THE COLONY ARCHITECTURE OF *PSEUDOMYRMEX SPINICOLA* ON THE ANT-PLANT *ACACIA COLLINSII*

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Abstract: The nesting space limitation hypothesis suggests that ant nest size may be an important factor in determining colony size and fitness. Nest size may be more important than food availability and territory size. We investigated the spatial layout of a *Pseudomyrmex spinicola* (Formicidae) colony living in *Acacia collinsii* (Fabaceae). Based on the nesting space limitation hypothesis, we predicted that the number of ant eggs and adults would be positively correlated with acacia thorn size (the domiciles of acacia ants), and thorns with more eggs would be more heavily defended. We also predicted the queen would reside in the largest thorn for optimal protection and productivity. Lastly, we studied thorn characteristics and distribution on a single acacia tree. We found a significant positive correlation between thorn size and both ant and larvae densities within thorns. The queen was located in the largest thorn measured. In addition, yellow mature thorns contained significantly more ants than either immature red or desiccated grey thorns. Our data suggest systematic thorn utilization by ants, supporting the application of space-limitation theory to the ant-acacia system.

Key Words: mutualism, nesting space limitation hypothesis, thorn capacity

INTRODUCTION

The nesting space limitation hypothesis in myrmecophytes (plants which provide a habitat for ants) predicts that nest size is an important variable in ant fitness (Fonseca 1999). Population growth of *Pseudomyrmex* colonies has been shown to be more limited by nest size than either food supply or territory size (Fonseca 1993). In addition, myrmecophyte space limitation can negatively impact the ability of the ant colony to care for their young (Fonseca 1999).

We investigated the concept that space limitation drives ant colony development. *Acacia collinsii* is known to be a myrmecophyte for several different ant species, including our focal species, *Pseudomyrmex spinicola*. In this mutualistic relationship, the ants provide the acacia with protection from herbivores and encroaching vegetation, while the acacia provides the ants with both a habitat and nutrients, in the form of nectar and Beltian bodies. While many studies address ant-acacia mutualisms (e.g. Raine et al. 2002; Evans et al. 2004), few studies have described the spatial organization of the colony on the tree (Janzen 1983). Because of potential space limitation, ant colony architecture could be an important variable in understanding the ant-

acacia mutualism and is investigated here.

We examined three main hypotheses: (1) a *P. spinicola* colony living on *A. collinsii* would be space limited, (2) queen survival is a crucial factor in colony fitness, and (3) thorn defense will vary according to the number of eggs within the thorn. From these hypotheses, we predicted: (1) the number of eggs and number of adult ants in a thorn would be positively correlated with thorn size, (2) the queen would be found in the largest thorn, and (3) a thorn with a higher density of eggs would be more heavily defended by adult ants. Lastly, we explored the natural history of the acacia and describe the layout of occupied thorns on a single focal acacia tree.

METHODS

We focused our study on one acacia tree colonized by *P. spinicola* with a range of thorn sizes. Our study took place in Palo Verde National Park in the Guanacaste province of Costa Rica, about 12 m from the access road. Over the course of two days (12-13 January, 2007), we haphazardly sampled 31 thorns of various sizes, colors, and locations on the tree. For each thorn, we noted its color (see description below) and measured its height from the base of the tree, distance along the branch (if applicable), and thorn length (all in mm). Thorn length was measured as the distance between the two thorn tips. Before cutting the thorn for adult and egg dissection, we observed ant movement into and out of the thorn for one minute as a measure of baseline ant defense of their larvae within each thorn.

We noted three distinct colors of thorns: yellow, red, and grey. Yellow thorns were fibrous and hollowed, often having leaf petioles with nectaries and Beltian bodies protruding from their apexes. Red thorns were commonly at the ends of branches consisting of newly formed plant tissue, often not yet hollowed by ants. Grey thorns were frail and appeared to have been cleared of their interior tissue long ago; they also had lost any previous protruding leaves or resources (such as nearby nectaries or Beltian bodies).

We then separated the thorn from the acacia and cut it open, recording the number of workers, nurse ants, queens, winged adults, larvae, and pupae inside. Workers were classified as those ants originally on the thorn body when it was cut, as well as those who came out of the opened thorn, presumably to defend it. Nurses were defined as those ants that stayed within the thorn even when it was cut open, guarding the larvae and often retreating to the corners of the thorns. The number of premature ants within each thorn was recorded and categorized into larvae, white

pupae, and orange pupae. Orange pupae are closer to the pigmentation of mature ants and closely resemble nurse ants, indicating they are closer to maturation than white pupae. After counting these larvae and pupae, we returned them by placing them at the base of the tree, where workers quickly collected them. We also recorded any queens and female winged adults ("virgin queens").

As a complement to these data, we randomly selected an additional 50 thorns and measured their length, as described above. We measured these additional thorns to map the placement of a wide variety of thorns along the trunk, primary branches, and secondary branches.

Statistical analyses. Along with mapping thorn locations, we used a variety of statistical analyses to test our initial predictions. First, we analyzed thorn length distribution for normality to help us understand the spaces available for use by ants and possible upper limits on available space. Second, a t-test assuming unequal variances was used to compare the lengths of thorns with and without premature ants. Third, we used pairwise correlations to look at the relationships between the number of mature ants in thorns, the number of premature ants in thorns, and the thorn length. We limited this analysis to thorns with larvae and pupae present because we wanted to focus on thorns with existing nurseries. Fourth, we used a one-way ANOVA to compare the number of premature ants between different colored thorns and a Tukey-Kramer Highly Significant Difference (HSD) test at $\alpha = 0.05$ to assess which thorn color(s) harbored the most premature ants.

To determine if there was a relationship between thorn size and thorn location on the tree, we performed a linear correlation of height of thorn from tree base and thorn length. We also used a one-way ANOVA and Tukey-Kramer HSD test (as above) to compare the height of differently colored thorns from the tree base.

RESULTS

The tree had a normal distribution of thorn lengths (mean ± SD = 47.30 ± 17.86 mm; interquartile range = 21.80 mm). All thorn length size classes had some proportion of thorns with larvae and pupae except the lower 20% of size classes, where there were no thorns with premature ants present. We found that the lengths of thorns with larvae and pupae were 59.5% longer than those without ($t_{15.10} = 3.61$ $P = 0.0013$; Figure 1).

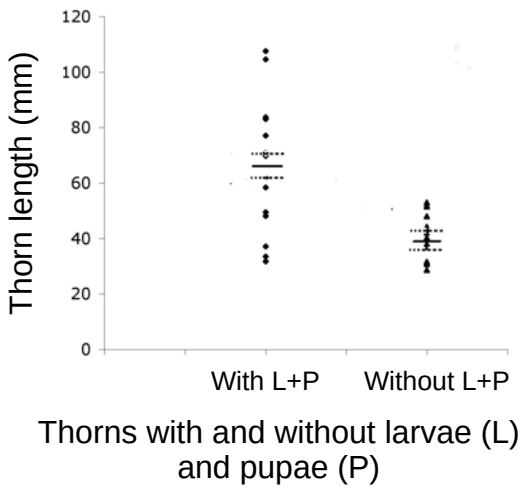

Figure 1. The mean length of thorns containing larvae (L) and pupae (P) was significantly higher than those without. Solid lines represent the mean, while dashed lines represent standard error. Sample size for thorns with larvae and pupae was N=14 thorns and for thorns without was N=16 thorns.

In thorns with premature ants present, we found that thorn length was positively correlated with both the number of premature ants ($r = 0.66$, $n = 13$, $P = 0.01$) and the number of mature ants ($r = 0.57$, $n = 13$, $P < 0.0001$) in the thorn. Furthermore, the number of mature ants was strongly positively correlated with the number of premature ants in the thorn ($r = 0.92$, $df = 13$, $P=0.0001$).

The number of premature ants in yellow thorns was 1200% greater than the number of premature ants in red and grey thorns ($F_{2,28}= 5.31$, $P = 0.01$; Figure 2).

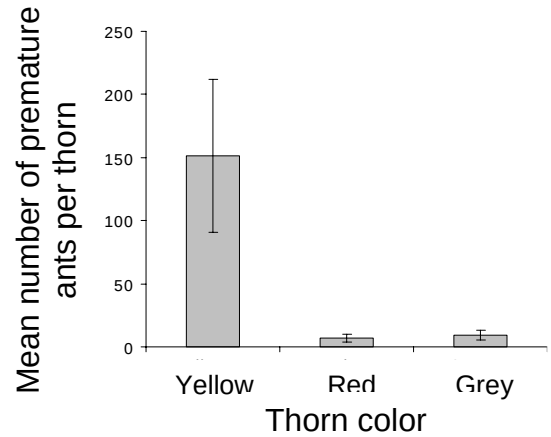

Figure 2. The mean number of premature ants (larvae and pupae) in yellow thorns was approximately 1200% higher than in either red or grey thorns. Sample size N = 16 for yellow thorns, N=5 for red thorns, and N=10 for grey thorns. Error bars represent ± SE.

We found a significant positive correlation between thorn length and distance up the tree ($r = 0.57$, $n = 54$, $P = 0.004$; Figure 3). Yellow thorns (mean ± SE = 2480 ± 230 mm) were 60% higher above ground than red thorns (mean ± SE = 1540 ± 340 mm) and 89% higher than grey thorns (mean ± SE = 1540 ± 120 mm; $F_{2,51} = 9.07$, $P = 0.0004$).

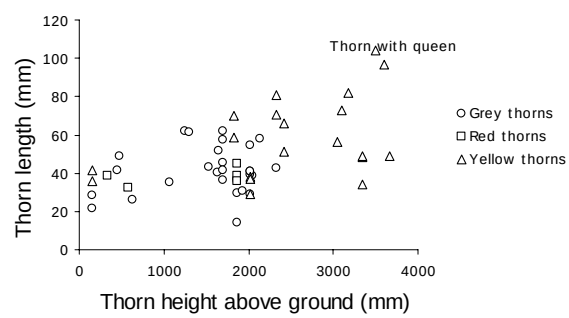

Figure 3. There was a positive correlation between thorn length (across all thorn colors) and thorn height above ground. Yellow thorns were found higher than red or grey thorns. Sample size was N = 54 thorns.

DISCUSSION

Spatial Limitation

By investigating the colony architecture of *P. spinicola* in a single *A. collinsii* tree, we supported our hypothesis that a myrmecophyte-inhabiting ant colony would use the largest thorns to house larger worker groups, larger nurseries, and the chambers of the queen and winged virgin queens. These data support the nesting space limitation hypothesis, which suggests the smallest thorns are not sufficient for daily social tasks, including the nurturing of offspring (Fonseca 1999). Nursery thorns require space for both larvae and nursing adults. Smaller thorns may require more growth before obtaining the ability to host these activities.

Because the largest thorns are generally on the trunk, size of thorn and location of the thorn on the tree are inherently confounded. Trunk sites may be more defendable and may be less susceptible to predation or disturbance. An experimental study could transplant all large thorns to other sites on the tree, including to secondary branches, in order to see if the ants continue to use the largest thorns as nurseries.

The grey thorns may be the exception to the use of the largest thorns for nurseries. The casing of grey thorns no longer contained pulp, which may increase the chance that larvae inside would dry out or be exposed to high temperature variation. Because larvae are temperature sensitive, these thorns may not be fit to support nurseries.

The colony architecture we report suggests that the larger, yellow thorns are essential in supporting *P. spinicola* fecundity and colony size, increasing the space for the carrying and nurturing capacity of the colony. Further investigation is required to examine techniques that ants may use to accelerate thorn growth.

Colony Architecture and Natural History

We profiled a single *A. collinsii* tree in order to map its reproductive centers for ants and chamber types in detail. Although focusing on one tree limits our inference space, detailed study of this tree allowed us to create a functional blueprint of its tree-dwelling ant colony. Further study may investigate the patterns of colony blueprints among many *A. collinsii* that host *P. spinicola*.

The tree contained a relatively normal distribution of thorn lengths, including red, yellow, and grey thorns. The red thorns were newly formed and may have still been full of pulp if the ants had not yet excavated them. The yellow thorns were the largest and most frequently used for reproductive centers. The grey thorns were withered and no longer had vascular tissue. A new

thorn could be used for the nursing of larvae and pupae of *P. spinicola* once they had reached approximately 30 mm in length. Among thorns with premature ants, thorns with more larvae and pupae contained increasingly more protective workers. The nurseries containing higher densities of larvae and pupae were more important to the fitness of the ant colony. Therefore, the ants' defensive resources were allocated in higher numbers to these nurseries.

The queen inhabited the largest thorn (107.7 mm) along with 54 nurse ants whose responsibility was to care for new larvae in their earliest stages. The queen's chamber contained 720 larvae and was also the absolute maximum of protective worker density. The queen's high production of larvae is likely limited by the space of her chamber, so workers likely created secondary reproductive centers in other large yellow thorns to care for and nurture the larvae. The redistribution of offspring into multiple thorns also decreased the concentration of offspring in one area, likely minimizing losses if one area was destroyed by biotic (e.g., herbivory or predation) or abiotic (e.g., storms) factors.

The youngest molted pupae in the secondary reproductive chambers remained in the thorn and became nurse ants before taking on other responsibilities as workers (Isbey et al., *personal observation*). In some of the nurseries, winged virgin queens were found, and they were always the last individuals we removed from the thorn because they huddled in the deepest thorn crevices. These winged virgin queens will later attempt to found a new colony (Janzen 1983). The cycle begins when a queen mates and then drops to the ground, leaving her old colony. After removing her wings, she begins to search for an acacia. When she finds a suitable tree, she hollows out a red, pulpy thorn and lays her first brood, which she must defend from predators and other colonizing queens until the birth of her population (Janzen 1983).

This study mapped a single *P. spinicola* colony in the thorns of *A. collinsii*. However, little is known about the development of this colony architecture through time. Future studies that establish *P. spinicola* colonies in a mimic host, using transparent panes to observe ant behavior and more fully map the use of thorn space, will provide additional ecological insights. The colonization of a mimic could be repeated using various distributions of thorn size and shape.

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