Anthers and stigmas

Fifteen riparian plots were sampled between February and March 2020. In each plot we observed all flowering understory plants up to 3 m in height, within 250 m of the plot up to ten meters long from the center line of each plot. Specimens of each plant species found were collected to be identified and deposited in the herbarium of the Instituto Nacional de Pesquisas da Amazônia.

All stigmas from open flowers were collected and at least one anther or floral bud from each plant. Plant parts were collected using tweezers and conserved in 70% alcohol for further analysis.

In the laboratory, slides were mounted in Kaiser's glycerinated gelatine stained with fuchsin (Kraus and Arduin, 1997). Slides with anthers were used to create a database for detection of heterospecific pollen on stigmas.

Pollen from the stigmas of both conspecific and heterospecific plants were counted and identified under a microscope. Subsequently, the percentage of heterospecific pollen on each plant collected was calculated.

Size and floral display

The size of each flower was described by the length of the corolla. Length was measured in the field using a 0.01 mm precision Vernier calliper. For the analyses, we used the mean length of the flowers in inflorescences per individual plant.

For each plot, we recorded the number of plant individuals with single flowers or inflorescences and the number of flowers in inflorescences was also related to the deposition of heterospecific pollen.

Symmetry

All flowers found on an individual plant were measured in each plot. The flower's symmetry was transformed into a continuous variable through an asymmetry index in which the higher the value of the index, the more asymmetric the flower. For each flower, the difference of the left side in relation to the right side was taken (Fig.3) in absolute values:

$$AI = \frac{\Sigma (|A - B| + |D - E| + |F - G|)}{Ni}$$

AI = asymmetry index

A = distance from the apex of the first petal to the farthest left petal

B = distance from the apex of the first petal to the petal furthest to the right

D = right width of first petal

E = left width of first petal

F = length of petal immediately to the left

G = length of petal immediately to the right

Ni = total number of flowers per individual

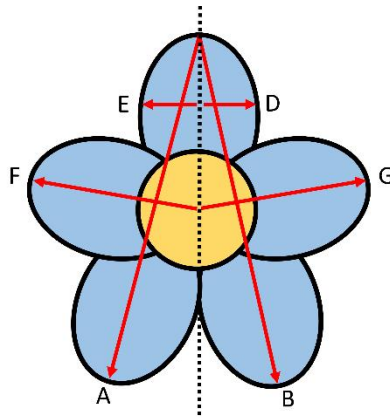


Figure 3. General scheme of the measurements that were used to measure flower asymmetry.

Distance between individuals

To determine the distribution of individuals from the same species in the plots, the number of flowering individuals in the same plot was counted and the distance between the individuals was measured. Individuals that were more than 50 m away from the next flowering individuals were considered isolated.

3.2.3 Statistical analyses

All statistical analyses were undertaken in the R software (R Core Team, 2021). Corolla size, floral display, number of flowers, index of symmetry and distribution of individuals were related to the proportion of heterospecific deposition through generalized linear mixed models (GLMM). Plant families and species were considered nested effects and plots were used as random variable. All variables were standardized to mean= 0 and S=1, and the effect of each variable was isolated.

3.3. Results

We collected 46 individuals, with a total of 156 flowers. These individuals were identified as belonging to nine families and 15 species, totaling 3.769 pollen grains, of which 375 were heterospecific (Table 1). From the 156 flowers, 45 (29%) had heterospecific grains. From the 46 individuals, 19 (41%) had heterospecific grains and of the 15 identified species, in two (13%) no individual had HPD. The individual average proportion of HPD was 5.8%.

Palicourea tomentosa (Aubl.) Borhidi was the species that had the highest amount of different grains in the stigma, with four types of grains.

The model fitted with all variables measured explained 77% ($R^2=0.77$) of the variance and the probabilities associated with the null hypothesis for each variable are given in table 2.

Table 1. Pollen load found by each flowering species (n= number of individuals).

| Family | Species | Conspecific grains | Heterospecific grains | Heterospecific proportion for the species (%) |
|-----------------|---|--------------------|-----------------------|---|
| Bromeliaceae | <i>Pitcairnia sprucei</i> Baker (n=1) | 525 | 38 | 6.48 |
| Gentianaceae | <i>Tachia grandiflora</i> Maguire & Weaver (n=5) | 61 | 25 | 29.07 |
| | <i>Voyria spruceana</i> Benth. (n=3) | 342 | 17 | 4.74 |
| Gesneriaceae | <i>Nautilocalyx pictus</i> (Hook.) Sprague (n=2) | 24 | 6 | 20 |
| Heliconiaceae | <i>Heliconia acuminata</i> L. C. Rich. (n=5) | 256 | 10 | 3.76 |
| Lamiaceae | <i>Amasonia lasiocaulos</i> Mart. & Schauer ex Shauer (n=1) | 7 | 2 | 17.14 |
| Marantaceae | <i>Goeppertia exscapa</i> (Poepp. & Endl.) Borchs. & S.Suárez (n=2) | 53 | 0 | 0 |
| | <i>Monotagma densiflorum</i> (Körn.) K.Schum. (n=1) | 101 | 1 | 0.58 |
| Melastomataceae | <i>Aciotis aequatorialis</i> Cogn. (n=3) | 581 | 13 | 2.19 |
| | <i>Aciotis purpurascens</i> (Aubl.) Triana (n=2) | 196 | 2 | 1.01 |

| | | | | |
|--------------|--|--------------|------------|------|
| | <i>Leandra secunda</i> (Pav. ex D.Don) Cogn. (n=2) | 39 | 1 | 7.93 |
| Rapateaceae | <i>Rapatea paludosa</i> Aubl. (n=5) | 53 | 3 | 5.36 |
| Rubiaceae | <i>Faramea platyneura</i> Müll. Arg. (n=1) | 289 | 0 | 0 |
| | <i>Palicourea gracilentata</i> (Müll.Arg.) Delprete & J.H.Kirkbr. (n=12) | 749 | 6 | 0.79 |
| | <i>Palicourea tomentosa</i> (Aubl.) Borhidi (n=1) | 118 | 251 | 68 |
| Total | | 3.394 | 375 | |

Table 2. Detailed result of the fitted model.

| Variables | Estimated | Std. Error | t value | p value |
|------------------------------|------------------|-------------------|----------------|----------------|
| Intercept | -1.1606 | 0.6621 | -1.753 | 0.0442 |
| Corolla size | -1.0178 | 0.4357 | -2.336 | 0.0126 |
| Number of flowers | 0.3128 | 0.1562 | 2.003 | 0.0264 |
| Floral Display | 1.7642 | 0.9271 | 1.903 | 0.0326 |
| Asymmetry | 0.1249 | 0.1968 | 0.635 | 0.2662 |
| Distance between individuals | -0.0210 | 0.3811 | -0.055 | 0.4801 |

Corolla size was negatively related to HPD ($t=-2.33$; $p=0.001$) thus individuals with larger flowers tended to receive less HPD than individuals with smaller flowers (Fig. 4-A). Individual flower number had a positive effect on pollen deposition ($t=2.003$; $p=0.002$), indicating an increase in HPD with the increase in number of flowers per individual (Fig. 4-B).

There was a significant effect for the types of floral display ($t=1.90$; $p=0.003$), with individuals with inflorescences having lower HPD than individuals with solitary flowers (Fig. 4-C).

Flower asymmetry had no significant effect ($t=0.63$; $p=0.26$) and seems to have little or no effect on the amount of HPD (Fig. 4-D). Likewise, the categorization of distance between individuals did not have a significant effect on the HPD of the individuals collected ($t=-0.055$; $p=0.48$), though there was slightly more variability among isolated individuals (Fig. 4-E).

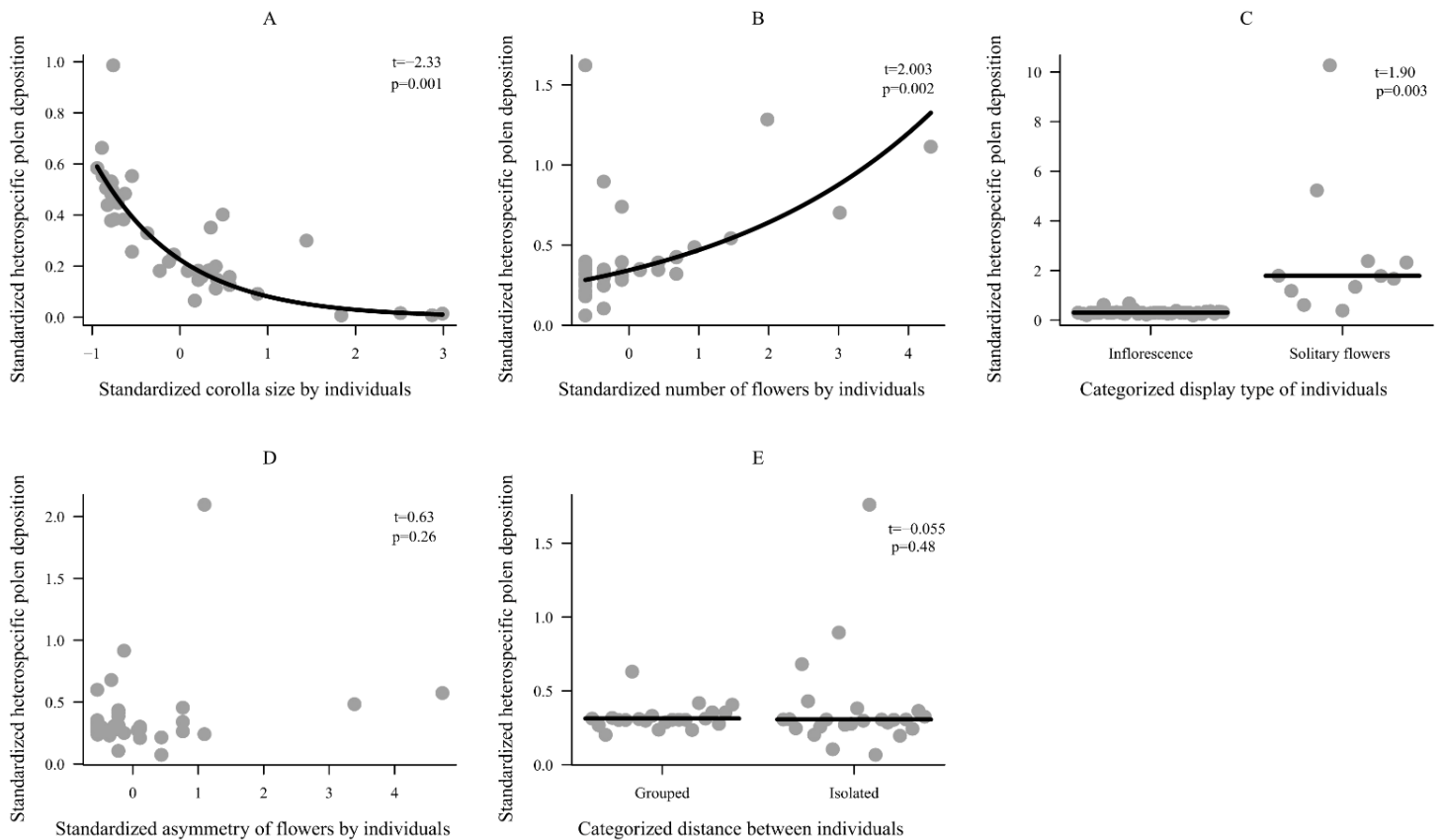


Figure 4. Effect of each measured variable. A – Corolla size; B – Number of flowers; C – Display type; D – Asymmetry; E – Distance between individuals.

3.4. Discussion

The deposition of heterospecific pollen occurs frequently in environments with shared pollinators (Fang and Huang, 2013). Arceo-Gómez et al. (2014) and Vamosi et al. (2006) suggested that areas close to the equator should tend to have greater chances of receiving heterospecific pollen, due to increased species richness, but Arceo-Gómez et al. (2019) showed that receipt of heterospecific pollen was lower in these areas than in non-tropical areas, where the studies by Tur et al. (2016), Ashman and Arceo-Gómez (2013) and Fang and Huang (2013), found HPD loads up to 70%. At our study site, we found an average of only 5.8% of heterospecific pollen on stigmas, supporting studies

that indicate a lower receipt of heterospecific pollen in areas of greater species richness, such as the Amazon.

We found a tendency for individuals with larger corollas to have lower HPD, which is the opposite of what is expected, since larger flowers tend to attract more pollinators (Montgomery and Rathcke, 2012; Arceo-Gómez et al., 2016). However, in co-flowering plant communities, being a generalist species, that is, receiving more visits from different pollinators (Maldonado et al., 2013) tends to lead to HPD and conspecific pollen loss (Ashman and Arceo-Gómez, 2013; Wei et al., 2021). Thus, individuals with larger flowers found in this study may have some degree of specialization for pollinators and, therefore, ended up receiving a smaller amount of heterospecific pollen, since most individuals with corollas considered large (greater than 2.5 cm) were of the species *Tachia grandiflora*, which is a species that is associated with ants, which use it as a shelter, and has only one type of pollinator - hummingbirds - registered (Struwe and Kinkade, 2013). Plant species that have associations with ants may receive fewer visits from floral visitors, including pollinators (Ibarra-Issassi and Oliveira, 2018; Martins et al., 2020). For other individuals with larger corollas, especially *Palicourea tomentosa*, the reception of heterospecific pollen was high (68%, n=251 grains), which may indicate that this is a more generalist species.

In our study individuals with more flowers tended to have greater HPD. Ohashi and Yahara (1998, 2001) showed that individuals with larger displays (more flowers) receive more visits from pollinators than individuals with smaller displays. However, there was no proportional difference in the number of flowers visited regardless of the size of the display (Ohashi and Yahara, 2001; Harder et al., 2004), so differences in pollen delivery could be related to the type and efficiency of pollinators that visit these plants, which may also be related to the availability of pollinators and flowering plants in the dry or rainy season.

Both plants and pollinators have mechanisms to avoid unnecessary energy waste in pollen delivery and foraging (Harder and Prusinkiewicz, 2013), such as limiting pollen for pollinators in the case of plants (Harder and Thomson, 1989), and in the case of pollinators, there is a short-term memory that helps to avoid flowers that have already been visited (Ohashi and Yahara 2002). However, this memory only prevents the revisiting of few flowers, and the visitation of many flowers of the same plant by the same pollinator can lead to crosses between flowers of the same individual, or geitonogamy (Harder et al., 2004).

Thus, to avoid resource limitation and to avoid revisiting a flower, pollinators may choose to visit other individuals, which could reduce the effect of geitonogamy, but would increase the chances of HPD, since more pollinators would be needed to visit the flowers of an individual. In this case, receiving heterospecific pollen could be a mechanism to avoid self-crossing, which could be more detrimental to the individual's reproductive success.

Inflorescences are perceived more quickly by pollinators than solitary flowers (Harder et al., 2013) and they receive more visits from pollinators. However, the same pollinator will often visit more than one flower of the same inflorescence, which despite increasing the chances of receiving pollen from the same individual, decreases the chances of receiving heterospecific pollen, confirming what we found in our study, in which inflorescences received less HPD than solitary flowers.

The asymmetry index did not show significant differences between individuals with more symmetrical or asymmetrical flowers in HPD. Several studies, such as those of Møller and Eriksson (1995), Rodríguez et al. (2004), Wignall et al. (2006) and Carvalho (2015), showed that pollinators prefer to visit symmetrical flowers regardless of whether they are radial or bilateral. Thus, more asymmetric flowers should receive fewer visits from pollinators and would be less likely to receive heterospecific pollen than symmetrical flowers. All studies that tested this preference of pollinators for a type of symmetry used only one species of plant in the experiments. In our study, we found 15 species, so perhaps this effect of pollinator preference has been diluted because there are other variables with a stronger effect on pollinators in selecting the flowers to be visited.

The distance between flowering individuals and/or individual patches is often considered essential in attracting pollinators, causing both competition and facilitation in the environments where they occur (Flanagan et al., 2010; Seifan et al., 2014; Ward et al., 2013). In our study, we did not find differences in HPD between isolated and aggregated individuals, which corroborates the results found by Jakobsson et al. (2015) in which distance effects increased pollinator visitation rates at distances up to 5m, but with no effect on total visitation. Seifan et al. (2014) considered that flowering individuals with showy displays attract pollinators to the surroundings, thus, even aggregated individuals may attract more pollinators as the distance effect is short, the total rate of visitation by pollinators is not affected by the distance between individuals, causing little difference in heterospecific pollen deposition between the two groups.

The Amazon basin has a diversity of plant species, and many may yet to be discovered (Hopkins, 2019). Consequently, there are diversity of floral types. Considering that more than 94% of plant species need a biotic vector for reproduction (Ollerton et al., 2011), many strategies are needed for ensure reproductive success. Arceo-Gómez et al. (2019) considered that the transfer of heterospecific pollen leads to specializations of pollination systems, since the transfer of pollen to flowers of different species can have a high reproductive cost. Thus, the low frequency of HPD found in Reserva Ducke may be a result of large-scale specialization to avoid HPD.

In summary, the deposition of heterospecific pollen in the Reserva Florestal Adolpho Ducke was, on average, 5.8%. Corolla size, number of flowers and display type were the most significant variables in the fitted model. The low frequency of HPD may result from the great diversity of floral types in the Amazon.

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4. CONCLUSÃO

A bacia Amazônica tem uma diversidade de espécies de plantas, e muitas ainda podem ser descobertas (Hopkins 2019). Consequentemente, existe uma diversidade de tipos florais. Considerando que mais de 94% das espécies de plantas precisam de um vetor biótico para reprodução (Ollerton et al. 2011), muitas estratégias são necessárias para garantir o sucesso reprodutivo. Arceo-Gómez et al. (2019) considerou que a transferência de pólen heteroespecífico leva a especializações dos sistemas de polinização, já que a transferência de pólen para flores de diferentes espécies pode ter um alto custo reprodutivo. Assim, a baixa frequência de DPH encontrada na Reserva Ducke pode ser resultado de uma especialização em larga escala para evitar DPH.

Em resumo, a deposição de pólen heteroespecífico na Reserva Florestal Adolpho Ducke foi de 5.8%. Tamanho da corola, número de flores e display floral foram as variáveis mais significativas no modelo ajustado. A baixa frequência de DPH encontrada na Reserva Ducke pode ser resultado de uma especialização em larga escala para evitar DPH.