The Effect of Distance From the Forest Edge on Airborne Pollinator Activity in Amazonian Regenerating Secondary Forest

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Abstract
Deforestation is a major issue all over the world and is increasing in prevalence in many areas, including in the Amazon rainforest. This practice is usually done to create patches of land for cattle through slash and burn techniques. However, this land is generally abandoned and is left as an open patch which can give rise to regenerating secondary forest over time. Pollinators are an essential part of how forests can regenerate as they can aid in the reproduction of plant species that are present in these open areas. This study looked at several different areas of regenerating secondary forest in the Amazon. Five different species of flowering plants were found in these areas with the activity of visiting airborne pollinators being observed at varying distances from the edge of the intact forest. Distance did not have a significant effect on the activity of all of the pollinators in this study, but it still impacted how they interacted with plants in these areas. Negative correlations were found between distance and the total number of pollinators, pollinator species and the amount of time spent by each pollinator type, which suggests that pollinators prefer to remain close to the edge of the forest. The time of day and the flower species present did not have a significant impact on most of the pollinators but did show that some species preferred to go further out in the morning and that there was a wider variety of species based on the flower species present. Based on the results of this study distance does have somewhat of an impact on pollinators. This may indicate that pollinators can aid in the regeneration of a forest by following the forest edge which may be why areas further away from the edge take longer to generate.

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Introduction

The Amazon rainforest is the largest rainforest in the world and contains a wide variety of plant and animal species. The Amazon is facing threats from deforestation and development which has put many species at risk. Pollinators are one of the groups of species that are under threat and have been affected by deforestation as this practice can lead to an increase in open areas. This human practice has caused many pollinators to lose their sources of food and have to choose new sources or adapt to new environments. These cleared areas create new habitats that may prove hostile to pollinators since it can potentially expose them to an increased abundance of predators and elements such as the wind and sun. This can impact how the pollinators move around in a certain area. One study examining the activity of bees near orchards found that the density of the population was lower in areas that had more orchards (Martins et al. 2014). These pollinators were active in areas that were a certain distance from wooded areas but were less likely to travel over a larger distance. The further away the food resource was from their nest, the less likely they were to travel to it and feed from it. Pollinators are willing to travel to food sources that are farther away but may prefer to remain closer to sheltered areas. A study done by Quesada et al. in 2004 was conducted in tropical forests in Mexico and Costa Rica and discovered that forest fragmentation did not affect the activity of generalist pollinators while specialist pollinators were more greatly impacted since they may only obtain nectar from certain plant species. Plants found in exposed areas may also have been visited more since they were producing more flowers as well (Quesada et al. 2004). Generalist pollinators can obtain nectar from a wider variety of plant species which may motivate them to move into more exposed areas to find food. In another study a species of bee was found to have traveled to a variety of different plants that were present at different distance intervals from the edge of a forest into a coffee crop field (Ricketts 2003). The bees made up the greatest percentage of pollinators that traveled from the edge

of the forest to these locations and collected more pollen from each flower as a result (Ricketts 2003). This same scenario could be present in the regenerating secondary forests of the Amazon as a greater number of generalist species would be willing to travel a greater distance from the edge of the forest. Expansive deforestation in the Amazon has occurred due to practices such as slash and burn which in turn have given rise to areas of regenerating secondary forest after these areas are abandoned. The activity of pollinators can be affected by these more exposed areas since it places them at greater risk of predation or heat.

While these studies were all done in different areas of the world they still have implications for the Amazon rainforest as they were conducted in environments that can be considered similar to regenerating secondary forests. The studies also have not looked at how these pollinator species may be affected by the presence of regenerative forest and juvenile plants, specifically, and how the distribution of these plants may impact how they move in regenerative forests. One such area where regenerative forests are common is the area of Finca las Piedras in the Madre de Dios region of Peru. The many different pollinators present in the forest are facing situations where they will have to travel to exposed and isolated plants in order to find food due to increased deforestation in the Finca las Piedras region as well as the Amazon as a whole. This study can be vital to forests all over the world as they face increased deforestation and as more plants are added to regenerating forests. The presence of pollinators is key to the reproduction of plant species, and if pollinators cannot get to them due to biotic or environmental factors then the biodiversity of plants in forests across the globe will decrease as well. It can be hypothesized that if there are plants that are further away from the edge of a primary forest, then they will be less willing to travel greater distances to obtain food due to threats from predation and heat from the sun. Based on all of this information the main question that can be asked for this region is: *How does distance from primary forest affect the movement and activity of airborne pollinators in regenerating secondary forest?*

Methods

Study Site

This study was conducted at the Finca las Piedras research station in the Madre de Dios region of Peru. This site contains several hectares of secondary regenerating forest surrounded by primary forest. This site is also situated in one of the most biodiverse regions in the country for pollinators which makes it ideal for studying how far they are willing to travel from primary forest and how they may interact with plants in exposed areas.

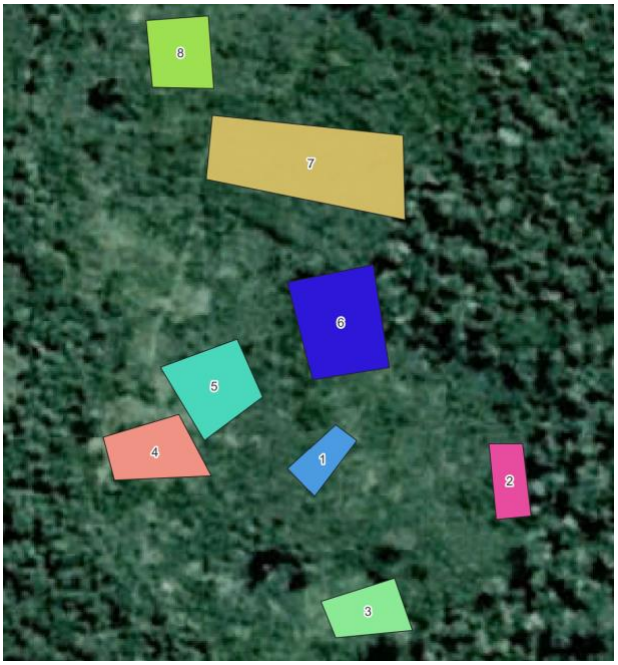


Figure 1. Map of the study areas around the property of Finca las Piedras. Areas are labeled in the order they were studied.

Data Collection

A total of eight areas of secondary regenerating forest were observed around the property of Finca las Piedras and were measured to have varying widths (21 m, 74 m, 77 m, 68 m, 68 m, 63 m, 62 m, and 63 m respectively). The widths of these areas were measured by marking the starting point of an area and then by walking to an end point with both of these points being marked in a GPS app. The edges of the forest were also marked to determine how large each area was. These points were entered into the geographical information system (GIS) software application QGIS where the edges of each area were traced and the area labeled (Figure 1). Two areas were observed each week for five weeks with each area being observed twice a week. One area was observed per week during the third and fourth week of the study due to unfavorable weather conditions. The flowering plants in each area were observed for 2 hours in the late morning and for 2 hours in the early afternoon to determine if there was any change based on time of day.

Pollinators were observed at flowering plants of the same species both near to and far away from the edge

of the intact forest with a total of five different plant species being monitored for pollinator activity. Each selected plant was monitored for five minutes and data about the visiting pollinators was recorded. The total number of individual pollinators and pollinator species was recorded. Pollinators were also monitored to determine how much total time they spent at the flowers and the total time they spent pollinating. A lack of pollinators at a flowering plant during a five-minute period was also recorded.

The data collected in the field was entered into a Microsoft Excel file where raw data was sorted numerically by flower number visited in each area and by distance from the edge of the intact forest. The data was then sorted into a separate spreadsheet where the data from all eight areas was categorized by time of day and then sorted by distance.

Distance Measurements

The locations of flowering plants in each area were recorded using a GPS app. Locations were marked during the data collection performed in the morning and then were revisited during the afternoon data collection. A photo was also taken of each flowering plant to aid in finding them in the afternoon. Data points were also marked at the forest edge in the GPS app to aid in distance measurements and in data analysis. The coordinates of these plant and forest edge locations were entered into the Excel spreadsheet.

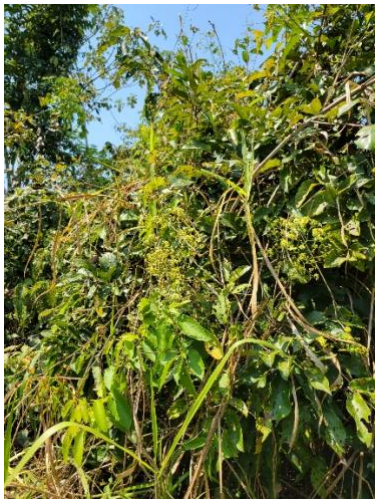
The plant locations were entered into QGIS and labeled to determine how far they were from the edge of the intact forest. Each flower plant species that was found was classified by color. The distances were measured in QGIS and then entered into the Excel spreadsheet.

A) 

B) 

C) 

D)



E)

Figure 2. The five flower species observed in the study. Flowers pictured are species 1 (A), species 2 (B), species 3 (C), species 4 (D), species 5 (E).

Statistical Analysis

Statistical analyses were performed on the R software coding app where several different tests were performed. Tests were performed on the activity of all pollinators as well as just on bees as they were the most abundant pollinator species. The effect of the time of day on pollinators and bees was done by running t-tests which were used to determine if any significant changes in activity were found in all of the pollinator types observed and in bees (Table 2). The effect of the flower species on the total number of all pollinators and bees that have pollinated them was found by conducting Type II ANOVA tests (Table 3). Spearman correlation tests were used to determine the effect of distance on the total number of pollinators present, the total number of pollinator species present, the total time that pollinators spent at each flower, and the total time that pollinators spent pollinating each flower. Spearman tests were also used to determine the effect of distance on these variables in each individual pollinator type observed. Pearson correlation tests were conducted on some of the data collected from butterflies as they were normally distributed. For the tests comparing distance to all pollinator types observed and bees, all distances that were under 160 meters were used to avoid any outliers. The p-values and other statistical values (W-values, F-values, S-values, and t-values respectively) were recorded. Graphs were created for the correlation tests comparing distance to the total number of pollinators and the total number of wasps as they had the most significant values for data. Tables were created in Excel using the data collected from the statistical analysis.

Results

There were five flower species observed in the study (Figure 2) along with five different types of pollinators. Bees were the most abundant and most biodiverse type of pollinator in each area and were found both near to and far away from the edge of the forest. Flies were the least abundant species found. The correlation tests conducted on the effect of distance on pollinator activity yielded results that were insignificant for some of the pollinators observed and significant for others (Table 1). Based on the tests, there was no significant correlation between distance and the activity of the pollinators (Table 1). This was also the case when analyzing the data for bees but there were significant results for the number of wasps present at each flower and the number of wasp species at each flower (p-value: 0.01232, S = 4294009, p-value: 0.01314, S = 4288973, respectively). Butterflies, moths and flies had some significant p-values but had far more insignificant values compared to the other types of pollinators since the sample sizes for these two pollinators were much smaller than the sample sizes of bees and wasps. P-values for other pollinators that were less insignificant were closer to 0.05 but were still above that value. Most of the tests looking for the correlation of distance on pollinator activity were found to be negative due to the rho values provided. The average time spent by pollinators and by each pollinator type did not depend on the distance as all of the p-values were insignificant.

Table 1

	p-value	S-value	rho value	Correlation
All Pollinators (Total)				
Number of Pollinators	0.5844	3098088	-0.03358804	Negative
Number of Pollinator Species	0.8534	3031801	-0.01147333	Negative
Total Time Spent at Flower	0.3984	589179	-0.06968752	Negative
Total Time Spent Pollinating	0.2825	600154	-0.08861512	Negative
Bees (Total)				
Number of Pollinators	0.5978	2899282	0.03273793	Positive
Number of Pollinator Species	0.4056	2842773	0.05159057	Positive
Total Time Spent at Flower	0.2901	437190	-0.09205872	Negative
Total Time Spent Pollinating	0.1738	3625579	-0.1181965	Negative
Wasps (Total)				
Number of Pollinators	*0.01232	4294009	-0.1488738	Negative
Number of Pollinator Species	*0.01314	4288973	-0.1475264	Negative
Total Time Spent at Flower	0.5353	2606	-0.1330718	Negative
Total Time Spent Pollinating	0.2835	2824	-0.2282167	Negative
Butterflies (Total)				
Number of Pollinators	0.4952	3889995	-0.0407788	Negative
Number of Pollinator Species	0.5019	3887638	-0.04014817	Negative
Total Time Spent at Flower	0.3828	80.168	0.3319328	Positive
Total Time Spent Pollinating	0.4492	85.21	0.289916	Positive
Moths (Total)				
Number of Pollinators	0.2892	3974287	-0.06333141	Negative
Number of Pollinator Species	0.2892	3974287	-0.06333141	Negative
Total Time Spent at Flower	1	2	-1	Negative
Total Time Spent Pollinating	1	2.24E- 16	1	Positive
Flies/Other (Total)				
Number of Pollinators	0.05128	4171849	-0.1161896	Negative
Number of Pollinator Species	0.05299	4168728	-0.1153547	Negative
Total Time Spent at Flower	0.5948	42	0.25	Negative
Total Time Spent Pollinating	0.8176	-0.2431[1]	-0.1080813	Negative

Table 1. Correlation between distance and recorded values for pollinators as found by the Spearman (S-value) and Pearson (t-value; indicated by [1] in table) correlation tests. Significant values are marked by an asterisk (*). Data are marked as NA if there was no data available.

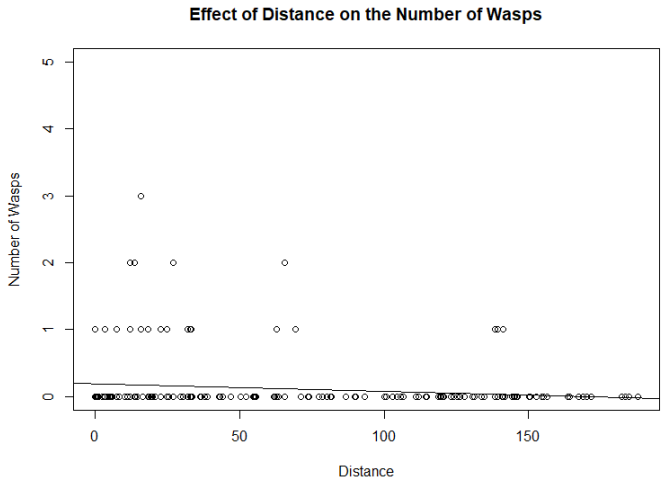


Figure 3. Graph depicting the correlation between distance and the total number of pollinators. All distances below 160 meters were used to avoid using outliers.

Table 2		
	p-value	W-value
Time of Day (All Pollinators)		
Number of Pollinators	0.5679	10314
Number of Pollinator Species	0.3313	10582
Average Time Spent at Flower	0.8228	10088
Average Time Spent Pollinating	0.8836	10036
Table 2 (continued)		
Time of Day (Bees)		
Number of Pollinators	0.8041	10100
Number of Pollinator Species	0.4471	10413
Average Time Spent at Flower	0.8444	9813
Average Time Spent Pollinating	0.7077	9701

Table 2. Data analyzing the effect of the time of day on the activity of the pollinators in the study. The p-values and W-values refer to the statistics produced by the Mann-Whitney U tests. Bees were analyzed as well as they were the most abundant pollinator species.

Time of day did not have a significant impact on the activity of the pollinators in the study. None of the p-

values in this table are significant. No other pollinator types were used as they were less abundant than bees.

There were some significant p-values obtained when looking at the effect of flower species on the number of bees and bee species (Table 3). Flower species did not have any significant effect on the amount of time that pollinators or bees spent at the plant or the time that they spent pollinating.

Table 3		
	p-value	**F-value**
All Pollinators		
Flower Species (Total)		
Number of Pollinators	0.121	2.426
Number of Pollinator Species	0.282	1.164
Average Time Spent at Flower	0.352	0.881
Average Time Spent Pollinating	0.223	1.493
Bees		
Flower Species (Total)		
Number of Pollinators	*0.015	6.056
Number of Pollinator Species	*0.007	7.492
Average Time Spent at Flower	0.145	2.14
Average Time Spent Pollinating	0.153	2.049

Table 3. Data analyzing the effect of flower species on the activity of the pollinators in the study. The p-values and F-values refer to the statistics provided by the ANOVA tests. Significant values are marked with an asterisk (*). Bees were analyzed along with the total pollinators as they were the most abundant pollinator species.

There were some significant p-values obtained when looking at the effect of flower species on the number of bees and bee species (Table 3). Flower species did not have any significant effect on the amount of time that pollinators or bees spent at the plant or the time that they spent pollinating.

Discussion

The results of this study provided many inferences of how distance may impact the activity of pollinators. While there was a general negative correlation between distance from intact forest and the number of pollinators and pollinator species these trends were not statistically significant. This data shows that distance did not have a large impact on the activity of most of the pollinators. These results may have been brought about by environmental factors that were not accounted for such as the weather or through other unknown reasons. The exception to this was the data for wasps which showed that distance did have a significant impact on them. This suggests that wasps are more sensitive to environmental changes than other pollinator types.

Negative correlations were also found for the average amount of time that wasps spent at the flower and how long they spent pollinating which suggests that they also preferred to avoid spending too much time away from the forest. This data was the opposite of what was found for bees, moths, and flies as distance did not significantly impact the activity of these other pollinator types. While distance may have had some impact there are likely other factors that could have influenced these pollinators. This could also be a survival instinct to stay closer to the forest to avoid danger and to avoid overheating. Butterflies were different from all other pollinators. Butterflies tended to spend longer periods of time further away from the forest. Butterflies may have better ways of surviving further away from the edge of the forest which may account for the higher quantity of time spent further away. There may be more predators of butterflies near the edge of the forest as well which may be why they choose to spend more time further away.

The time of day did not have a significant impact on the activity of the pollinators in this study. Based on the results it can be inferred that pollinators preferred to be active in every part of each area during all times of the day regardless of whether or not there may have been anything that changed during the day. While it can be hypothesized that there may be a rise in temperature from the morning to the afternoon this did not seem to affect the activity of any of the pollinators in this study. The activity of bees followed the activity of the other pollinators as they were not impacted by the time of day either. This data suggests that there were other factors that could have impacted the activity of the pollinators during these times.

The three most abundant flower species did not have a significant impact on the activity of all of the pollinators in this study. The p-values comparing all of the pollinator types to flower species were insignificant while some of the p-values looking at the effect of flower species on bees were significant which suggests that most of the pollinators had no significant preference for any particular flower species in the study while bees did. Bees seemed to gather in higher numbers at particular flower species and showed a wider range of species diversity at these flowers but

they had no preference for how long they spent at a particular flower species.

There were some limitations to this study, mainly in the number of individuals of each flower species. There was a higher number of individuals for flower species one compared to the others which may have swayed the results when comparing the activity of pollinators to the flower species. Conversely, the sample sizes for flower species four and five were much smaller which caused them to be excluded from the ANOVA tests and which could have swayed the data for the other tests. The areas were also all different sizes which could have swayed the results since the flowers in some of the areas could have been unknowingly excluded and caused significant data to be missed. There may have been some error in the distance measurements as well. Patches of regenerating forest may have been mistaken for the edge of the intact primary forest which may have caused errors in initial data collection.

Conclusions

This study has shown that the distance of flowers from the edge of intact forest can potentially impact the activity of pollinators. As distance from the edge of the forest increases, the number of pollinators and pollinator species generally decreases, which could be due to environmental factors such as heat or the threat of predation. Other factors such as wind and rain may also play a role in this. Bees are the most durable pollinator type and can gather in greater numbers farther away from the forest especially if the environmental conditions are less harsh such as in the morning. This study can have major implications for developing reforestation and conservation efforts in the Amazon and in the world. It can be said that pollinators move with the forest edge as it slowly overcomes an open area. Because of this it is important to implement protections for pollinators as they play an important role in the regeneration of a forest. It is particularly important to protect bees as they seem to be the pollinator type that can travel further away from the forest edge and may be essential in maintaining the reproduction of individual plants that may be further away from the forest edge. Further studies can examine which particular bee or other pollinator species are most abundant in regenerating secondary forest. A wider variety of plant species can be observed as well and if there is one particular plant species that is the most important to pollinators in terms of forest regeneration.

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