Change in biodiversity in the inflorescence of *Xanthosoma robustum* (Araceae) during the flowering period

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ABSTRACT

Inflorescences of the species *Xanthosoma robustum* (Araceae) create various microhabitats during the flowering period. This thermogenic plant heats its inflorescence for the purposes of pollination and many organisms such as scarab beetles, mites, and flies come to the flowers to mate and feed during the heating process. Most of these organisms stay in the inflorescence for two days and then fly to the next inflorescence. The purpose of this study is to see if species diversity changes during the three day flowering period and to investigate the relationship between pollinating beetles *Cyclocephala* (Scarabaeidae) and mites (Acari) that visit these plants. Inflorescences were collected the first and second days following heating, and for one day after heating was complete, for a total of three groups of inflorescences. Species diversity was recorded for each inflorescence day. Results showed that day two inflorescences had the greatest species diversity. There was a higher abundance of both *Cyclocephala* beetles and mites on day one than day three inflorescences. There was a positive correlation between beetle abundance and mite abundance. These results show that species partition the inflorescence and inflorescence day in different ways. They use different parts of the inflorescence for feeding and mating. This study shows species interactions and niche differentiation in small habitats such as phytotelmata.

RESUMEN

Las inflorescencias de la especie *Xanthosoma robustum* (Araceae) crean varios micro-hábitats durante el período de floración. Estas plantas termogénicas calientan las inflorescencias con el proposito de polinización y varios organismos como escarabajos, acaros y moscas visitan las flores para aparearse y alimentarse durante el proceso de calentamiento. La mayoría de estos organismos se quedan en la inflorescencia por dos días y luego se mueven a otra diferente. El proposito de este estudio es determinar se la diversidad de especies cambia durante los tres días de floración e investigar la relación entre los escarabajos del género *Cyclocephala* (Scarabaediae) y acaros (Acari) que visitan estas plantas. Las inflorescencias fueron colectadas durante los primeros dos días del calentamiento y un día despues del mismo, para un total de tres grupos de inflorescencias. Los resultados muestran que el Segundo dia de calentamiento las inflorescencias muestran una mayor diversidad. Hay una alta abundancia tanto de escarabajos como de acaros en las inflorescencias durante los dias uno y tres. Hay una correlacion positiva entre la abundancia de acaros y escarabajos. Estos resultados muestran que la reparticion de especies dentro de las inflorescencias y tambien durante el tiempo. El estudio muestra la interaccion de especies y la diferenciacion de nicho en habitats pequeños como la fitotermata.

INTRODUCTION

For many years biologists have been intrigued by how many species there are on earth and how these organisms live with one other. One of the main reasons this coexistence is possible is niche partitioning (Shoener 1974). Each species has its own niche where it lives, eats, drinks, and mates. A niche can be defined as the position or role a species has in a community and the distributional relation of a species to a range of environments and communities (Whittaker *et al.* 1973). An organism's niche can be very large but it can also be something as small as the inflorescence of a plant. According to the Principle of Competitive Exclusion, two species cannot coexist in exactly the same niche at the same time (Hardin 1960). Therefore, organisms partition niches through facultative or obligate niche partitioning. Facultative niche partitioning is when resources are divided only in the presence of a competitor while obligate niche partitioning is an evolved response of specialization to a long evolutionary history of competition (Levine 1980). Obligate niche partitioning is when resources are divided in the absence of a competitor (Levine 1980). In the tropics, there is very fine niche partitioning frequently due to the high productivity and/or competition, which may aid in the high species diversity seen in tropical habitats (Levine & HilleRisLambers 2009).

Species diversity can vary over large scales (ecosystems and landscapes) or small scales (single flowers or pools of water). Small scale habitats, or microhabitats, are interesting for studying changes in species diversity over time and often times have high species diversity due to competition and partitioning of habitat space (Anderson 1978). Microhabitats can last for weeks at a time, or in the most extreme cases, for just a few days (García-Robledo *et al.* 2005). Inflorescences of the plant family Araceae are a place where ephemeral microhabitats are present (Greeney 2005). The inflorescences of one species of Araceae, *Xanthosoma robustum*, have inflorescences that contain three different microhabitats over the flowering period. The plant is thermogenic and on day one of the inflorescence opening, it heats and creates a habitat that attracts pollinators and other arthropods. On the second day of heating, a separate microhabitat is created and the species composition of visitors to the inflorescence changes. By the third day, the inflorescence stops producing heat, and by the fourth day, has created another microhabitat called a phytotelmata which are small aquatic habitats in terrestrial plants (García-Robledo *et al.* 2005)).

It has been shown that organisms that live in microhabitats can change over time, creating different niches for various organisms to coexist without competition (Naeem 1988)). One study (Naeem 1988) looked at resource heterogeneity between two competing species in a microhabitat. Both species depended on the same resources in the plant. In the study there was a negative correlation between the densities of the two species so one species was more dominant. Based on competition theory, one species should out compete the other but in the field both these species coexist. This shows temporal and spatial heterogeneity in resources and that species may coexist in this habitat.

Because of the large number of species that can live in inflorescences and the habitat that they create, *X. robustum* inflorescences are the ideal place to obtain information about niche partitioning, species diversity, and the complex interactions that occur between organisms. The main questions that this paper addresses are: 1) What is the effect of time on the species diversity in inflorescences?; and 2) What is the effect of *Cyclocephala* abundance on mite abundance? I predict that species diversity will stay the same on all three days but species composition will change, and that day one inflorescences will have the greatest abundance of *Cyclocephala* beetles and mites. For my second question I predict that inflorescences with a greater abundance of *Cyclocephala* beetles will have a greater abundance of mites.

METHODS

Study system

Xanthosoma plants are thermogenic in order to attract pollinators. The first night the inflorescence opens, it heats to 40-42^{o}C (Seymour & Schultze-Motel 1997). This heating is a reproductive strategy of the plant. It is beetle pollinated and beetles need high temperatures in order to be active (Ghose 2009). These plants and insects have coevolved with each other and have a mutualistic relationship. The first night the inflorescence heats up, *Cyclocephala* (Dynastinae) beetles land on the spadix and go down to the lower chamber. There, they mate and eat the ring of sterile flowers, remaining in the flower until the next day (Goldwasser 1979). The second day, the inflorescence heats again to about 34^{o}C and it starts to shed pollen, which is white and covers the spadix. The beetles then crawl out of the spadix and fly to the next flower carrying pollen with them. This way the plants are pollinated and the beetles have a place for mating and feeding. By the third day of the cycle, the spadix turns a grayish color then eventually falls off, becoming a fruit.

There are other organisms that visit the inflorescences of *X. robustum,* such as mirids (Miridae which mate and suck the juices out of the spadix, various species of flies (Odonata), which lay their eggs in the inflorescence and mites (Macrochelidae). (Goldwasser 1979). Nitidulidae beetles also lay their eggs in the spadix damaging the seeds (García-Robledo 2005). A *Cyclocephala* beetle can contain up to 30 mites on it at a time. The mites climb onto the beetles and are thought to ride back and forth between inflorescences on the beetles. Once at a new flower, the mites go into the lower chamber of the inflorescence and feed on the nectar of the plant (Goldwasser 1979).

Collection of inflorescence

This study was conducted in Monteverde, Costa Rica, during the month of November, 2010. The site used for this study was Fred Morrison's property located near the Monteverde Cloud Forest Reserve at 1550 meters above sea level. This property contained 214 *X. robustum* plants (Ghose, 2009) which were almost all flowering and/or fruiting during this time. Eleven day one inflorescences, eleven day 2 inflorescences, and eleven day three inflorescences were collected in ziplock bags. The day one inflorescence's spadix was white and did not have pollen on it, the day 2 inflorescence could be identified by a white spadix with pollen along it, and a day three inflorescence was slightly gray and the spathe was slightly brown (Figure 1).

Data Collection

Inflorescences were taken out of zip-lock bags and opened to take out *Cyclocephala* beetles. The beetles were put into separate jars labeled with the inflorescence that they came from. Bags were then quickly closed and (96%) ethanol was put into each bag so that the organisms could be counted more accurately without moving. The bags were shaken to make sure the ethanol entered the inflorescence and then re-opened. The abundances of each species found in the inflorescences were recorded. The number of mites in the jar and on the beetles was also counted and then divided by the number of *Cyclocephala* there were to find the average amount of mites per beetle.

RESULTS

Flower day and species richness

Day two inflorescences had the greatest species diversity (Figure 2). There was a greater species diversity in day two flowers than day one flowers; H'1=1.04 and H'2= 1.32 (t=4.91, df=807.3, p<0.001). There was also a greater species diversity in day two inflorescences than day three inflorescences; H'3=1.08 (t=3.04, df=602.8, p=0.002). There was no significant difference between the species diversity of day one and day three flowers (t=.49, df=499.2, p=0.621). Means and standard deviations for the most common species are listed in table 1.

There were more *Cyclocephala* beetles in day one inflorescences than day three inflorescences (Figure 3; $F_{2,30}$=4.25, p=.024). Day one inflorescences had a mean number of 1.73 $^{+/-}$ 1.84 beetles, day 2 inflorescences had a mean number of .90 $^{+/-}$ 1.3 beetles, and day three inflorescences had a mean number of .09 $^{+/-}$ .3 beetles.

There were more mites in day one inflorescences than day three inflorescences (Figure 4; $F_{2,30}$=3.7018, p=.037). Day one inflorescences had a mean number of 44.09 $^{+/-}$ 44.2 mites, day 2 inflorescences had a mean number of 30.18 $^{+/-}$30.9 mites, and day three inflorescences had a mean number of 11.00 $^{+/-}$15.7 mites.

Cyclocephala and mite abundance

The more beetles that were in the inflorescence, the more mites were present (Figure 5; $F_{1,31}$=10.6, p=.0027).

DISCUSSION

The main purpose of this experiment was to see if inflorescence age would have an effect on the species diversity inside the inflorescence. The species diversity was highest in day two inflorescences. Different organisms have different niches so each day of this inflorescence makes a slightly different microhabitat. On the first day beetles *Cyclocephala sexpunctata* and *Cyclocephala nigerrima* are in the inflorescence from the heating the night before and can remain there for a couple of days. Upon their arrival at heating, beetles of the family Nitidulidae also arrive and lay eggs and mites appear as well. Day two is also when more organisms come such as fruit flies and mirids. Therefore, on day two inflorescences the species that arrived at heating are still in the inflorescence but new organisms come as well to feed on nectar and sterile flowers. This makes species diversity highest in day two inflorescences. Some of the other observed species in the inflorescences were, a spider, a grasshopper, and a *Similisca puma.* This is an ideal example of niche partitioning and how no organism shares the exact same niche. Although many of these organisms feed and mate in the inflorescence, they do so in different parts and at different times so although there are many species in the inflorescences, they all interact with it differently. One hypothesis as to why species diversity would be highest in day two inflorescences is the “mid-domain effect” (Schulte *et al*. 2010). This hypothesizes that species diversity would be highest in the middle gradient due to overlapping boundaries. Few species can live in an upper or lower extreme but both can live somewhere in the middle so you have an overlapping of habitats. Species that come to the inflorescence the first day may not be able to survive there the third day but can remain on the second. Other species visit on the second day and the habitat on the third day may be suitable for them so species diversity is greatest on the second day due to this overlap in boundaries.

Because *Cyclocephala* beetles coevolved with *X. robustum*, it was appropriate to see if inflorescence age had an effect on *Cyclocephala* abundance. On day one I observed the greatest amount of beetles, and on the third day the least number of beetles. One reason for this is that heating allows beetles to reduce their basal metabolism by 1/8 while they are mating and feeding so this is somewhat of a reward for coming (García-Robledo 2005). When the inflorescence heats it also releases a sweet odor, which attracts many of the organisms that come to it. There were still beetles present on the second day inflorescences because they feed on the sterile flowers and the combination of this, the still elevated temperature, and the sweet scent act as an aphrodisiac to the beetles (Ghose, 2009). On the third day, the beetles have already mated and eaten the sterile flowers so almost all of them have already left. Goldwasser (1979) observed that only a few beetles were present in day three inflorescences and many of these seemed almost dead.

Mite abundance was also predicted to be greatest on day one and least on day three inflorescences. During the study, observations were made that whenever beetles were present, mites were present versus when there were no beetles present, there generally very few mites present. Results showed that mite abundance was greatest for day one and lowest for day three inflorescences. There was also a positive correlation between beetle abundance and mite abundance.

The mites in *X. robustum* drink the nectar of the inflorescence, riding the beetles from inflorescence to inflorescence so that they can obtain the nectar they feed on. With mites and beetles together it causes interspecific competition in the system. Although the mites drink small amounts of nectar, this nectar would otherwise be available to the scarab beetles (Venkatesan 2001). In this case, in can be considered exploitative competition where one species is indirectly limiting the resources of another organism. This is because the mites also do not play a role in pollination since they are too small to carry pollen so the reward of nectar that should be for pollinators such as the beetles instead goes partly to the mites. The mites drink a small amount of this but it still means less of a reward for beetles that have to use a high amount of energy to fly from inflorescence to inflorescence.

It would be very interesting to see if patch size has an effect on species diversity because larger patches have more resources and would therefore be able to sustain a greater amount of species. This study shows how habitats as small as phytotelmata can have a copious amount of species diversity and even within a very short amount of time the organisms within this habitat can change. The significance of this study is it supports the “mid-domain effect” and shows how organisms can live together and partition their resources in the niche they occupy.

FIGURE 1. Different ages of inflorescences. a. Day one inflorescence, 1st day after heating, b. day two inflorescence with pollen on spadix, c. day three inflorescence with slightly gray spadix and slightly brown spathe.

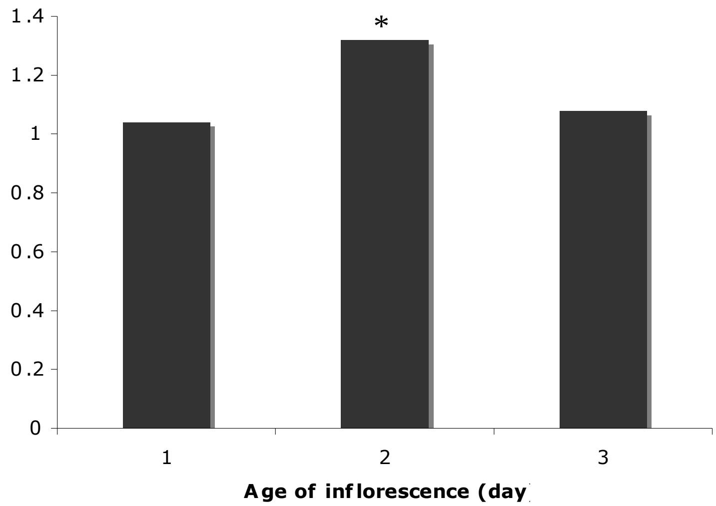

FIGURE 2. Species diversity in inflorescences one, two, and three days after opening. Shannon Weiner Diversity index shown, measuring species richness and species eveness. Asterisks represent a significant difference. There was a significant difference between the diversity of day one and day two flowers ($t=3.04$ $df=602.83$, $p=.00245$) and a significant difference between day two and day three flowers ($t=3.04$ $df=602.83$, $p=0.00245$).

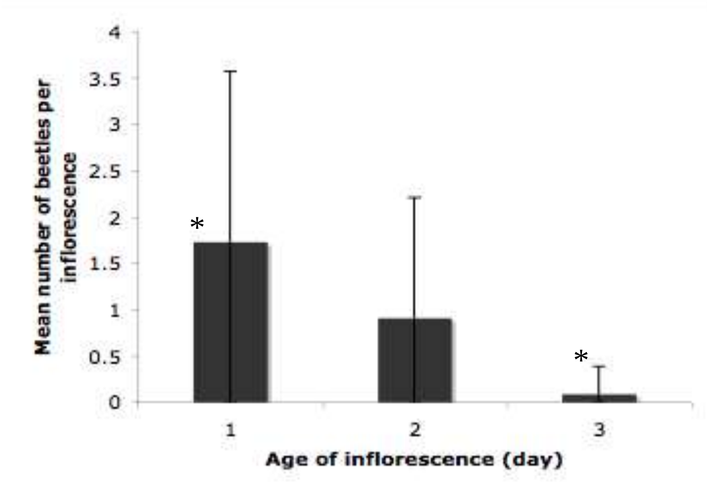

FIGURE 3. Mean number of *Cyclocephala* beetles in inflorescences one to three days after opening. Standard deviation are shown. Asterisks represent a significant difference. There was a significant difference between day one beetle abundance and day three beetle abundance ($F_{2,30}$=4.2483, p=0.0237).

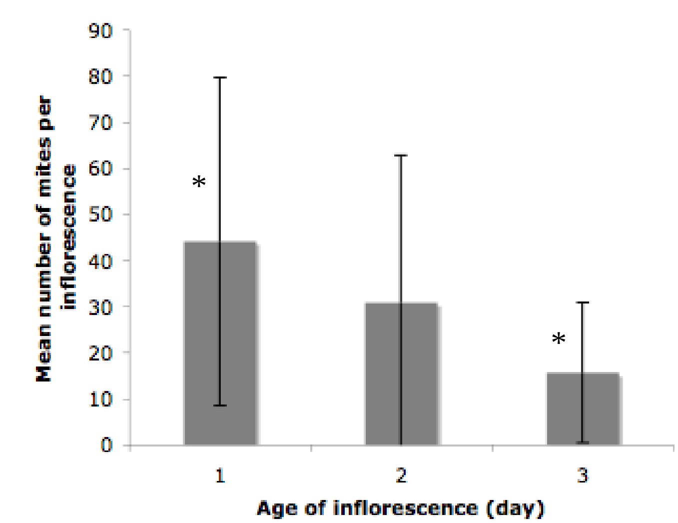

FIGURE 4. Mean number of mites in inflorescences one to three days after opening. Standard deviation bars are shown. Asterisks represent a significant difference. There was a significant difference between day one mite abundance and day three mite abundance ($F_{2,30}$=3.7018, p=0.0366).

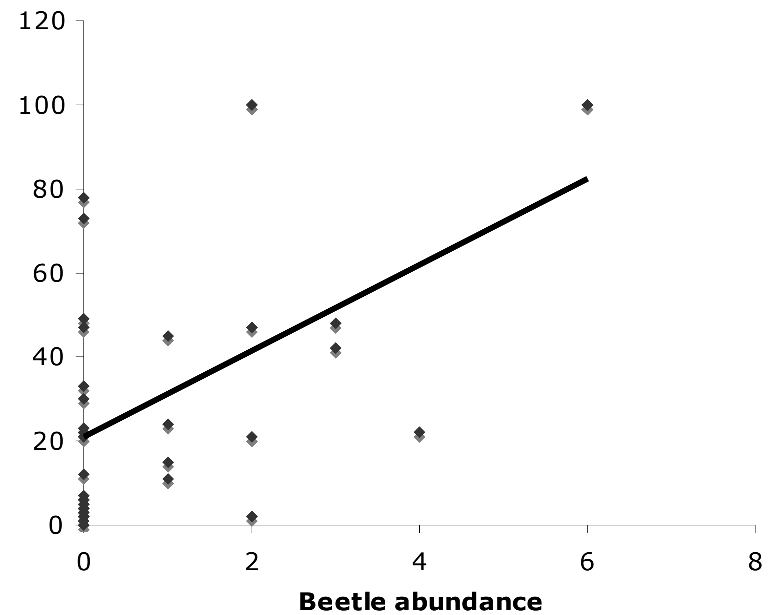

FIGURE 5. The relationship between *Cyclocephala* beetles and mites in the inflorescences of *Xanthosoma Robustum.* There was a correlation between beetle and mite presence ($F_{1,31}$=10.6, p=.003).

TABLE 1. Mean number of each species per inflorescence. Standard deviation shown.

Species	Day 1	Day 2	Day 3	SD day 1	SD day 2	SD day 3
Cyclocephala nigerrima (Scarabaeidae)	4	5	1	0.67	0.82	0.3
Cyclocephala sexpunctata (Scarabaeidae)	15	5	0	1.36	0.93	0
mites (Macrochelidae)	485	332	168	35.65	32.3	15.6
black mirid (Miridae)	56	27	36	3.83	2.49	4.1
brown mirid (Miridae)	1	53	3	0.3	0.4	0.46
fruitfly (Tephritidae)	70	59	37	5.83	5.37	2.44
larvae	0	1	54	0	0.3	10.25

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