

Native and Invasive Ants Affect Floral Visits of Pollinating Honey Bees in Pumpkin Flowers (*Cucurbita Maxima*)

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Research Article

Keywords: Pollination, Mutualism, Ants, Plant-Animal interaction, Pumpkin, India, Vegetable crops

Posted Date: December 1st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-113766/v1>

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Version of Record: A version of this preprint was published at Scientific Reports on February 26th, 2021.
See the published version at <https://doi.org/10.1038/s41598-021-83902-w>.

Abstract

Global pollinator decline is a major concern. Several factors – climate change, land-use change, the decline of flowers, pesticide use, invasive species – have been suggested as the reasons. The effect of ants on flowers, despite being a potential reason, received less attention. The consequences of ants being attracted to nectar sources in plants vary depending upon factors like the position of the nectar source, the identity of ants, and other mutualists interacting with the plants. We studied the interaction between floral ants and pollinators in *Cucurbita maxima* and compared the competition exerted by native and invasive ants on the pollinator with the hypothesis that the invasive ants exacerbate more interference competition for pollinators than native ants. The pollinator's choice, visitation rate and time spent/visit were assessed. Regardless of species and nativity ants negatively influenced all the pollinator visitation traits, such as visitation rate and duration spent on flowers. The invasive ants exerted a higher interference competition on the pollinators than native ants did. Ants despite performing pollination in flowers with generalist pollination syndrome, can be a threat for plant-pollinator mutualism in specialists like monoecious plants. A better understanding of factors influencing pollination will help in implementing better management practices.

Introduction

Humans brought together what continents isolated. One very conspicuous evidence of this claim rests in the rearranged biota. With the age of exploration, migration and commerce, the previously unlikely event of organisms crossing the biogeographical and ecological barriers became typical¹. Sometimes introduced species establish themselves very successfully in the new place, and impact on native flora, fauna, ecosystems, and human welfare². One of the most successful taxa that invaded islands, mainland and continents around the globe is ants³. With their sociality and colony structure backing their success, they cause widespread damage in the introduced area⁴. Their consequences are detrimental to native ants, other invertebrates, reptiles, birds, and mammals^{5,6}. Managing invasive ants has become an important conservation tool⁷.

Despite causing a direct effect on biodiversity, the invasive species pose deleterious consequences to the community structure and ecosystem⁸. Invasive ants participate in a range of interactions. One such interaction is between ants and the honey-dew producing arthropods in which the ants are attracted to the “honey” produced by aphids. To payback this, ants tend the aphids and protect them from parasites and parasitoids⁹. When the invasive ants interfere with the native seed-dispersers-fruit interaction, the reproduction of the plant is affected, which alters the plant community structure in terrestrial ecosystems^{10,11}. Argentine ant (*Linepithema humile*) displaced native seed dispersers of myrmecochorous plant, *Dendromecon rigida*, decreased the seed dispersal, and increased seed predation¹². There are cases when invasive ant interactions contribute to plant fitness as well^{13,14}. However, the cost-benefit balance of such interactions are subtle and often result in negative repercussions¹⁵.

The interference of pollinators by invasive ants is another critical outcome of the interaction that directly deters plant reproduction. Although some studies support the argument that invasive ants protect the plant against herbivores, this aggressiveness wards off some useful mutualists too^{16–18}. When the pollination of a shrub was studied in the uninvaded area and an Argentine ant-invaded area, the pollinator visit was found to be significantly lower in the latter¹⁹. Similarly, Hanna *et al.*²⁰ also reported the Argentine ants reducing the pollination and seed set in *Calystegia macrostegia*. *Anoplolepis gracilipes*, the invasive yellow crazy ant, was previously discovered to harass the pollinators, decreasing the visitation frequency and duration of pollinators¹⁸. Thus, invasive ants are a taxing competitor for the pollinators. Although there are few studies on the effect of invasive ants on pollinators, a comparative study between native and invasive ants have not been done yet¹⁵. In this study, we have attempted to compare the effects of ants on pollinators, and also, to compare the competition exerted by non-native and native ant on the pollinators of *Cucurbita maxima* – a monoecious globally-important crop plant.

To understand the effects of ants on pollinators, we took three attributes of pollinators and observed how these were affected by floral ants. The three attributes were the choice of pollinator entry, the frequency of pollinator's visit (visitation rate) and the time the pollinators spent inside the flowers. With their aggressive behaviour ants generally ward off other floral visitors^{15,16}. Thus, we expected the visitation rate and visit duration to be negatively affected by the presence of ants²¹. Since invasive ants are known for displacing biodiversity and interrupting species interactions, we hypothesize that the invasive ants exert more pressure on plant-pollinator interaction than the native ants do. Since the plant is monoecious we also asked whether the ant-pollinator interaction was affected by flower sex as well.

In a comparative study of competition exerted by the two ant categories, we took certain exploitative and interference competition for our study. Lach²² defined interference and exploitation competition in the context of floral ants and pollinators. Exploitation competition is considered dependent on how much a competitor can access and utilize a resource. In our case, we consider the ant's ability to occupy the maximum number of flowers and maximising the number of individuals occupying a single flower as the parameters for assessing the exploitation competition. Interference competition is the efficiency with which one competitor ward off the other. For this, we considered the visitation frequency and duration of a visit by the pollinators as characters that were directly influenced by the ants. To compare the interference competition offered by native and non-native ants, we compared these two characters of pollinators that entered flowers occupied by either invasive or native ants. The higher foraging ability and aggressiveness are considered as key features of invasive ants²³. Moreover, invasive ants are said to locate carbohydrate sources more efficiently²⁴. Therefore, we hypothesised that invasive ants would exhibit a higher exploitation and interference competition to pollinators than native ants.

Materials And Methods

Study Site

Our study was conducted at Kasaragod, the northernmost district of Kerala state in the peninsular India. The district is located at 12.5°N 75.0°E with an average elevation of 19 m above sea level. The highest and lowest temperature is 37 °C and 17°C respectively. The district enjoys a tropical and sub-tropical climate, with winter (January and February), Hot summer (March to May), and two rotations of Monsoon, the Southwest Monsoon (June to September) and Northeast Monsoon (October to November) giving an average annual rainfall of approx. 2000 mm. The district exhibits a topographic division into three, the lowland bordering the sea, the mainlands, and the forest highlands on the eastern side. The soil type reciprocates this natural division. The coastal strips are sandy, the lowland has red ferruginous loam with a mixture of clay and sandy soil, and the highland region is laterite. Agriculture is a chief income source for the population of Kasaragod. The district follows an organic mode of cultivation with organic manuring and non-chemical pest management practices due to endosulfan-related human casualties in the recent past.

The study took place in seven different farmers-managed vegetable gardens in Kasaragod. These sites stood 3-10 km apart. Within each of these sites, we chose 2-10 vegetable patches separated by a distance not more than 500 meters. The farmers of these sites cultivated paddy as their main crop, sowing in June to August, and harvesting in November to January months of the year. Farmers often practice heterogeneous intercrop vegetable farming during the fallow period. After rice cultivation, they cultivate vegetables of Amaranthaceae, Cucurbitaceae, Malvaceae, Solanaceae, and Fabaceae families.

Study System

We took pumpkin (*Cucurbita maxima*) as our study system. It is a monoecious plant with separate staminate (male) and pistillate (female) flowers. Both flowers are borne on different nodes with a disproportionately high number of staminate flowers per field²⁵. Both the staminate and pistillate flowers are large showy yellow with abundant floral nectar—the pistillate flowers producing a slightly higher quantity of quality nectar²⁶. The quality of nectar produced by staminate flowers averaged 29.4% sugar concentration by weight, and 31.1% in pistillate flowers. The quantity of nectar was significantly higher in pistillate flowers. The pistillate flowers produced an average of 5.2µL nectar and the staminate flower produced an average of 2.8µL nectar immediately following the anthesis, which continued through the entire anthesis period.

A large ovary at the base of the flower and three stigma lobes inside the flower distinguishes the pistillate flower. The staminate flower has its anthers united into a single long filament of about 10cm. The pollen grains are large and sticky; thus, pollination is dependent on the animal pollinator. Flower longevity is one day. The flower opened between 0630 and 0700 h and remained open for four to six hours. Native honey bee of *Apis cerana* is the major pollinator of *C. maxima* in our study area²⁵. Farmers raised plants from the seeds collected from their production of the previous year with “Ambili” being the predominant variety in field populations. Four to six seeds are sowed in a pit of about 60 cm diameter and 30-45cm depth, mixing it with neem cake and cow dung. The field is irrigated daily morning either using the traditional pot method or water hose²⁶.

Sampling method

The observations for our study was carried out from 28th December 2018 to 15th February 2019, at the peak season of pumpkin flowering. During the study days, the data were collected during our visits from 0700 h to 0800 h. We counted a total of 1664 flowers to record the number of pistillate and staminate flowers in seven sites. From this, we studied the activity of the pollinator and ants in 348 randomly chosen flowers (206 ant-infested flowers (93 staminate and 113 pistillate flowers) and 142 ant-less flowers (33 pistillate and 109 staminate flowers)). For observing the pollinator and ant activity, we spent 15 min per flower during the early hours of the anthesis and peak activity of pollinators (0700-0800 h). We observed both, the flowers in which ants were present as well as the flowers in which ants were absent in the fields having ants in flowers. During this observation window, we assessed the visitation rate, the duration of the visit, and the hovering activity of pollinators. We observed the visitation rate as the number of times the pollinator entered the flower. We recorded the time of entry and exit of a pollinator, which is essentially the duration the pollinator spent inside the flower in a single visit. This time was recorded using a stopwatch. In cases, when the flower was colonised by a large number of ants, the pollinators did not enter the reproductive part of the flower; the pollinator rather hovered around the flower or landed on the tip of petals for very less time (<3secs). Such activity by the pollinator was recorded as 'hovering'. In flowers occupied by ants, we collected the ants in 5 mL vials with ethanol and identified later from the lab. Based on the identification and the invasive ant list²⁷, we grouped ant-infested flowers into native ant flowers and invasive ant flowers. We also counted the number of ants occupying a single flower. Whenever the ant colony was too large in number, the flowers were cut open to facilitate this counting.

Data analysis

We took three main attributes of pollinator – visitation rate, visit duration, and the choice of entry to identify the effects ants have on flowers. The choice of entry of pollinators on ant-infested and ant-less flowers was tested using a generalized linear model with binomial distribution as error factor, visit-no visit data of pollinators on flowers as the dependent variable, flower type as the fixed factor, and logit as a link function. We studied the effect of flower type (ant-less and ant-infested) on visitation rate and the time the pollinators spent in flowers using two generalized linear models with negative binomial error as an error type, flower type and flower sex (staminate and pistillate) as the fixed effects and visitation rate and time spent by the bees as the response variables. We studied the effects of ant type (invasive/native), number of ants, and flower sex on visitation rate and the time the pollinators spent on ant-infested flowers using another glm with the ant type, number of ants on flowers, and flower sex as fixed factors, visitation rate and the time the pollinators spent on flowers as the response variables and negative binomial error as the distribution type. The significance of the models is tested using Wald Chi-square test available in R-package *car*. All analyses were carried out using R version 3.5.2.

Results

The native honey bee, *Apis cerana*, was the primary pollinator of pumpkin flowers (92% of the total sample). In 8% of the flowers, another native bee species, *Apis dorsata*, also visited the flowers along with *A. cerana*. Since *A. dorsata* was a minority, we used pooled data of visitation characteristics of both the species in the analyses after realizing that the response of both the species of ants was the same towards the ants.

Nine species of ants visited the flowers of pumpkin as soon as the anthesis occurred (Fig. 1); three were non-native – *Anoplolepis gracilipes* (Smith, F.), *Paratrechina longicornis* (Latreille), *Solenopsis geminata* (Fabricius) – and six – *Tapinoma melanocephalum* (Fabricius), *Camponotus* sp, *Polyrhachis* sp, *Monomorium* sp, *Pheidole* sp, *Tetramorium* sp – were native species. The composition of ant species was different between sites, but both the native and invasive ants were present in all the seven sites studied. The fire ant *S. geminata* occupied the highest number in a single flower, a maximum of 137 individuals. *P. longicornis*, the black crazy ant, occupied the maximum number of flowers (53 flowers). While the average number of individuals per ant-infested flower was 11.13 for native ants, it was as high as 32.4 for exotic ants (est±se=-0.91±0.17, z=-5.45, p<0.00005). The invasive ants also occupied a higher number of flowers in field populations. About 73% of the flowers of a given ant-infested site had invasive ants in them. The number of foraging ants in ant-infested staminate flowers was lesser than that in ant-infested pistillate flowers (-0.41±0.16, z=-2.59, p=0.009).

The pollinators preferred to visit ant-less flowers (75%) over ant-infested flowers (62%; 0.60±0.24, z=2.48, p=0.013). The visitation rate of pollinators on ant-less flowers was significantly higher than on ant-infested flowers (0.55±0.14, z=3.82, p=0.0001). Within the antless flowers, the visitation rate of pollinators on staminate flowers was lower than that on pistillate flowers (-0.67±0.31, z=-2.21, p=0.026). This suggests that ant-pollinator interaction is affected by flower sex. It was found that (a) when the number of ants increased in flowers, the visitation rate of pollinators significantly reduced (-0.02±0.003, z=-5.83, p<0.00005), (b) pollinators did not distinguish between invasive ant-infested and native-ant infested flowers (p=0.66), (c) but visitation rate of pollinators reduced by the native ants, when their number increased in the flowers (-0.02±0.01, z=-1.66, p=0.09). The results of the models are summarized in Table 1. The effect the nine ant species had on the visitation rate of pollinators was not different (Wald $X^2=13.5$, df=8, p=0.09). The flower sex had a weak effect on the visitation rate of pollinators on ant-infested flowers (Wald $X^2=3.53$, df=1, p=0.06).

The pollinators spent more time foraging on ant-less flowers than on ant-infested flowers (0.16±0.06, z=2.56, p=0.01). The duration the pollinators spent on ant-infested flowers decreased when the number of inhabiting ants was more (-0.02±0.002, z=-6.39, p<0.00005). Flower sex had a clear effect on time the pollinators spent on ant-infested flowers; on native ant-inhabiting flowers, the pollinators spent more time for foraging (0.35±0.16, z=2.18, p=0.02), which however got reduced when their number increased in staminate flowers (-0.07±0.03, z=2.4, p=0.02) (Table 1).

Discussion

Ants, though sometimes help in pollinating flowers of some species²⁸⁻³¹ have a complex interaction with the plants¹⁴. This interaction has a narrow cost-benefit border¹⁵. Invasive ants are particularly known to give an exploitative competition and predation pressure on foraging legitimate pollinators (see Sinu *et al.*¹⁸ and references therein). They predate on pollinators¹⁸ and decrease the chances of pollinator visit by exhausting the floral rewards³². There are quite a handful of studies exploring the impacts of invasive ants on pollinators. However, a comparison of invasive and native ants concerning their interaction with floral visitors are scarce¹⁵.

In this study, we attempted to explore the effects of ants on pollinators, as well as to compare these effects in cases of invasive and native ants. The study found that ants regardless of the nativity gave a competitive pressure for honeybees in pumpkin flowers. Both the visitation rate and the duration they spent foraging floral resources was significantly low in ant-infested flowers. Although the bees tend to spend more time on native-ant occupied flowers, when the number of ants is high, they avoid such flowers.

With its showy flowers with a reasonable amount of floral rewards in the form of both pollen and nectar, pumpkin (*Cucurbita maxima*) is a noteworthy lure for both ants and bees. We found that ants occupied more than 75% of the flowers during the early hours of anthesis in different fields. The ratio of staminate flowers to pistillate flowers counted in our study agrees that the number of staminate flowers is mostly higher in a field^{25,33,34}. As the nectar amount varies between the staminate and pistillate flowers, this might be a major determinant of ant infestation of flowers. This is a possible justification of why a larger margin of pistillate flowers was found to be occupied by ants than staminate flowers.

Ants generally repel pollinators by its aggressive nature³⁵. Sometimes, the pollinator might choose not to enter a flower due to the presence of ants¹⁸. The scent of ants negatively influences the choice of pollinator's entry into flower²¹. In our study, we found that the pollinator entry was significantly lower in the case of ant-occupied flowers. We also observed several cases of hovering, wherein the bee hovered around the flower or landed on petals but flew off. The bees in such cases avoided contact with ants and did not touch the reproductive structures of the flower. This may be a huge loss for the plant as pollination is likely to be directly affected. The pollination frequency was also significantly lowered in case of ant-occupied flowers¹⁸. Some studies suggest that the pollinator might frequently reposition in ant-occupied flower, and have more pollen deposited on them, which might increase the seed set¹⁵. Although there were cases of predation attack by yellow crazy ants previously¹⁸, we found the native ant, *Diacamma sp.* was opportunistically preying upon honey bees in the present study. The duration of each visit was also significantly higher in the case of an antless flower. This is an indication of direct interference competition that ants offer to the pollinators. The duration of the visit was significantly lower in the case of *Solenopsis geminata* and *Tetramorium sp.* The presence of sting could be a possible defence to drive off pollinators. Although there were other ants with a sting, only these two stinging ants occupied a considerable number of flowers to draw a possible conclusion.

There are several cases of ant-pollinated plants. In the Western Ghats, ants were recently reported as a pollinator of *Syzigium occidentale* plant inflorescence²⁹. In the Atlantic rainforest system, ants have been found to be a major pollinator of aggregated inflorescence²⁸. Though there is a possibility that ants might pollinate pumpkin, it will have to be tested through a seed set experiment to be certain. During our observation, we did find some amount of pollen sticking onto the ants. However, as most of the characters of pumpkin flower did not resonate with the characters of ant pollinated flowers^{18,36}, ants might not play a major role in the pollination of pumpkin flowers. Additionally, exposing flower to ant secretions seems to deter viability. When subjected to ant secretions, the pollen vitality, the germination rate and the length of pollen tubes significantly reduced³⁷. While they feed nectar, ants sometimes damage the base of pistil affecting the seed set³⁸. Although ant-visited or pollinated flowers are not rare in tropics^{29,39} they are mostly bisexual and have either generalist pollination syndrome or have no bee pollinators on them. Pumpkin is a major floral resource for specialist bees including honey bees, bumblebees, and squash bees, which perform cross-pollination in their monoecious flowers. Because they are monoecious, the visitors should travel from staminate to pistillate flowers for effecting pollination. It is very unlikely that the ants switch visits between staminate and pistillate flowers in pumpkin fields as staminate flowers are abundant and pistillate flowers are not necessarily to be closer to staminate flowers. Although not investigated on all the nine species of ants in this study, Sinu et al. (2017)¹⁸ found that none of the *A. gracilipes* visited flowers sets fruits.

The efficiency with which ants used up resources determined the exploitation competition offered by ants to the pollinators. The main parameters of the ants that we used to measure this were the number of individuals that occupy a single flower and the number of flowers occupied by a single species of ant. *S. geminata* was found with the highest number of individuals per single flower. This mass recruitment, although not particularly common in *S. geminata* is a key character of invasive ants²³. Similarly, invasive ants, in general, had a higher number of individuals recruited to a single flower compared to the native ants. The black crazy ant, *P. longicornis* occupied the highest number of flowers. This implies that they are highly efficient in locating resources. *P. longicornis* has a complicated modular recruitment system as well as polydymous nesting^{23,40}. This minimises the distance between the nest and food resource and increases their foraging efficiency. The other globally-important invasive species colonized in pumpkin flowers, *A. gracilipes*, is also known to make supercolonies and exploit resources quickly⁴¹. Thus, invasive ants exerted a higher exploitation competition on pollinators than native ants. Both the visitation frequency and the duration of visit decreased with the increasing number of ants; thus, a higher number of invasive ants indirectly influenced the pollinator attributes. The interference competition offered by invasive and native ants seemed to be similar. The visitation frequency and the duration spent by the pollinators are affected by the attack and aggression of ants on pollinator directly. These were considered key attributes for comparing the interference competition. In our studies, there was no considerable difference in these characters irrespective of which ant (invasive or native, or any of the nine species) occupied the flower. The choice of the pollinator entry was not affected by whether the flower was

occupied by an invasive or native ant. Bees did not particularly show any discretion between the scents of an invasive ant occupied and native ant occupied flower.

Our studies further confirmed the negative effect of ants on pollinators of pumpkin¹⁸. We found that ants negatively influenced most crucial visitation characters of the pollinators. However, concluding from this is quite complicated as the interactions between ants, plants, other herbivores, and pollinators are quite complex¹⁴. Unlike many of the previous studies, we compared the competition offered by invasive ants and native ants on the pollinators. The exploitation competition is largely offered by invasive ants owing to their supercolonies and high population size. However, the interference competition can be offered by any native and invasive ants which can show aggressive and predatory behaviour to pollinators. But it is not possible to draw a fine border between these two competitions. Information on the effects of invasive ants on pollinators will help in implementing better pollination management practices in agricultural lands^{15,42}.

Declarations

Acknowledgements

We thank the farmers for their cooperation in the study. The cost of the fieldwork was supported by a core grant of Science Engineering and Research Board, New Delhi awarded to PAS. SM would like to thank Indian Academies of Science for a 2-month summer research fellowship.

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Tables

Table I. Predictors of pollinators' visitation rate and time spent on flowers of pumpkin, *Cucurbita maxima*.

Factor	Visitation rate			Time spent/visit		
	Chi-square	df	p-value	Chi-square	df	p-value
Ant abundance (n)	45.37	1	<0.00005	95.18	1	<0.00005
Ant status (native/invasive)	3.12	1	0.07	0.27	1	0.6
Flower sex (staminate/pistillate)	2.56	1	0.1	5.78	1	0.01
n*ant status	0.54	1	0.46	1.34	1	0.24
n*flower sex	0.97	1	0.32	9.05	1	0.002
n*ant status*flower sex	0.001	1	0.99	5.08	1	0.02

Figures

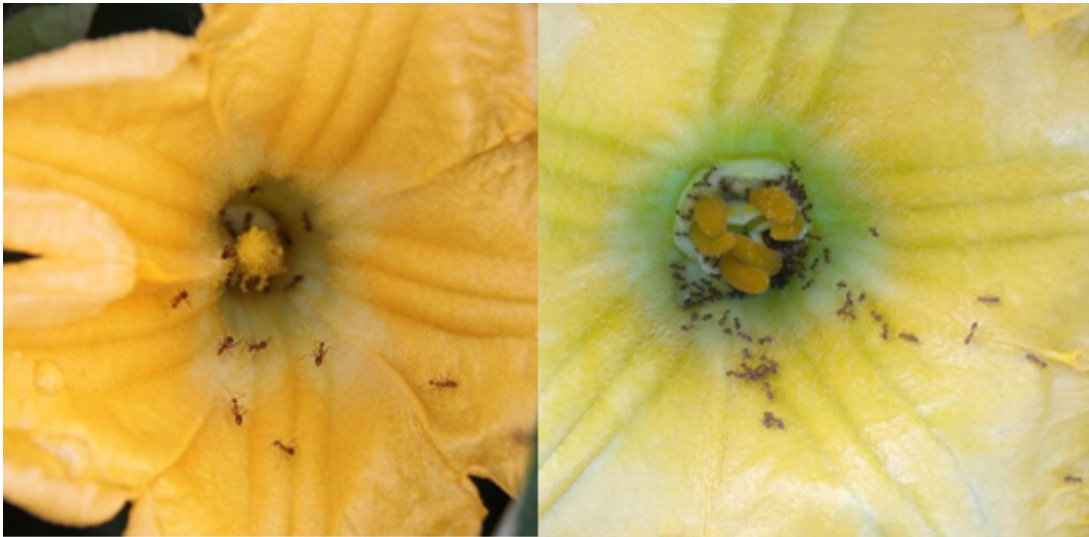


Figure 1

A staminate (male) and pistillate (female) flower infested by the ants