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# **The effect of distance from forest edge on flower visitor frequency and fruit set; a case study of *Cucumis sativa* around Kakamega forest**

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Master of Science in Ecology





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a case study of *Cucumis sativa* around Kakamega forest**

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In loving memory of Atieno and Awuor.

## **Abstract**

Pollination is a key ecosystem function that is important for food production. This service is maintained by natural habitats which provide a dwelling place for these pollinators. However, these natural habitats are on the decline due to various human activities such as conversion of forests to arable land.

This study analyzed the frequency of flower visits on cucumber flowers with increasing distance from the forest edge and subsequent pollination success of these plants. The plants were grown in the nursey and transferred to the field at the onset of flowering. Sixteen study sites were located along four 300m transects over 1km apart.

Data was collected through direct observation. Observations were done in the morning from 7.30am and in the afternoon from noon for a five-day period. Each plant was observed for ten minutes and the number of insects landing on its reproductive parts counted. At the end of the five-day observation period, the number of fruits formed were counted.

In total 504 flower visits were observed over the five-day period. Distance had a negative effect on frequency of flower visits; there was a decrease in flower visits with increasing distance from the forest edge. Visits declined by 34% (+9.4%) with every 100m meter increase in distance from forest edge. However, distance and visitation frequency had no detected effect on fruit set.

This study demonstrates that tropical forest provides a source population for flower visitors and thus the need to conserve them.



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## **Introduction**

### *Pollinators importance and threats*

Pollination is a key ecosystem function important for food production. It is estimated that the pollination of about 35 percent of food crops depends on animals (Klein et al. 2007). The economic value of pollination worldwide has been estimated to be about \$235-\$577 billion annually (IPBES, 2017). Without natural pollination from various pollinators such as honeybees, the cost of agricultural production for many commodities would be more expensive as crop production would depend on human managed pollination (Aizen & Harder, 2009). This service is maintained by natural habitats which provide a dwelling place for these pollinators. However, in most areas around the world the natural habitats have been destroyed and degraded by various human activities (such as land conversion and timber cutting) leading to pollination problems (Gemmill-Herren & Ochieng, 2008; Steffan-Dewenter & Westphal, 2007). Even though forest and nature reserves have been created around the world to conserve pollinator habitats and mitigate their decline, more measures are still necessary to keep up with the ever-accelerating land use change.

### *Pollinator and food security*

It has been established that generally the yield in pollinator dependent food crops increase with the increase of pollinators presence (Klein et al, 2007); however, certain limitations like pollination efficacy and climatic conditions may curtail this. Pollination success has been noted to increase in areas nearby natural habitat (Carvalho & Seymour et al, 2010; Ghazoul, 2005). Fruit set has also been noted to increase with increased visitation frequency for instance a study in Greece observed that fruit set in watermelon increased with increased pollinator visitation (Garantonakis et al, 2016). Also, cucumber yield was observed to increase with high pollinator presence in a greenhouse than in open field: greenhouses with bee colonies had better cucumber yield than greenhouses with no bee colonies and also than the open field where there was no pollinator augmentation (Santos, Roselino, & Bego, 2008). The positive dependence of crop yield on pollinators further supports the importance of maintaining pollinators.

### *Pollinators in changing landscapes*

The loss and fragmentation of natural habitats has been noted to be a threat to pollinator diversity (Steffan-Dewenter & Westphal, 2007). Insects such as bees, moths, hover flies and butterflies provide pollination to crops and their abundance have been observed to increase in areas with more natural vegetation cover (Cartar, 2005). For instance the frequency of bee visit to eggplant was noted to decline with increasing distance from wild habitat (Gemmell-Herren & Ochieng, 2008). Also, the abundance and diversity of flower visitors to camphor basil was also observed to decrease with increasing distance from the forest edge in a study conducted around Kakamega forest (Mandela et al., 2018). It is evident that natural habitats provide source population for these pollinators. Even though pollinators are mobile and able to move over long distances, they do require nesting and foraging sites which are provided by natural habitats (Patrício-Roberto & Campos, 2014).

It is projected that human population will increase by 34% by 2050 with the highest growth being in the developing countries; as such, food production will subsequently increase by 70% to meet the population demands (FAO 2009). To meet this target, it forecasted that there would be need for expansion of arable land for farming. This would lead to further fragmentation of the already fragmented land. Such changes on the landscape are inevitable changes. It is therefore important to consider how to maintain the key ecosystem service -pollination- needed for food production, in the face of these changes.

This study was conducted around Kakamega forest; a tropical forest found on the western part of Kenya. The forest has significant economic and ecological value to the people. However, the forest has been logged over the years for various human activities (Schleuning et al., 2011). The main economic activity around the forest area is farming. Various food crops are farmed, and these mainly depend on natural pollination. This provides a good set up to assess pollination services provided by insects in a multi-use landscape.

### *Objectives*

In this study, I quantified the number of flower visits to cucumber (*Cucumis sativa*). *C. sativus* is an important food crop in the family Cucurbitaceae. The plant is monoecious with bright yellow flowers. *C. sativus* is highly dependent on pollinators such as bees, wasps, flies and butterflies for pollination to occur because the large and sticky pollen produced by male flower cannot be easily moved by wind (Gingras, Gingras, & De Oliveira, 1999). I assessed how distance from the

forest edge affects availability of flower visitors and in turn fruit set. I addressed two questions: i) how does the visitation frequency to cucumber flowers vary with increasing distance from the forest? ii) how does the fruit set of cucumber vary with increasing distance from the forest edge? A further consideration that I explored was how the environmental variables such as temperature, humidity and cloud cover affect the number of flower visits.

## **Materials and Methods**

### *Study area*

This study was carried out around Kakamega forest, in the western part of Kenya ( $0^{\circ}07' - 0^{\circ}27' \text{ N}$ ,  $34^{\circ}46' - 34^{\circ}57' \text{ E}$ ). Lying at an altitude of 1500m to 1700m, Kakamega forest is a montane rainforest of the Congolese origin (Schleuning et al., 2011). The average annual temperature in Kakamega is 20.4 degrees with an average annual rainfall of between 1200mm to 1700mm that is bimodally distributed. It experiences a wet season in April/May and August/September. The dry season is between December to February

Kakamega forest is made of a main block stretching over 9,500ha and six other forest fragments of different sizes (40- 1,348 ha) (Schleuning et al., 2011). The forest has a wide species diversity and is home to various endemic species. The undisturbed forest parts have an immediate surrounding of tea plantation stretching for about 100meters. These tea plantations were established in 1986 to act as a buffer to the natural forest and prevent the likely human encroachment into the forest

Surrounding the forest are commercial and agricultural areas which are densely populated. Farming is the main economic activity of the people living around Kakamega forest. Cereals and vegetable farming are more prevalent and interspersed with livestock farming.

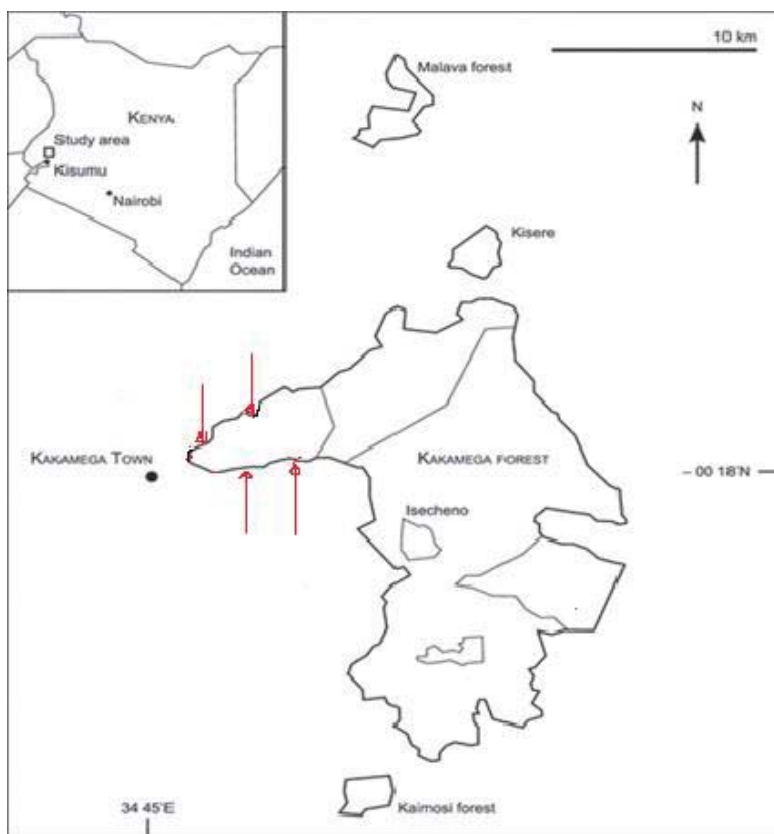
### *Experimental design*

Four transects were selected for the study. All the transects were on the western side of the forest (figure 1). Two transects were placed at Lianungu and the other two at Lurambi; the distance between the two stations was 6km. The two transects at each station were spaced by 1.5km. Each transect was 300meters long. The plants were placed at distances of 0m, 100m, 200m and 300m along each transect (Steffan-Dewenter & Tschardtke, 1999). The two transects at Lianungu

passed through the tea plantation stretch at the first 100 meters and the surrounding area consisted of grass, shrubs, and farmlands. Lurambi station is near the Kakamega town and the two transects were mainly surrounded by residential buildings.

### *Experimental plants*

Cucumber seeds (*C. sativus*) were soaked in lukewarm water for six hours prior to planting. The soil used for planting was prepared by mixing five portions of forest soil, two portions of organic manure and one portion of pure sand. The soil was potted in plastic containers then sprayed with insecticide. After ten minutes, the seeds were planted. The seeds were kept at the nursery until they started flowering (from 26<sup>th</sup> of January to 10<sup>th</sup> March 2018). Watering was done thrice a week for the whole period the plants were in the nursery. At the onset of flowering, the plants were transferred to the field. No watering was done in the field as it was a rainy period.



*Figure 1:* Map of Kakamega forest. Red lines on the map are the location of the transects. These are however not drawn to scale.

### *Observation of flower visitors*

Observations of flower visitors were done between 11<sup>th</sup> to 15<sup>th</sup> of March 2018 when all plants had started flowering. Each plant along each transect was observed for 10 minutes and the number of insects landing on the reproductive parts of the flowers counted. The observations were carried out in the morning from 7.30am and in the afternoon from noon. The order of which the sites were observed were decided randomly: the four field assistants each picked a transect to work on in the morning and at every transect they randomly choose which site to start observing. On all the days of flower visitor observations, there was no rain and temperatures were above 15 degrees. Due to lack of measuring instruments, recordings of temperature, cloud cover and humidity were obtained from the local weather station.

### *Fruit set*

Fruit set was used to determine the pollination success of the cucumber plants. Fruit set is the relationship between the number of flowers and number of fruits formed. A flower was considered to have been successfully pollinated when it formed a fruit. At the end of the observation period, each plant was assessed, and the number of fruits formed were counted and recorded.

### *Statistical analysis*

Statistical analysis was performed using R version 3.5.1 (R Core Team, 2018). Data exploration was first done to assess trends between predictor and response variables, correlations and outliers in the data; following the procedures of Zuur et al., (2007). All the models were generated using the “glmer” function in lme4 package version 1.1 (Bates et al., 2015). All the graphs were plotted using the R package ggplot2 version 3.1.0 (Wickham, 2016).

The data was first scaled due to the varying magnitudes in the variables. In analyzing the variations in pollinator visit frequency, I used a generalized linear mixed model (GLLM) with a Poisson error distribution. The response variable was the number of flower visits and the offset variable was the number of flowers, following (Reitan & Nielsen, 2016). I used the transects as the random effect. Table 1 in the appendix shows the various GLMMs generated.

I also used a GLMM with a Poisson error distribution in analyzing the fruit set. The response variable was number of fruits on the last day of observation. Transect was used as the random effect and distance forest and average visitation frequency over the five days of observation was included as fixed effects. Table 2 in the appendix shows the various GLMMs generated.

For the model building, I used stepwise model selection. I ran a series of models with different combinations of the explanatory variables. I first started with a few covariates that I identified as important to explain the variation then varied the number of covariates in different models. For the model selection, I used the Bayesian information criterion (BIC), to identify the most parsimonious model. The model with a low BIC is more parsimonious and was thus favored over a model with high BIC (Schwarz, 1978).

#### *Challenges during data collection*

In the preliminary set up of this study, I had planned to use watermelon as my reference plant. I set up my study plants in the nursery in early December 2017. Just as my plants were starting to flower in mid-January 2018, they all died. An assessment of the plants showed that they had bacterial wilt which might have been favored by the cool and wet conditions in the nursey where I had grown them. I therefore opted to plant cucumber which was more common in Kakamega. It was costly keeping the plants in the field and having someone look after them by the day and night to prevent damages. For this reason, I could not keep the plants till they developed mature fruit as I had originally intended to. The transects were far spaced between each other and this also made it costlier to ensure a randomized data collection with my field assistants.

## **Results**

#### *Flower visiting insects*

In total, 504 flower-visits were observed over the period of five days. The flower visitors observed were bees, moths, hoverflies and butterflies. An insect was considered a flower-visitor only if it landed on the reproductive parts of a flower (figure 2). The maximum flower visits per plant in a ten-minute observation period was sixteen and the maximum visitation frequency per flower was 2.33.



*Figure 2: Illustration of pollinating insects on a cucumber flower. The picture was taken at one of the study sites.*

#### *Flower visitor frequency*

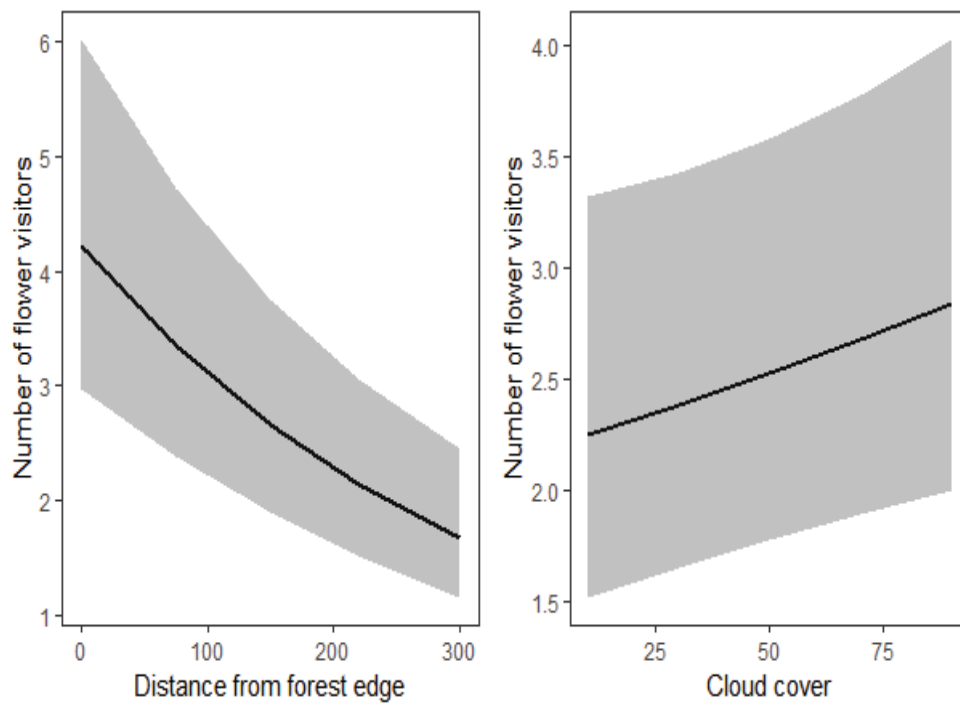
The frequency of flower visitors declined with increasing distance from the forest edge; there were more flower visitors at the edge of the forest than at 300 meters from the forest (figure 4). Visits declined by 34% (+9.4%) with every 100m meter increase in distance from forest edge.

The average temperature during the period of observing pollinator activity was 22<sup>0</sup>C (with a range of 16<sup>0</sup>C – 27<sup>0</sup>C). The average humidity was 69.7% (range of 37 – 89%). The mean cloud cover was 67.5% (range of 11 – 89%). The frequency of flower visitors was higher in the afternoon when the temperatures were higher than in the morning when the temperatures were lower (figure 5). More flower visitors were observed when it was more overcast than when the skies were clear (figure 3).

Although humidity was measured, it was excluded from the model because it was highly negatively correlated with temperature (appendix C). Time of the day and temperature were also highly correlated; thus, temperature was excluded from the model too. The model with time had a better fit than the model with temperature.

*Table 1:* Output for the GLMM that best explains pollinator visit frequency to flowers. Distance is distance from the forest edge measured in meters. SE = Standard Error. The random effect is day of observation. Scaled variables were used in running the GLMM (n=160)

	<b>Estimate</b>	<b>SE</b>	<b>z. value</b>	<b>p-value</b>
(Intercept)	-0.642	0.179	-3.587	<0.001
Distance	-0.346	0.048	-7.256	<0.001
Cloud cover	0.084	0.046	1.813	0.0698
Time. Morning	-0.438	0.092	-4.782	<0.001



*Figure 3:* Graph plotted from the output of the best GLMM for flower visit frequency.

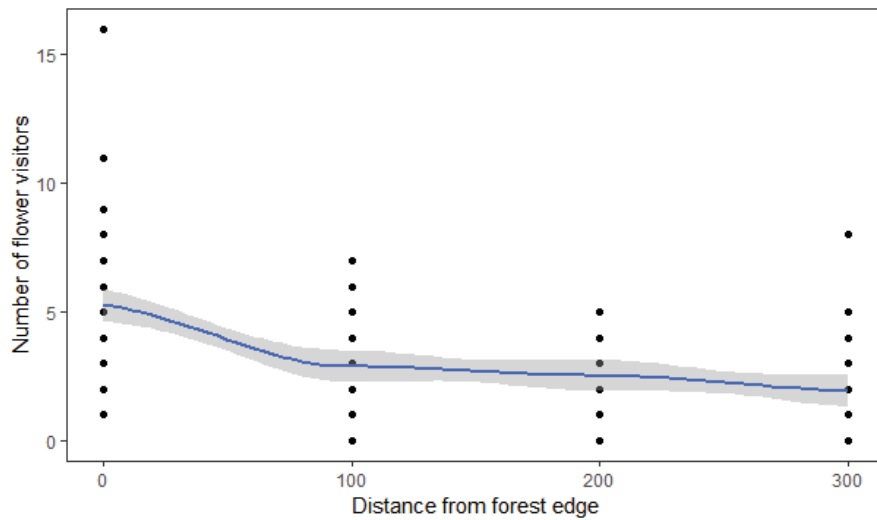


Figure 4: Graph showing the frequency of flower visitors with increasing distance from the forest edge with the confidence interval band. The graph is plotted from the raw data.

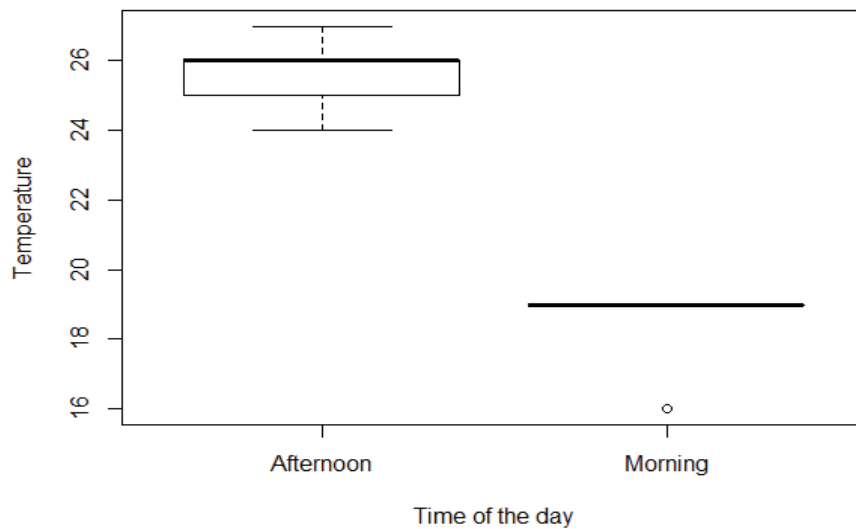


Figure 5: Graph relating temperature and time of the day.

### Fruit set

There was no significant effect of distance to forest or visitation frequency on the fruit set in cucumber (table 2). The average fruit set realized was 0.32. The maximum number of fruits produced per plant was 6 and the total

Table 2: Output for the GLMM model that best explains the variables accounting for fruit set in cucumber plants. SE = Standard Error. The random effect is the transect. Both distance and visitation frequency were scaled. (n=160).

	Estimate	SE	z. value	p. value
(Intercept)	-1.2075	0.1805	-6.688	<0.001
Distance	-0.1080	0.2401	-0.450	0.653
Visitation frequency	0.1501	0.2314	0.649	0.517

## Discussion

### *Distance from forest edge*

The flower visitation frequency decreased with the increase in distance from the forest edge; there were fewer insects at 300 meters than at the edge of the forest. The same pattern has been found elsewhere, for example, bee richness and their visitation rate was found to be higher in sites near forest edges than in sites farther away in coffee plantations in Costa Rica (Ricketts, 2004). Another study found similar results with an increase in bee abundance and activity in habitat islands of mustard and radish near natural grasslands than further away from these natural habitats (Steffan-Dewenter & Tschardt, 1999). Also, bee abundance was negatively affected by distance from forest edge in oilseed rape fields in France (Bailey et al., 2014). The abundance of ants and flower visitors to mango plants in South Africa were observed to decline significantly with increasing distance from natural habitat (Carvalho et al., 2010). This relationship between distance from forest edge and the abundance or frequency of flower visitation indicate that natural habitats act as a dwelling place for insect and thus provide pollination services. Such natural habitats also act as a mating and breeding ground for insects and are useful in maintaining different ecosystem functions.

### *Fruit set*

The frequency of flower visitation had no detected effect on fruit set. This can be attributed to the fact that when sampling the flower visitors, they were not grouped into morphological groups. The flower visitors recorded (bees, butterflies, hover flies, wasps) were all grouped

together. It was therefore hard to tell whether the insect landing on the reproductive parts of the flower were pollinating it or not. Various studies have shown that although various insects visit cucumber, bees are the most common and effective pollinators of cucumber plants ( eg Hanh 2008; Kauffeld and Williams 1972; Santos et al., 2008).

A study done in India by Hanh (2008) showed that even though several insects landed on the reproductive parts of the cucumber plants, some do pollinate while others only collect nectar; of the total foragers counted only 16.54 percent were pollen collecting while 83.46 were nectar collecting. In my study, it might have been that the insects visiting the flowers were not necessarily pollinating it. Grouping of the insects into their functional groups and analysis of the data as such would have given a better indication as to whether the visitation frequency significantly affect fruit set. Grouping of insects was not possible in this study because the field assistants that were helping in data collection had little background knowledge in biology to be able to help identify and group the insects accurately.

It is important to note that in these studies, the sample sizes were considerably higher than that in my study. It is therefore possible that due to the low plant number in my study, the flower visitors did not show fidelity to the plants resulting in no detected effect on fruit set. At each study site, I had only two plants. With this, the probability of a visit occurring by an insect without any cucumber pollen on its body is high. This may have resulted to the low fruit set observed. Furthermore, it is not only the abundance of flower visitors that is important in providing pollination services but rather their efficiency in carrying pollen between the reproductive parts.

Other studies have however shown that an increase in pollinator presence increase the fruit set on entomophilous plants; and also a significant effect of distance from natural habitat to fruit set. For example fruit set in macadamia and longan in Australia was found to be higher when orchards were near to the rainforest where pollinator presence was high (Blanche, Ludwig, & Cunningham, 2006). The fruit set and yield in sweet cherries in Germany was noted to be increased in semi natural habitats with high wild bee population (Holzschuh, Dudenhöffer, & Tscharrntke, 2012). Also, another study carried out in Thailand found out that rambutan fruit set was significantly influenced by distance to forest, the pollinators were stingless bees (Sritongchuay, Kremen, & Bumrungsri, 2016). In addition to this, the fruit set of the highland

coffee in Indonesia was affected by bee diversity which was in turn affected by distance from the forest (Klein, Steffan-Dewenter, & Tschardtke, 2003). It is worth noting that in these studies, the pollinator groups were categorized, and the main pollinator group investigated were bees. This further points out the necessity of grouping the flower visitors when making observations.

#### *Weather variables*

The frequency of flower visits was increased at warmer temperatures. This is in line with other studies that have observed the same pattern (Arroyo, Armesto, & Primack, 1985; McCall & Primack, 1992). However, this is at observed temperature range. Even though this study suggests that increased temperatures are good for pollination, this is not necessarily the case. We expect the relationship to be bell shaped, with an optimal temperature where an increase will result in lower visitation frequencies. The linear relationship observed in this study is because the temperature recording during the days of observation were relatively low for the study area (maximum temperature of 27 degrees). During the dry seasons, the temperatures in Kakamega go as high as 35 degrees. Had the study been replicated in the season with higher temperature, the expected bell shape relationship might have been observed.

Although the full effect of climatic factors is not explored in this study, these results provide an insight into the expected effects that adverse climatic change may have on Kakamega forest ecosystem and agricultural systems depending on natural pollination in the areas surrounding it. With global temperatures expected to rise due to anthropogenic factors, more studies are needed to assess the ecological effects these would have on the pollinator community in the Kakamega forest ecosystem.

#### *Synthesis and application*

The flower visit frequency was higher near the forest than farther away. This is evidence that Kakamega forest provides a habitat and a source population for pollinators. It is therefore important that the forest is conserved to maintain the habitat used by pollinators for nesting, breeding and foraging. A failure to conserve the pollinator habitat may lead to the disruption of pollinator community, their loss and eventual pollination limitation in the area which may subsequently increase the cost of agricultural production. More studies are however needed to accurately quantify the composition of insects in the Kakamega forest and how their abundance

vary across the landscapes. Further on, such studies should look at how the abundance of these pollinators affect fruit set and pollination success of various entomophilous plants.

#### *Pitfalls and recommendations for future work*

In this study, I grouped all the flower visitors together and analyzed the data as one. Previous studies have indicated that bees are the most effective pollinators of cucumbers. Therefore, grouping the insects into morphological groups would have been necessary in this study to assess the most frequent insect visiting the cucumber plant and the realized fruit set. It's been noted that while an insect may land on the reproductive part of a flower, they may not necessarily be effective pollinators but only carry nectar from the flower (Hanh, 2008). Future studies should consider the frequency of visit of each insect group.

The plants should have been grown to maturity and an assessment done on the quality and size of the fruit. This would have given a clear indication on the economic value of the pollination services provided by Kakamega forest. A larger sample size of the plants and a temporal replication of the study would have been desirable for proper assessment of pollinator activity in the Kakamega forest ecosystem. Future work should also include a control treatment with flowers that have been extra pollinated. This will give data on whether the plants are pollen limited in the Kakamega forest ecosystem.

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## Appendices

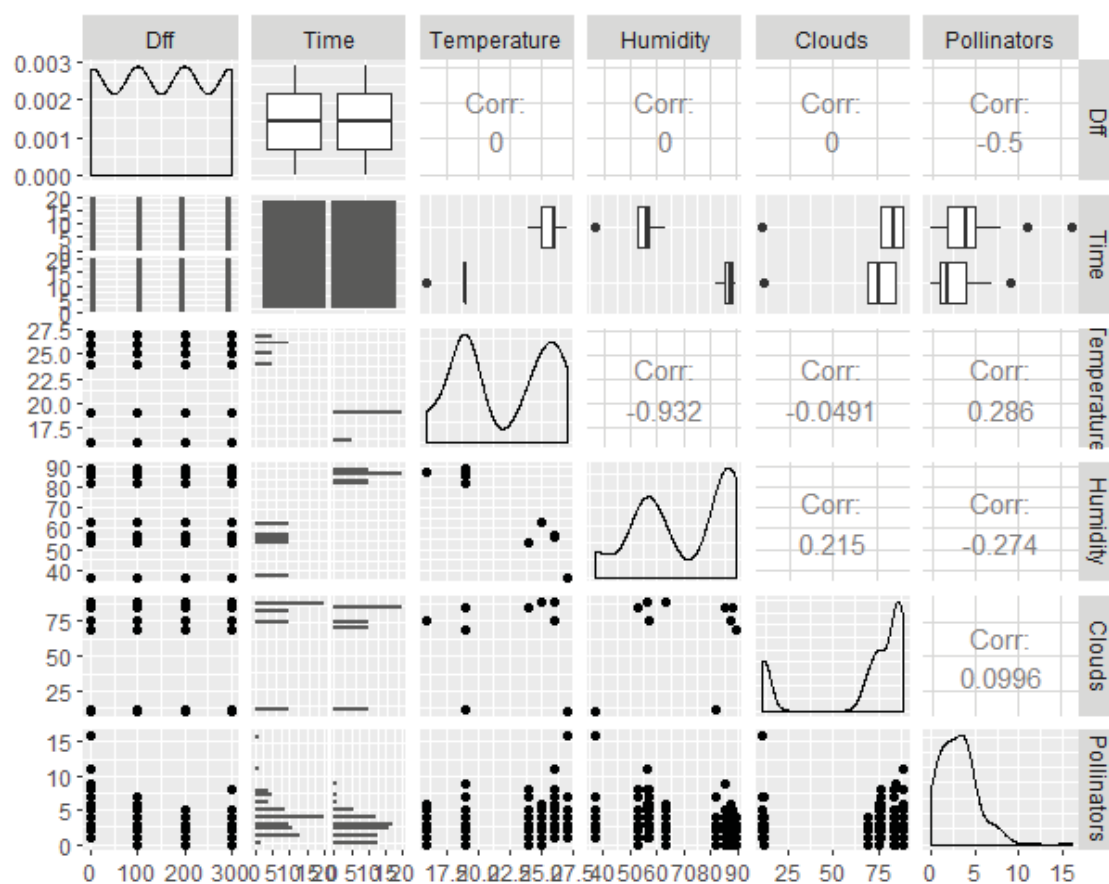
- A. Alternate GLMMS considered in model selection for flower visit frequency
- B. Alternate GLMMS considered in model selection for pollination success
- C. Correlation plot of various variables
- D. Final models

### Appendix A: Alternate GLMMs considered in model selection for model 1

Distance from forest edge	Time	Temperature	Clouds	Observation day	Transect name	BIC	Change in BIC
x	x		x		x	646.0	-
x		x	x		x	646.9	0.9
x	x		x	x	x	651	5.0

x		x	x	x	x	651.8	5.8
x	x		x	x	x	650.2	4.2
x	x	x	x	x	x	655.2	9.2

Appendix B: Correlation plot of the various variables



Appendix C: Final models

## Model 1

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) [

```
glmerMod]
Family: poisson ( log )
Formula: Pollinators ~ s.Distance + s.Clouds + Time + (1 | Transect_name)
Data: kk
Offset: log(Flowers)
```

AIC	BIC	logLik	deviance	df. resid
630.6	646.0	-310.3	620.6	155

Scaled residuals:

Min	1Q	Median	3Q	Max
-2.1632	-0.7501	-0.0759	0.5673	4.7908

Random effects:

Groups	Name	Variance	Std. Dev.
Transect_name	(Intercept)	0.1134	0.3367

Number of obs: 160, groups: Transect\_name, 4

Fixed effects:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-0.64174	0.17892	-3.587	0.000335 ***
s.Distance	-0.34580	0.04766	-7.256	3.99e-13 ***
s.Clouds	0.08385	0.04624	1.813	0.069806 .
TimeMorning	-0.43788	0.09157	-4.782	1.74e-06 ***

---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	s.Dstn	s.Cl ds
s.Distance	0.093		
s.Clouds	-0.036	-0.006	
TimeMorning	-0.201	0.000	0.088

## Model 2

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) [

```
glmerMod]
Family: poisson ( log )
Formula: Fruits ~ s.Distance + s.VisFrq + (1 | Transect_name)
Data: fruitData
Offset: log(Flowers)
```

AIC	BIC	logLik	deviance	df. resid
58.1	61.2	-25.0	50.1	12

Scaled residuals:

Min	1Q	Median	3Q	Max
-1.4216	-0.4160	-0.1244	0.6370	1.5158

Random effects:

Groups	Name	Variance	Std. Dev.
Transect_name	(Intercept)	3.552e-15	5.96e-08

Number of obs: 16, groups: Transect\_name, 4

Fixed effects:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-1.2075	0.1805	-6.688	2.26e-11 ***
s. Distance	-0.1080	0.2401	-0.450	0.653
s. VisFrq	0.1501	0.2314	0.649	0.517

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)	s. Dstn
s. Distance	0.128	
s. VisFrq	-0.045	0.617







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