August 2008

Patterns of petiole abundance and diversity in bark beetles (Scolytinae) of Cecropia trees in Monteverde, Costa Rica

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ABSTRACT

Cecropia trees regularly shed their woody, large petioles year round. This provides bark beetles from the family Scolytinae with a dependable and stable microhabitat. Bark beetles bore into the petioles, lay their eggs, and feed on the pith (Wood 1983). This study was conducted in Monteverde, Costa Rica. It examined the abundance and diversity of bark beetles between *Cecropia polyphlebia* in the Monteverde Cloud Forest (1550 meters) and an intermediary phenotype at Bajo del Tigre (1400 meters). It also investigated the percent of petioles colonized, species richness, evenness, and the influence of petiole moisture and length on the abundance and diversity of beetles. I analyzed 90 dead, woody petioles from the ground and from hanging vegetation from each site. Petioles were dissected and beetles were removed and identified by one of six Scolytinae morphospecies. Differences in the abundance and diversity of beetles between the two species were then compared. This study suggests that there was no difference in species diversity between the two tree species ($p > 0.05$). However, a difference in abundance between the hybrid phenotype and *C. polyphlebia* was observed. The hybrid phenotype possessed a higher abundance of beetles. Also moisture content (ANCOVA, $F = 4.93$, $df = 2$, $p = 0.0083$), the species of *Cecropia* (ANCOVA, $F = 11.2$, $df = 1$, $p = 0.001$), and petiole length with *Cecropia* species (ANCOVA, $F = 5.16$, $df = 1$, $p = 0.0244$) had an influence on the abundance of beetles. The higher abundance of beetles in the hybrid phenotype could indicate differences in interspecific competition due to phenotypic traits of the *Cecropia* species. Differences in moisture content and petiole length between the two *Cecropia* species may reflect petiole preference and favorability of tree hosts. Furthermore, the higher abundance of beetles in the lower elevation hybrid phenotype could indicate that the majority of bark beetles may not be affected by climate change unlike many other species of animals and plants that are being severely impacted by the increasing temperatures.

RESUMEN

Los árboles de Cecropia sueltan regularmente sus largos y leñosos durante todo el año. Esto proporciona corteza escarabajos de la familia Scolytinae de un microhábitat fiable y fijo. Los escarabajos de la corteza habitan en los pecíolos, colocan sus huevos, y comen la médula (Wood 1983). Este estudio fue realizado en Monteverde, Costa Rica. Examinó la abundancia y la diversidad de escarabajos de corteza entre Cecropia polyphlebia (1550 metros) y un fenotipo intermedio en el bosque nuboso de Monteverde y un fenotipo intermedio en del de Bajo Tigre (1400 metros). También investigó el porcentaje de pecíolos colonizados, riqueza de especie, la equidad, y la influencia de la humedad de pecíolo y longitud en la abundancia y la diversidad de escarabajos. Analicé a 90 peciolos leñosos del suelo y de la vegetación colgante de cada sitio. Los pecíolos fueron disecados y los escarabajos fueron sacados y fueron identificadas como seis diferentes especies de Scolytinae. Las diferencias en la abundancia y la diversidad de escarabajos entre la dos especie entonces fueron comparadas. Este estudio sugiere que no había diferencia en la diversidad de la especie entre la dos especie de árbol ($p > 0.05$). Sin embargo, se encontró una diferencia entre C. polyphlebia y el híbrido. El fenotipo híbrido poseyó una abundancia más alta de escarabajos. También humedad contenida (ANCOVA, $F = 4.93$, $df = 2$, $p = 0.0083$), la especie de Cecropia (ANCOVA, $F= 11.2$, $df = 1$, $p = 0.001$), y la longitud de pecíolo con la especie df de Cecropia (ANCOVA, $F = 5.16$, $df = 1$, $p = 0.0244$) tuvo una influencia en la abundancia de escarabajos. La abundancia más alta de escarabajos en el fenotipo híbrido podría indicar las diferencias en la competencia intraespecífica debido a rasgos fenotípicos de la especie de Cecropia. Las diferencias en el contenido de la humedad y la longitud de peciolo entre la dos especie de Cecropia pueden reflejar la preferencia de peciolo y favoritismo por árboles hospederos. Además, la abundancia más alta de escarabajos en la elevación más baja podría indicar que la mayoría de escarabajos de corteza no puede ser afectada por el cambio del clima a diferencia de muchas otra especie de animales y plantas que son impresionados severamente por las temperaturas crecientes.

INTRODUCTION

Woody petioles are rarely considered a habitable location for any subcortical-feeding insects, but beetles from the family Scolytinae use several substrates such as xylem, petioles, and the pith of dead twigs as breeding grounds (Jordal and Kirkendall 1998). Interestingly enough, several species from the family Scolytinae have a unique ecological relationship with *Cecropia* trees. These beetles, known colloquially as bark beetles, breed within the fallen, dead, woody petioles that Cecropia trees offer year-round (Jordal and Kirkendall 1998). Therefore, these petioles can provide information about the diversity and abundance of bark beetles found in types of *Cecropia*.

Each *Cecropia* tree can be considered a mini-ecosystem that is colonized by several wood-boring beetles. With high competition, the various genera of bark beetles demonstrate niche stratification in order to coexist. Competition can be greater when sharing a petiole (Larimer et al. 2006). So, it is not uncommon to find two or three species of bark beetles in each petiole due to niche partitioning. For example, *Scolytodes aratus panamensis* is notorious for having one to four pairs of adults per petiole (Wood 1983). This is a relatively high number of beetles per petiole.

Bark beetles are extremely unique in their breeding and habitat locations. They colonize the leaves around three to fifteen days after they have fallen (Wood 1983). Also, their breeding systems can have eight different inbreeding origins (Larimer et al. 2006). Bark beetles breed by having the adult male Scolytine beetle bore an entrance hole through the hard cortex into the soft pith of the petiole. Then a female bark beetle assumingly joins the male within the petiole by following a pheromone trail. After meeting, the female begins to burrow in an irregular pattern and lays one to twenty eggs through the tunnels. The pupae eat away at the pith and escape to find another petiole to inhabit (Wood 1983).

The goal of this study is to determine if some species of Scolytine beetles are exclusive to one species of *Cecropia*. It has been hypothesized that bark beetles will not be able to easily colonize another species of *Cecropia* because they are highly specialized and have unique breeding systems. Abundance was predicted to be greater in the hybrid species otherwise known as the intermediary phenotype due to physiological traits that may favor bark beetles over other species. For instance, the Mullerian bodies are less distinct in the hybrid phenotype. This results in a drop of *Azteca* colonies. However, higher diversity will be found in *C. polyphlebia*. *C. polyphlebia* is the only species of *Cecropia* found at high altitudes (Longino 2000). This would lead to a concentration of bark beetles preying upon these *Cecropia* woody petioles, which can lead to higher diversity due to high competition for similar resources. However in the hybrid range, more than one species of *Cecropia* can coexist, which may lead to lower competition of resources and thus less diversity (Longino 2000).

METHODS

Two study sites were used in Monteverde, Costa Rica to obtain *Cecropia* petioles for the research of the Scolytine beetles from July 17, 2008 to August 1, 2008. The high elevation species *C. polyphlebia* was collected from the Monteverde Cloud Forest (1550m), and the lower elevation hybrid species was collected from the Bajo del Tigre of the Children's Eternal Rainforest (1400 m). At each site, 30 petioles per tree were collected, thus 90 petioles at each site. Petioles were arbitrarily collected from a variety of locations from the ground to hanging vegetation up to 4 meters high, and a variety of petioles were selected. Petioles were placed in plastic bags for transport.

Petiole moisture and petiole length were determined for each petiole. Petiole moisture was determined using a procedure outlined by Jordal and Kirkendall (1998). They ranked each petiole on a scale of zero to two. Zero was desiccated, one was wet, and two was soft and moist. Petioles were dissected, and all the beetles were removed and placed in containers. Beetles were initially placed in containers filled with ethanol, but classification of color may have been compromised with prolonged time in the containers. Containers without ethanol were used afterwards. Scolytine bark beetles were identified by morphospecies. A dissecting microscope was used to distinguish physical characteristics of color, presence of hair on abdomen, presence of hair on elytra, size, and color.

A modified t-test was used to compare diversity. I then calculated abundance, evenness, and species richness for both sites. A two-way ANCOVA analysis was performed to see if the type of species, moisture content, or length of petiole influenced the abundance of beetles.

RESULTS

A total of 180 petioles were dissected from two different species of *Cecropia.* 90 petioles were from *C. polyphlebia* and 90 petioles were of the intermediary phenotype. 44% of the petioles were inhabited in the hybrid but only 20% of the petioles were colonized in *C. polyphlebia*. 32% of all the petioles were colonized.

A statistically significant difference in abundance was found between the two *Cecropia* species (Table 2). The hybrid species had a total number of 304 beetles, whereas *C. polyphlebia* had 85 beetles (Table 2). In both species of *Cecropia*, the most abundant beetle species was Morpho 3 and second most abundant was Morpho 1 (Table 2). The least abundant beetle species found in *C. polyphlebia* was either Morpho 5 or 6 (Table 2). The least abundant beetle species found in the hybrid *Cecropia* was Morpho 2 (Table 2). No significant difference in Shannon-Weiner diversity index was found between *C. polyphlebia* (H' = 1.26) and the hybrid (H' = 1.23) (Modified t-test, df = 127.9, $p > 0.05$; Table 2). Six species of beetles were found in *C. polyphlebia* and five species in the hybrid (Table 1, Table 2). No significant difference in evenness was found. *C. polyphlebia* had an evenness of 0.76, and the hybrid *Cecropia* had an evenness of 0.77 (Table 2). S'marg for *C. polyphlebia* was 1.13, and the hybrid *Cecropia* S'marg was 19.21 (Table 2). Moisture content was found to increase as the abundance of beetles increased (ANCOVA, $F = 4.93$, $df = 2$, $p = 0.008$; Figure 3). Also, the hybrid species had a greater abundance of beetles compared to *C. polyphlebia* (ANCOVA, $F = 11.2$, $df = 1$, $p = 0.001$; Figure 2). Furthermore, as the petiole length increased within each species of *Cecropia*, the abundance of beetles increased (ANCOVA, $F = 5.16$, $df = 1$, $p = 0.024$; Figure 1). Several larvae were observed in many of the occupied petioles. Furthermore, there were many insects and non-beetle species found in the Monteverde Cloud Forest.

DISCUSSION

Based on results from this study, it was concluded that bark beetles were able to successfully colonize and reproduce in the hybrid species of *Cecropia* better than in *C. polyphlebia*. This is supported by the fact that there was also a higher abundance of beetles found within the hybrid *Cecropia*. This indicates that there was not a significant difference in diversity, but there was a significant difference in abundance of beetles colonized.

Also, 44% of the hybrid petioles were colonized and 20% of the *C. polyphlebia* petioles were colonized. One possible explanation for a higher colonization percentage in the hybrid *Cecropia* is that the hybrid species offers a better habitat for bark beetles than *C. polyphlebia.* Through observation, there were significantly less insects other than beetles found in the hybrid petioles than in the *C. polyphlebia* petioles. This may reduce inter-specific competition between species and allow for bark beetles to thrive in the hybrid *Cecropia*. Also, the intermediary phenotype may inhibit other species that generally colonize *Cecropia* petioles. For example, hybrid Müllerian bodies, similar to extrafloral nectaries, are less distinct and are covered in long hairs. Furthermore, colonies of *Azteca* that shares a mutualistic relationship with *Cecropia* drop out around the elevation where the hybrid species is found (Longino 2000). These factors may explain a higher abundance of beetles in the hybrid *Cecropia*.

Bark beetles regularly inbreed and generally mature and mate with their sisters before even leaving the petiole (Beaver et al 2001). In bark beetles, usually one to four pairs of adults was found per petiole (Wood 1983). However the hybrid *Cecropia*, had up to 26 beetles per petiole in this study. Bark beetle reproductive fitness may be greatly increased in the hybrid phenotypes due to reduced inter-specific competition (Jordal and Kirkendall 1998). Additionally, bark beetles are colonizing organisms and regularly inbreed. Therefore, they are not affected by inbreeding depression that may affect their reproductive fitness (Beaver et al. 2001).

Even though there was a difference in abundance between the hybrid and *C. polyphlebia*, there was not a significant difference in diversity. Most beetle species may be able to colonize both of the *Cecropia* species, but beetles may be able more successful in the hybrid species of *Cecropia*. This would lead to higher abundance of beetles in the hybrid *Cecropia*. Also, altitude may play a role in affecting abundance of bark beetles but may not prohibit species from traveling to different altitudes. Studies done by Wilkinson (2002) and Strauss (2007) both found that all species were found at both of the sites separated by altitude. Further studies should be completed to specifically observe the altitudinal effects on bark beetles.

Interestingly enough, Morphospecies 6 was found only in the Monteverde Cloud Forest. Although this is contradictory to previous studies, this may be due to a variation in sample sizes and the time in which the study took place. Furthermore, a high number of petioles studied were filled with larvae, and there were many more beetles per petiole. Collection of petioles may have occurred during a community turnover time, where many bark beetles were emerging for their migratory phase to locate new hosts for breeding material (Berryman 1983).

In addition, differences in the species of *Cecropia* with varying petiole lengths affected the abundance of bark beetles. Therefore, bark beetles may prefer specific habitats and petiole lengths. Overall, as petiole length increased in each species of *Cecropia*, the abundance of beetles increased as well. Larger petioles would have less intense competition for similar resources. Also spatial partitioning would be more successful in longer petioles. Jordal and Kirkendall (1998) found that many bark beetles were found in a specific tissue of the *Cecropia* petioles. For instance, *S.maurus* colonized the base of the petiole, but *S. acares* and *S. cecropiavorus* colonized the fibrous tissue. With longer petioles, there are more resources to share thus allowing a greater abundance of beetles to be supported.

As petiole moisture increases, abundance of beetles increases. Bark beetles may prefer petioles that are in the shade and moist. Therefore, bark beetles favor certain tree sites. This coincides with the study done by Jordal and Kirkendall (1998). They found moist petioles in shaded forest sites had ten or more egg tunnels per petiole in comparison to dry, sun exposed sites, which were characterized by *Hypothenemus* species.

In this study, there were several petioles that were not colonized at all. Only 32% of the petioles studied were colonized. In the study done by Jordal and Kirkendall (1998) 83% of their petioles were colonized. The difference in colonization may be counterbalanced by the high amount of bark beetles found per petiole. Bark beetles complete their whole life cycle protected within the petiole (Rudinsky 1962). However, they may leave to search for a new host or reproduce. My results may indicate that collection of data may have been during an inbreeding season, where many are drawn to petioles to reproduce. This can explain why there were many beetles per petiole and why few were colonized.

With the high abundance of bark beetles found in lower elevations, climate change may not be a factor that affects bark beetles. However, *Scolytinae* beetles prefer moist petioles, which means they may have a harder time finding petioles that are not desiccated in the future. However, there was one species found in the higher elevations that was not found in the lower elevations (Morpho 6). This specific species of bark beetle may suffer from climate change if this species is only found in higher elevations. Further investigation may want to study the effects of climate change on bark beetles. For example, the ever-increasing dry days in Monteverde may have significant effects on bark beetle habitats.

As the Earth warms and continues to alter the face of our landscape, it is essential to see the impacts climate change will have on biodiversity. Biodiversity is threatened as global warming changes the very basics of our ecosystems. The carbon-enriched atmosphere has wounded species and ecosystems for millions of years. As biodiversity is rapidly declining due to climate change and anthropogenic factors, this study shows that bark beetles may not be affected by climate change and may even benefit.

ACKNOWLEDGEMENTS

I would like to thank Tania Chavarria Pizarro for advising me throughout this entire research project. I would also like to thank Karen Masters for helping and supporting me throughout the planning process as well as with any follow-up questions I ever had. I would like to thank Pablo and Moncho for helping me with any questions or supplies I needed. I really want to thank Nathan Spaht and Caitlin Loooby for helping me collect data and keeping my sanity. Finally, I want to thank every person on the Summer 2008 program, my trip and life will not be the same without you!

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Table 1. The abundance of each morphospecies found in *C. polyphlebia* and the hybrid

Species	C. *polyphlebia*	Hybrid
Morpho 1	25	106
Morpho 2	6	13
Morpho 3	40	142
Morpho 4	8	19
Morpho 5	3	24
Morpho 6	3	0

Table 2. The abundance (N), evenness (E), Shannon-Weiner Index (H'), Species Richness (S), and S'marg (S'marg) between *C. polyphlebia* and the hybrid (Modified t-test, df=127.85 p=0.1-0.2).

	C. polyphlebia	Hybrid
E	0.76	0.77
N	85.00	304.00
S	6.00	5.00
H'	1.36	1.23
S'marg	1.13	19.21

Table 3. Degrees of Freedom, F Ratio, and Probability > F for Species of *Cecropia*, Moisture content, and Species of *Cecropia* and Length using 2-way ANCOVA test.

Source	DF	F Ratio	Prob > F
Species	1	11.2419	0.001
Moisture	2	4.9336	0.0083
Species and Length	1	5.1569	0.0244

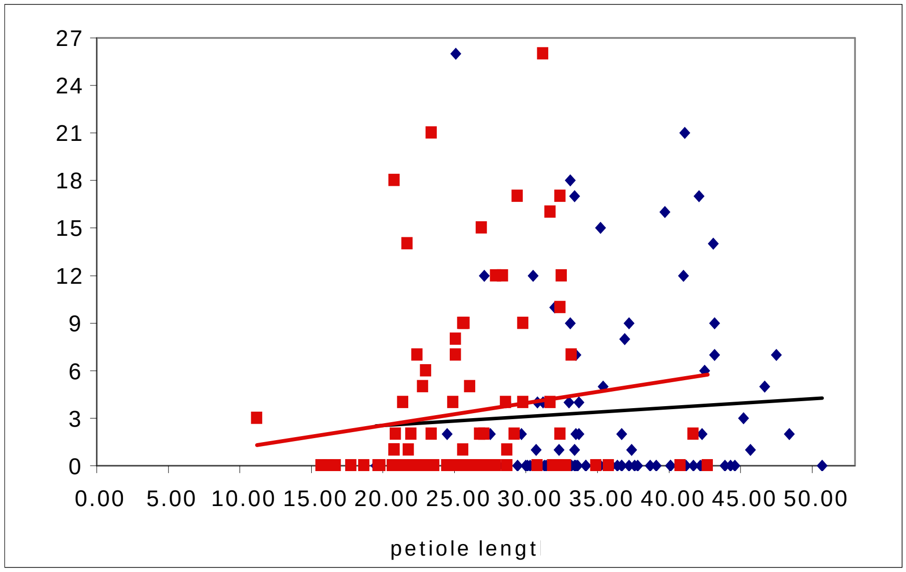

Figure 1. Petiole length (mm) in *C. polyphlebia* (blue) and hybrid (red) in relation to the abundance of beetles (ANCOVA, F = 5.16, df = 1, p = 0.0244)

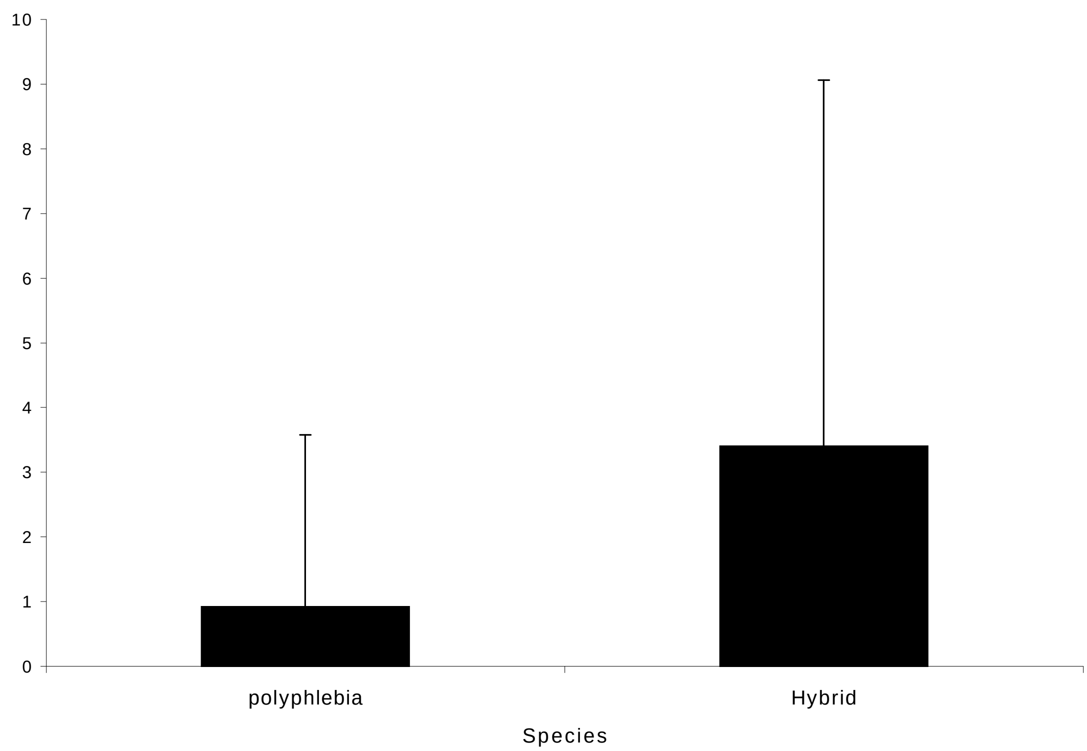

Figure 2. The abundance of beetles in *C. polyphlebia* and the hybrid *Cecropia* (ANCOVA, F = 11.2, df = 1, p = 0.001)

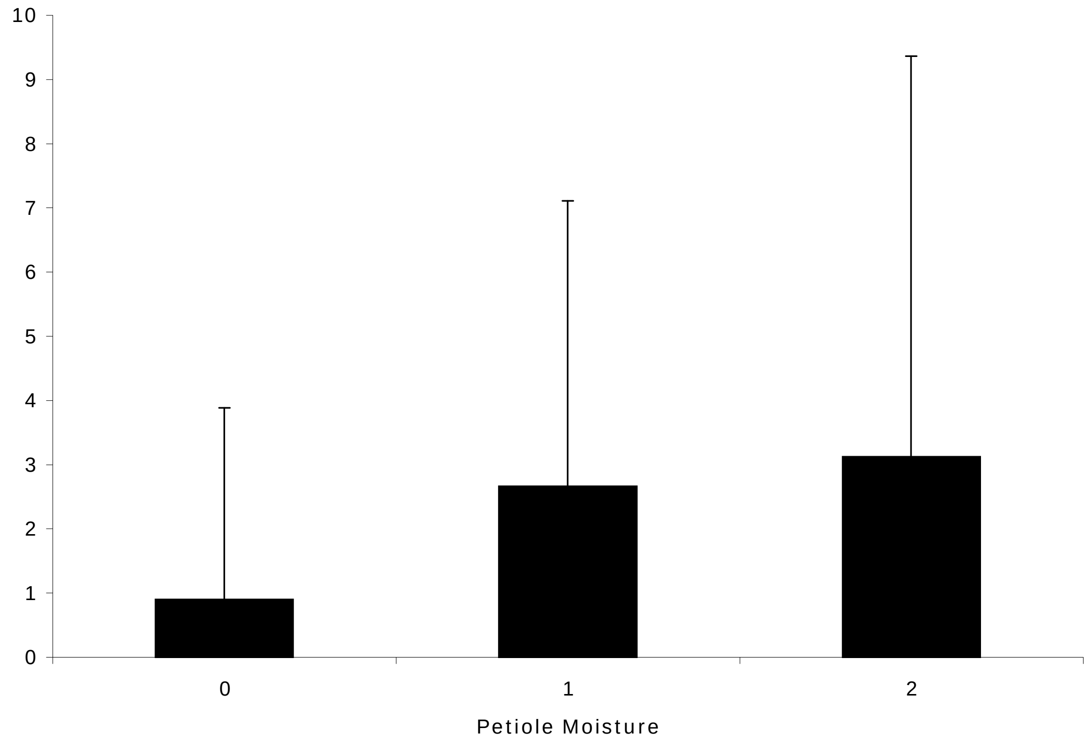

Figure 3. The abundance of beetles versus moisture content in *Cecropia* (ANCOVA, F = 4.93, df = 2, p = 0.0083)