

Distribution of *Azteca* spp. associated with *Cecropia obtusifolia*

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Abstract

Many plants exhibit sex-biased reproductive investment, in which females invest more energy into reproduction than males. This theory is easily studied in *Cecropia obtusifolia*, a common dioecious tree at La Selva. *Cecropia obtusifolia* has a mutual relationship with *Azteca* ants; the tree provides nesting sites and food bodies for the ants, and the ants provide the tree protection from herbivores and epiphytic growth. The *Azteca* species at La Selva can be categorized into aggressive and timid species based on previous studies. Because female *Cecropia* are investing much more energy in reproduction, the females should also invest in more aggressive ant species to protect their expensive reproductive structures. We tested this by sampling and identifying ant species from 27 male and 31 female *Cecropia obtusifolia*. Contrary to our predictions, we found that there was no significant relationship between the category of *Azteca* present on the tree and the tree's sex. A possible cause for this pattern is a difference in resource allocation between males and females during reproduction. Females may not be able to provide the proper rewards required for the more aggressive ant species to inhabit them. Tree location did have a mildly significant effect on the category of ants present, and we propose that habitat type and disturbance level may play a role in determining the distribution of ant species on *Cecropia*.

Introduction

Sex-biased reproductive investment exists in most organisms and females tend to invest more energy in reproduction than males (Lloyd and Webb 1977). Since so much energy is devoted to reproduction, many organisms have developed mechanisms to defend themselves against predation (Cornelissen and Stiling 2005). These mechanisms

may involve secondary compounds, structural defenses, escape methods, or mutualistic relationships (Coley and Barone 1996).

In plants, herbivory decreases overall fitness, thus affecting sexual reproduction (Cornelissen and Stiling 2005). In dioecious plants, females may be inclined to invest more in defense methods to protect their reproductive structures (Lloyd and Webb 1977). However, reproductive investment in females could also cause a decline in investment in defenses, causing males of the same species to be relatively better defended against herbivores (Cornelissen and Stiling 2005). In this case males may grow faster, have less chemical defenses, and have a higher nutritional quality than their counterpart (Cornelissen and Stiling 2005).

Cecropia obtusifolia, an easily recognizable, abundant tropical tree, is well-suited to test sex-biased reproductive investment since it is dioecious and is involved in an ant-plant mutualism with *Azteca* (Longino 1989). There are three different species of *Azteca* ants found in the mutualism with *C. obtusifolia* in Costa Rica (Longino 2009). Each of these ant species has been rated on an aggression scale based on how well they protect the *Cecropia* from herbivores (Longino 2009). We expect that female *C. obtusifolia* will have associations with the more aggressive species of *Azteca* ants, since they need to protect their large reproductive investments more so than males do.

Methods

Our study was conducted from 22 –23 April 2009 at the La Selva Biological Station in Heredia Province, Costa Rica (10° 26' N, 83° 59' W). La Selva is classified as lowland tropical wet forest (*sensu* Holdrige 1947) and receives approximately 4000 mm of rain annually (Roberts 1994).

Trees in the *Cecropia* genus are uniquely adapted to harbor ant mutualists for three reasons: one, they provide highly nutritious Mullerian bodies at a modified petiole base; two, their stems are hollow between nodes, providing safe homes for ants; three, unusually thin spots exist in the wall of every internode allowing the ants an easy entrance and exit from the internode (Skutch 1945).

The *Azteca* genus of ants is a group of aggressive, arboreal species, most of which rely on plant species with hollow stems or nodes that are used as nests (Carroll 1983). They are easily recognized by their distinctive odor, and occur only in the Neotropics.

We sampled 58 adult trees, 27 of which were male and 31 of which were female, from a road and a path inside the biological station. The road location was more open and disturbed than the path location. Only flowering *C. obtusifolia* were sampled because the sexes were easily identifiable. Tree height was calculated using a meter tape and Suunto® clinometers. We attracted ants from each tree using canned Splash® tuna as bait, and if the tuna did not succeed in attracting the ants, the tree was shaken and hit vigorously. Only ants that came from the top of the tree were collected in order to avoid intruder ants from the surrounding area.

We performed ant species identification using an *Azteca* dichotomous key (Longino 2009). We grouped *Azteca constructor* and *Azteca xanthochroa* together as “aggressive” *Azteca* species and *Azteca alfari* and *Azteca coeruleipennis* together as “timid” ant species (Longino 1991). For statistical analysis we assumed that the trees at which we could not collect ants were inhabited by “timid” ants, and so pooled those trees into our “timid” ant category.

To determine the effects of tree sex, height, and location on the aggression level of the inhabiting *Azteca* species we first conducted a nominal logistic multiple regression with ant category as the response variable and tree sex, height, and location as the explanatory variables. We determined if tree sex or location individually affected ant category and if tree location affected sex using Fisher’s exact tests between each pair of variables. We determined if tree height affected ant category using a nominal logistic simple regression. All statistical analyses were conducted using JMP v. 5.1.2 software (SAS Institute 2004).

Results

Of the 33 trees on which we were able to collect *Azteca* species, 28 had *A. xanthochroa*, three had *A. constructor*, one had *A. alfari*, and one had a non-specialist *Azteca* species. We were not able to collect specimens of *Azteca* species on 25 of the sampled trees. We measured height for 44 of the sampled trees.

The whole model produced by the nominal logistic multiple regression was significant ($n = 44$, $DF = 3$, $\chi^2 = 8.89$, $R^2 = 0.15$, $p = 0.031$), although none of the individual effects of tree height, sex, or location had a significant effect on ant category (respectively, $DF = 1$, $\chi^2 = 1.46$, $p = 0.23$; $DF = 1$, $\chi^2 = 2.53$, $p = 0.11$; $DF = 1$, $\chi^2 = 2.48$, $p = 0.12$). Tree sex did not significantly affect ant category (Figure 1; $p = 0.12$) or tree location ($p = 0.43$). The relationship between ant category and tree location was marginally significant (Figure 2; $p = 0.062$), with more aggressive ant species being more common on the path. Tree height did not significantly affect ant category ($n = 44$, $DF = 1$, $\chi^2 = 3.00$, $R^2 = 0.049$, $p = 0.083$).

Discussion

Since female individuals of dioecious species typically allocate more resources to their reproductive strategies than males do (Cornelissen and Stiling 2005), it is logical that they would try to protect these investments to the best of their ability without compromising the integrity and vitality of their reproductive structures. Sex-biased defense allocation has been seen in numerous dioecious species and females have often been found to experience lower levels of herbivory than males due to secondary compound production and other methods of protection (e.g. Jing and Coley 1990, Elmqvist et al. 1991). We had proposed that since *Cecropia* were in a mutualistic relationship with *Azteca* and since there were different species of *Azteca* with differing aggression levels, the female *Cecropia* would allocate resources to attract the more aggressive *Azteca* in order to protect their reproductive structures.

Contrary to our hypothesis that female *C. obtusifolia* would have more aggressive ant species in order to protect their sexual investments, there was no significant relationship between the category of *Azteca* present on the tree and the tree's sex. It could be that since the females allocate more energy to reproduction than the males do, they have less energy left over from a finite source to attract the ants that would be more beneficial to them. These results call into question the accuracy of the assumption that typical female resource allocation strategies are valid in *Cecropia*, or at least in *C. obtusifolia*. In both sexes of *C. obtusifolia*, large inflorescences are produced; however, only the female trees are responsible for devoting resources to producing and maintaining

fruit. However, the presence of aggressive ants on these fruits could deter bird dispersers, so a lack of aggressive ants could be a strategy of female plants for effective seed dispersal. This situation is similar to what is commonly seen in the bird-dispersed *Acacia* (Janzen, 1966).

When tree sex, height, and location were put into a nominal logistic multiple regression model, the whole model was significant. However, none of the individual effects were significant, so the only conclusion we can draw is that, together, these three factors somehow explain the distribution pattern of ant species in *C. obtusifolia*. Individually, tree location had a mildly significant effect on the category of ants present, while tree height had no effect. Previous studies have shown that location may affect the ant species present in *Cecropia* individuals (Longino 1989, Yu and Davidson 1997), and we propose that habitat type and disturbance level may play a role in determining the distribution of ant species on *Cecropia*.

According to previous studies, repeated, vigorous shaking and tapping could not bring *A. alfari* and *A. coeruleipennis* out of the canopy (e.g. Longino 1991). For this reason, we chose to categorize the trees at which we could not collect any ants as having “timid” ants. Since *Cecropia* have not always been found to be inhabited by *Azteca* (Janzen 1973), our results may be strongly influenced by our assumption of “timid” ant presence at these trees. However, it is difficult to hypothesize the direction of the influence of the unknown number of trees that could be uninhabited.

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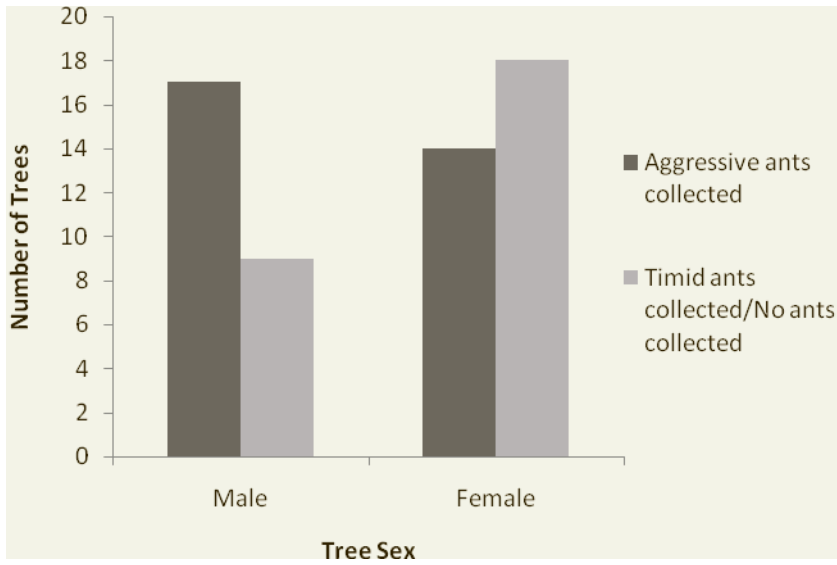


Figure 1. The number of *Cecropia obtusifolia* trees at which we collected aggressive *Azteca* species compared with the number at which we collected timid *Azteca* species or did not collect *Azteca* species, within male and female individuals, at La Selva Biological Station, Costa Rica.

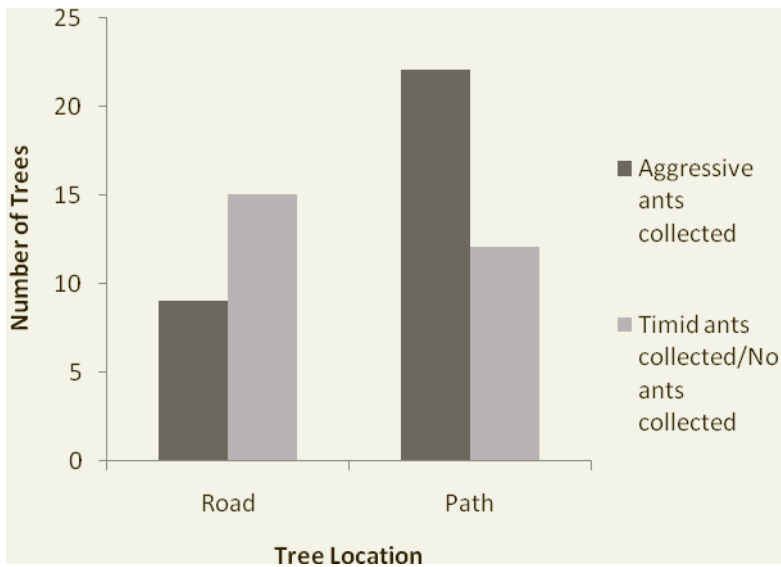


Figure 2. The number of *Cecropia obtusifolia* trees at which we collected aggressive *Azteca* species compared with the number at which we collected timid *Azteca* species or

did not collect *Azteca* species, within individuals located on a disturbed site along a road or a more densely forested site along a path, at La Selva Biological Station, Costa Rica.