POSITIVE FEEDBACK BETWEEN *ACACIA COLLINSII* AND *PSEUDOMYRMEX SPINICOLA* COLONIES

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Abstract: Mutualisms play prominent roles in tropical ecosystems. However, the maintenance of obligate mutualisms is still poorly understood. In this study, we looked for signs of positive feedback between two common mutualists in a tropical dry forest in Costa Rica: *Acacia collinsii* trees and *Pseudomyrmex spinicola* ant colonies. To examine the mutual benefits experienced by plants and ants, we assessed the leafiness of each plant as well as the size of the associated ant colony. We found a weak positive correlation between plant productivity and ant colony size, but no correlation between plant productivity and ant colony response to simulated herbivory. Finally, we observed an eight-fold greater recruitment of *P. spinicola* to the trunk of the plant as compared to the auxiliary branches in response to disturbance.

Key Words: mutualism, neotropic, Costa Rica, Palo Verde, dry forest

INTRODUCTION

Mutualisms are reciprocally beneficial inter-specific interactions that are ubiquitous in nature, especially in tropical environments (Bronstein, 1998). The evolution and ecology of mutualistic interactions between ants and plants has been particularly well studied because ants are both very common and prone to entering mutualisms (Bronstein, 1998). A common ant-plant mutualism between the tree *Acacia collinsii* (Fabaceae) and the ant species *Pseudomyrmex spinicola* (subfamily Pseudomyrmecinae) occurs in the Costa Rican tropical dry forest (Janzen, 1966). While the costs and benefits of this obligate mutualism are well studied, the mechanisms through which the interaction evolved and how and why it continues to flourish have not been well-studied (Bronstein, 1998). Although it is often assumed that the evolution and persistence of mutualisms involve a positive feedback cycle, most studies focus solely on the costs and benefits of the interaction for one partner in the relationship (Bronstein, 1994). In this study, we tested whether there was evidence of positive feedback in the obligate mutualism between *A. collinsii* trees and *P. spinicola* colonies.

Acacia collinsii provides ants with shelter in the form of hollowed thorns and food in the form of extrafloral nectaries and lipid-rich Beltian bodies (Janzen, 1966). In

return, *P. spinicola* protects *A. collinsii* by attacking herbivores and clearing away competing vegetation (Janzen, 1966). *Acacia* species without thorns must invest a greater amount of metabolic energy in secondary compounds (Zuchowski, 2005). Ant mutualisms allow the *Acacia* to invest in Beltian bodies and nectaries as indirect methods of defense, rather than secondary compounds, some of which may be more metabolically costly (Zuchowski, 2005). Thus, the mutualism between ants and acacias may result in a positive feedback cycle: well-protected trees may grow larger with more leaves (Janzen, 1966) and therefore more resources to provide food and shelter for ants, subsequently increasing ant colony size. Additionally, in dry climates, acacias drop their large leaves and only produce small young leaves containing Beltian bodies to feed their obligate ants (Janzen, 1966). The number of small young leaves produced is therefore a representation of the energy investment the acacias put toward maintaining their ant colony with extra lipids.

We examined positive-feedback patterns in the mutualism between *A. collinsii* and *P. spinicola* by evaluating the relationships between tree leafiness, ant colony size, and ant defensive behavior. We hypothesized that a positive feedback between ants and acacias would result in positive correlations between tree leafiness and ant colony size. In addition, ants might more aggressively defend trees that are leafier, and thus more valuable to the ants (Burnaford et al., 1992). Thus, we also considered the hypothesis that trees might be leafier if ants were simply more aggressive, and not necessarily greater in number.

Specifically, we predicted that (1) leafier trees would have larger ant colonies and (2) ant colonies on leafier trees would defend their trees more aggressively. Finally, during our field observations, we noticed that ants had a tendency to respond in greater numbers to the trunk of the tree than the branches of the tree during simulated herbivory. We tested this pattern by comparing ant behavioral response to simulated herbivory on tree trunks and auxiliary branches.

METHODS

We conducted our study at Palo Verde National Park, located in the Guanacaste province of northwestern Costa Rica. This 20,000-hectare park is a seasonally dry tropical forest dotted with limestone cliffs, bordered by wetland vegetation along the Tempisque River.

We haphazardly selected 38 *A. collinsii* trees that were colonized by *P. spinicola,* located 1-10 m from

either side of the dirt road leading to the Palo Verde field station. We counted the number of leaves on one 30 cm section of branch per tree (hereafter referred to as tree leafiness) as an estimate of a tree's potential energy available for maintaining an ant colony. We counted the initial number of ants on the branch segment and then simulated herbivory by tapping the branch segment for 10 sec. After tapping the branch, ants entered the branch from the thorns. We counted the total number of ants on the 30 cm branch segment after simulated herbivory as a measure of the total ant colony size. The final number of ants on the 30 cm branch segment reflects the initial number of ants on the branch, as well as those in the thorns. We measured the ant behavioral response as the change in ant numbers 10 sec after the simulated herbivory. To justify using a single branch measurement to represent the entire tree, we tested the variation in ant response between two auxiliary branches on the same tree. On 10 trees, we chose two auxiliary branches from the same section of the tree and sampled ant colony size and response to simultaneous disturbance simulations, as described above. For all trees, we recorded the time of day that the simulated herbivory was conducted.

To test if ant response was greater on the trunk of the tree than the auxiliary branches, we haphazardly sampled 10 trees that were 1-2 m tall. We chose a 30 cm section on the main trunk of each tree and a 30 cm section on an auxiliary branch above the trunk segment and measured ant colony size and response to simultaneous disturbance simulations, as described above.

We tested the relationships between acacia leafiness and our estimates of ant colony size and ant response to simulated herbivory with correlation analyses. We also tested the correlation between time of day and ambient ant numbers to see if the response of ants varied over the course of our data collection period (0800 – 1100). We performed a two-tailed, paired t-test to determine if there was a difference in ant response to tapping between two branches within a tree. We performed a one-tailed, paired t-test to test our *a priori* hypothesis that ant response would be greater on the trunk than on an auxiliary branch.

RESULTS

The number of ants present after tapping was weakly positively correlated with acacia leafiness (leaves per 30 cm of branch; $r = 0.30$, $n = 37$, $P = 0.07$; Fig. 1). However, there was no relationship between leafiness and number of ants responding to the branch after tapping ($r = 0.02$, $n = 37$, $P = 0.90$; Fig.

2). Thus, ant colonies in leafier trees were larger, but did not respond to simulated herbivory more aggressively.

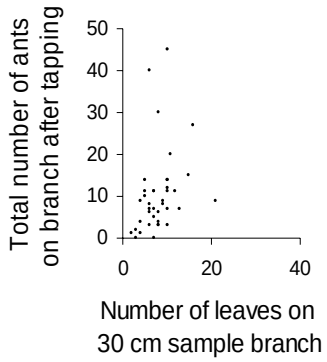

Figure 1. Correlation between the number of *Acacia* leaves on a 30 cm sample branch and number of ants on the branch after tapping to simulate herbivory (n = 38 trees). Each point represents a different tree.

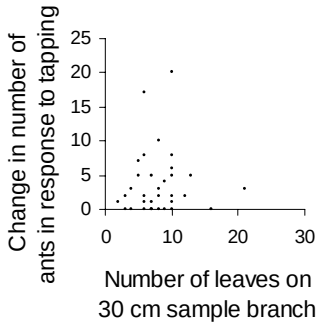

Figure 2. The density of *Acacia collinsii* leaves (number of leaves on 30 cm sample branch) and change in number of *Pseudomyrmex spinicola* ants on a branch in response to tapping. Each point represents a tree (n = 38 trees).

There was no difference between ant response to the tapping on two branches within a tree (paired-t_9 = -0.37, P = 0.72), suggesting that one branch was a representative sample of the entire tree. However, there was a strong positive correlation between the initial number of ants and the time of day; initial ant activity increased as the morning progressed (r = 0.58, n = 37, P < 0.01). Finally, the mean number of ants responding to tapping on the trunk was eight times greater than the mean number of ants responding to the auxiliary branch (paired-t_9, = 5.86, P < 0.01; Fig. 3).

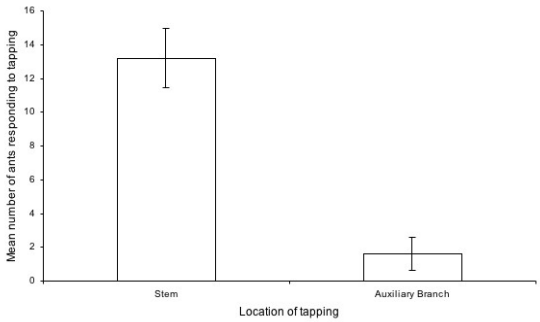

Figure 3: Mean number of *Pseudomyrmex spinicola* ants responding to tapping on the trunk (n = 10 trees) and on the auxiliary branch of *Acacia collinsii* (n = 10 trees). Error bars represent ± 1 SE.

DISCUSSION

In our study of the potential positive feedback between *A. collinsii* and *P. spinicola*, we found a positive correlation between acacia leafiness and ant colony size. Our first hypothesis, that *P. spinicola* colony size would be positively correlated with leafier trees, was supported; leafier trees had more ants. Although the leaf-ant relationship was weak, it corroborates the hypothesis that there may be positive feedback between *P.*

spinicola colony size and *A. collinsii* leafiness. To further elucidate the observed correlation, future studies should attempt to separately discern the effect of leafier *A. collinsii* on *P. spinicola* colony size and the effect of *P. spinicola* colony size on *A. collinsii* leafiness. If it could be demonstrated that leafier trees cause bigger ant colonies, and that bigger ant colonies cause bigger trees, the degree of this positive feedback cycle could be quantified.

Our second hypothesis, that the *P. spinicola* colonies would defend leafier trees more aggressively, was not supported; there was no relationship between the number of ants after a disturbance and the number of leaves, both on the sample branch. However, we may have failed to detect a response because we only sampled auxiliary branches, and our third experiment demonstrated that ants responded mainly towards the main trunk of the plant. In other words, we may have failed to detect a correlation between tree leafiness and ant aggressiveness, not because no such correlation exists, but because the trunk effect overwhelmed our measurements at the auxiliary branch.

The ants may more actively defend the trunk because that may be where the reproductive future of the colony is located. After cutting open three large thorns from the trunk of three separate *A. collinsii* trees, we found large quantities of *P. spinicola* eggs, larvae, pupae, and winged adults. Quantifying the correlation between the location of the juvenile *P. spinicola* and the location of the *P. spinicola* response to simulated herbivory may therefore be a fertile ground for future research. A subsequent study found that the large, central thorns of the tree contained the ant eggs, larvae, pupae and the queen of the colony, while the smaller thorns on auxiliary branches did not, further supporting our hypothesis that ant response will be greatest to the trunk of *A. collinsii* (Isbey et al. SIFP1 2007).

Our study supports the hypothesis that the ant-acacia mutualism exhibits positive feedback. This evidence for positive feedback between ants and acacias may help explain how mutualistic interactions are maintained. If mutualistic partners are tightly linked through positive feedback, then declines in one partner could result in two outcomes. First, the beneficial partner may be able to help a declining partner regain strength. Alternatively, if the health of the second partner is closely linked to the first, the second might weaken as well, leading to a more rapid decline of the entire mutualistic relationship. Future studies should experimentally test the strength of the mutual benefits between *A. collinsii* and *P. spinicola* to determine if the positive correlative

patterns we observed are causal and how quickly partners will respond to changes in each others abundance.

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APPLYING OPTIMAL DEFENSE THEORY TO ANT-ACACIA MUTUALISMS

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Abstract: Optimal defense theory predicts greater resource allocation to the defense of plant tissues that are more valuable to plant fitness. We tested an extension of this theory to indirect mechanisms of plant defense, namely the mutualism between *Acacia collinsii* and three species of its ant colonizers. We observed ant response to simulated herbivory on fruits, flowers, and leaves. We tested acacia defensive allocation by studying which structures were more highly defended by ants. Based on optimal defense theory, we hypothesized that ants would defend fruits and flowers more heavily than leaves due to their reproductive value for plants. Prior to trials simulating herbivory, the baseline (control) conditions had significantly more ants present on undisturbed leaves than on undisturbed flowers. During treatment trials simulating herbivory, there was no significant difference in ant response between leaves and reproductive structures (flowers or fruits). Possible explanations for our results include 1) ants may only respond to direct threats to the fitness of their colony, 2) herbivory was poorly simulated, and 3) optimal defense theory may not apply to indirect defenses. We conclude that while ants mutualistically protect acacias from leaf herbivory, they do not preferentially defend reproductive structures, as optimal defense theory would predict.

Key Words: Acacia collinsii, *plant defense,* Pseudomyrmex spinicola, Pseudomyrmex flavicornus, Crematogaster brevispinosa

INTRODUCTION

Optimal defense theory (ODT) for plants states that a plant will allocate defensive resources to tissues based on the value of the tissue to plant fitness, the probability of damage to that structure, and the ecological and allocation costs of defending the tissue. ODT is the result of studies on secondary compound distribution within and among plants (McKey, 1974) and was elaborated on by studies of plant responses to herbivory (Zangerl and Rutledge, 1996). Because reproductive structures are often more important to plant fitness than leaves, secondary compounds are expected to be found in higher concentrations near and in plant reproductive structures (McKey, 1974).

ODT has been used to understand the distribution of chemical defenses in plants (McKey, 1974). However, some plant species rely on indirect defenses, such as predators of herbivores, as a source of protection against herbivory. For example, *Acacia collinsii* (Fabaceae) relies on ants to deter herbivores (Janzen, 1983). Because the presence of ant mutualists on acacias relieves

some of the defensive burden, plant secondary compound production decreases and plant fitness increases after ant colonization (Janzen, 1966). Here, we extended predictions of ODT to indirect defense mutualisms to ask whether predictions from ODT could be used to understand how plants defend different tissue types. In only a limited number of cases have predictions from ODT been successfully applied to indirect plant defenses, such as ant mutualists (Wackers and Bonifay, 2004).

We examined acacia defensive resource allocations by studying which plant structures ant mutualists defend most strongly. Assuming that acacia fruits and flowers are highly valuable structures to the tree for reproduction, we predicted, under ODT, that ants would defend these structures more strongly than they would leaves.

METHODS

Study system.

Acacia collinsii (hereafter referred to simply as acacias) benefit from a mutualistic relationship when colonized by some ant species. Eight ant species in the Guanacaste Province of Costa Rica are known to enter in this mutualism. Each individual tree is colonized by one species. Acacias provide ants with thorn habitat, nectar, and Beltian bodies in exchange for protection against herbivores and competing plants. We focused on trees colonized by three common ant species, *Pseudomyrmex spinicola*, *P. flavicornus*, and *Crematogaster brevispinosa*. Ant species vary in the anti-herbivore protection they provide to acacias; *Pseudomyrmex spinicola* is the most aggressive at defending the plant (Janzen, 1983).

Field methods.

We collected data midmorning on 8 January, 2007, at Palo Verde National Park in the Guanacaste region of Costa Rica. We haphazardly selected 18 acacia trees with leaves and either pods or flowers. Each tree contained one of the three species of ant mutualist. To estimate plant defense to different tissue types, we quantified ant activity following simulated herbivory to leaves, pods, or flowers.

Before simulating herbivore behavior, we ran control trials for one leaf and one reproductive structure (either a pod or a flower). We observed the structures for two minutes at ten second intervals, noting the number of ants on both structures and on the inter-nodal branch area, also recording the ant species present (Figure 1). The control was used to determine the normal activity levels of ants on each of the three structures.

We simulated herbivore behavior by tapping simultaneously

on one leaf and one reproductive structure located on different branches of the tree for one minute. We observed the ant activity in the area for one additional minute after tapping. Response variables recorded for the treatment were the same as for the control trials.

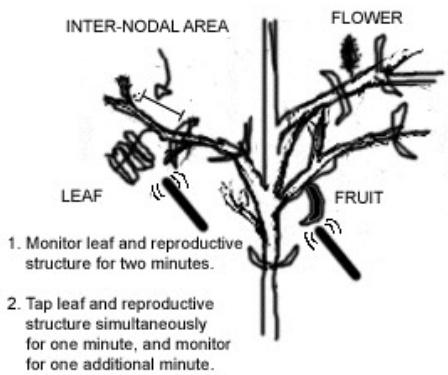

Figure 1. We monitored ant activity on acacia plant structures (flower, fruit, and leaf) across (1) control and (2) treatment methods.

Because observations of the leaves and reproductive structures were paired for each tree, we used paired t-tests to compare the mean (square-root transformed) and maximum number of ants on leaves to their numbers on reproductive structures.

To determine where acacias place incentives for their mutualists, the number of nectaries was counted at the base of haphazardly selected flowers, pods, and leaves on 17 trees. We sampled up to three replicates of each structure present per tree. We calculated the mean number of nectaries for each structure.

RESULTS

In the control treatment, the mean number of ants on leaves was 140% higher than the mean number of ants on flowers ($t_7 = 2.54$, $P = 0.039$, Figure 2). Moreover, the maximum number of ants on leaves was 84% higher than the maximum number of ants on flowers ($t_7 = 5.18$, $P = 0.0013$, Figure 3). However, there was no significant difference between leaves and pods for mean ($t_8 = 1.18$, $P = 0.27$) or maximum number of ants ($t_8 = 1.20$, $P = 0.26$).

In the herbivore (tapping) simulation treatment, there were no significant differences in mean and maximum numbers of ants between leaves and flowers and between leaves and pods (Table 1).

In our nectary survey, the bases of leaves contained a mean ± 1 SD of 2.61 ± 0.46 nectaries. No nectaries were present on the bases of flowers or fruits.

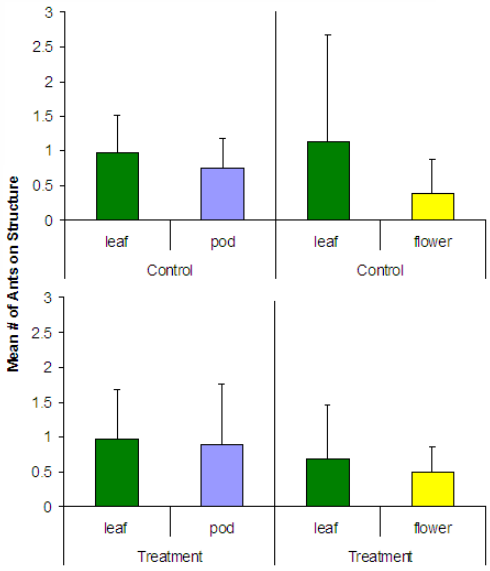

Figure 2. Mean number of ants counted on acacia leaves and flowers or leaves and pods for (a) control and (b) treatment trials. Error bars show SE. In both control and treatment trials, N = 8 paired leaves and flowers; N = 10 paired leaves and pods.

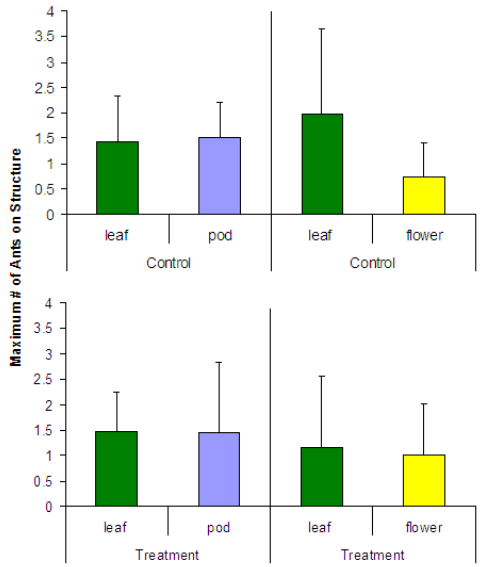

Figure 3. Maximum number of ants counted on acacia leaves and flowers or leaves and pods for (a) control and (b) treatment trials. Error bars show SE. Sample sizes as in Figure 2.

Table 1. Results of paired t-tests in the treatment trial. Each pair of acacia structures (leaves/flowers, leaves/pods) is shown for each variable.

Treatment	Mean ± SE	t-ratio	P >|t|	df
Mean # Ants: Flowers and Leaves	0.49 ± 0.84	0.60	0.566	7
Mean # Ants: Pods and Leaves	0.09 ± 0.26	0.38	0.923	8
Max # Ants: Flowers and Leaves	0.38 ± 1.76	0.31	0.762	7
Max # Ants: Pods and Leaves	0.50 ± 0.76	0.099	0.923	8

DISCUSSION

ODT predicts that tissues more closely tied to plant fitness, such as flowers and fruits, should be more heavily defended. However, ODT has rarely been applied to indirect plant defenses. Our data failed to support the hypothesis that more ants would be found on the flowers and pods of acacia trees than leaves after simulated herbivory. Tapping on plant structures did not cause preferential defense, but when undisturbed, ants spent more time on leaves than flowers. These results suggest different priorities for the ant and the acacia. While the flowers and pods are important to acacia reproduction, the leaves are

probably more important to the ants because they contain nectaries and Beltian bodies on which the ants feed. Because the ants and acacias likely value leaves and reproductive structures differently, ODT may not be applicable to indirect defense mutualisms in this system.

A number of mechanisms, none of which are mutually exclusive, may explain our results. First, acacias may discourage ants from patrolling flowers to decrease the number of potentially negative ant-pollinator interactions (Ghazoul, 2001). Similarly, ants may be less abundant on fruit pods to decrease the possibility of deterring mutualist seed dispersers (Carey et al., 2005). Second, our initial assumption that fruits and flowers would be more closely tied to plant fitness may have been erroneous for this particular species. ODT was developed based on studies of short-lived species (McKey, 1974). Because acacias are long-lived species, annual plant reproduction may be less important to their fitness than postulated by ODT. Third, our simulation of herbivore presence may have been too vigorous, and therefore unrealistic. Given the size of the tapping implement and the force with which we tapped, the ants may have been overwhelmed and unable to respond as they would to a real herbivore. Four out of the five main acacia herbivores are larvae that feed specifically on leaves, indicating that ant abundance on the leaves during the control trials could be a form of preemptive defense (Janzen, 1983). Thus, the threat of herbivory on leaves may be higher and more detrimental than originally expected.

Another limitation of our study is an incomplete consideration of optimal defense theory's premises. Our study contained a bias toward the premise of tissue value and lacked investigation into probability of damage and cost of defense, two of the three facets of ODT. To explore the importance of the other two tenets, potential areas of further study include experimental manipulation of nectar availability on different tissue types and investigation into potential deterrent compounds in pods and flowers (Ghazoul, 2001). Sampling damage on leaves, flowers, and pods to determine herbivory levels would also help determine which structures are at the highest risk of damage.

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ENVIRONMENTAL EFFECTS ON NECTAR PRODUCTION AND POLLINATOR INTERACTIONS WITH THE FLOWERING PLANT, *RUELLIA NUDIFLORA*

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Abstract. Plant-pollinator interactions can vary across environmental gradients. *Ruellia nudiflora*, a wild petunia, is an early successional plant with flowers visited by several bird and insect pollinators. We examined nectar production and consumption per flower in a shadier site versus a sunnier site. We did not find a difference in per flower nectar production or consumption between the two sites. Plant-pollinator interactions may not vary at the level of individual flowers across environmental gradients in this species, but instead may vary at a larger scale.

Keywords: nectar, pollinator preference, neotropical flower

INTRODUCTION

Flowers and the nectar they produce are valuable resources for many flowering plants and their nectar-consuming pollinators. As a result, plants invest heavily in floral display to attract pollinators (Galen 2000) and expend as much as 37% of their daily photosynthetic energy in nectar production (Southwick 1984). Nectivorous pollinators, such as hummingbirds, spend much of their day foraging and require daily nectar volumes far above their body weight (Powers and Conley 1992). Although the nature of plant-pollinator interactions is well studied (reviewed in Proctor *et al.* 1996), the effect of environmental variation on these relationships is less well understood.

Our study focused on per-flower nectar production and consumption in two sites to understand how the rewards offered by a flowering species differed across environments. We chose *Ruellia nudiflora* (Acanthaceae), a wild petunia with a large range across the Americas. *Ruellia nudiflora* is a ubiquitous, opportunistic plant that can grow across a variety of soil moisture, sunlight, and temperature conditions. We compared nectar production and consumption per flower between two sites that received different amounts of sunlight in the morning.

The tropical dry forest habitat at Palo Verde National Park provided an ideal site to examine the effects of sunlight on both nectar production and consumption. Some

neotropical plants are known to have low intraspecific variation in per flower nectar production (McDade and Weeks 2004). In addition there is a preference for warmer nectar by some pollinators due to the decreased energetic cost of warming this nectar to their body temperature (Lotz *et al.* 2003, Dyer *et al.* 2006). We hypothesized that in different environmental conditions, per flower nectar production in conspecifics would remain constant while pollinator nectar consumption would vary. We predicted that (1) the per flower nectar production in *R. nudiflora* would be the same at both sites and (2) nectar consumption would be lower at the shadier site, assuming shadier sites would have lower nectar temperatures.

In addition to evaluating plant-pollinator interactions through per flower nectar production and consumption between sites, we also considered the interactions at the level of the plant. The floral display of a plant is a prominent visual cue for pollinators and might be more influential in attracting pollinators than the actual amount of nectar produced by a flower. In addition, more flowers per plant might indicate more nectar per plant, if flowers contain similar quantities of nectar. We predicted that the sunnier site would have a lower number of flowers per plant due to lower soil moisture and extensive cattle disturbance. Because of potential lower number of flowers per plant, we also predicted that the sunnier site would have less total nectar per plant.

METHODS

We chose two patches of *R. nudiflora* with different abiotic conditions on 10 January, 2007 at Palo Verde Biological Research Station, Costa Rica. The first site was in direct sun throughout the day and had drier soil that was highly disturbed. Our second site was damper, received moderate sun, and had less disturbed soil.

To collect and measure nectar, we extracted nectar from each flower with 10 μL capillary tubes. Nectar volume per flower was calculated by using the ratio of the length of the capillary tube to the measured length of nectar: [(nectar length / capillary tube length)* 10 μL] = μL of nectar per flower.

To determine the amount of nectar produced per flower throughout the morning, we haphazardly bagged two flowers from each of ten plants at both sites at 0700 and extracted the nectar at 1100. We averaged the amount of nectar from two flowers on each of the ten bagged plants at both sites, and we counted the total number of flowers open on each of these plants. At 1100, we measured the nectar standing crop of one flower from

each of forty un-bagged plants. We assumed the difference in nectar between the bagged and un-bagged flowers at each site to be due to pollinator nectar consumption and not due to other factors, such as evaporation or re-absorption of the nectar. Using flowers per plant and nectar production per flower, we calculated the approximate nectar available per plant for each *R. nudiflora* examined.

We compared nectar production and pollinator nectar consumption (square-root transformed) between sites using a one-way ANOVA followed by a Tukey Highly Significant Difference (HSD) test (α=0.05). Based on our *a priori* hypothesis that the sunnier site would have a lower number of flowers per plant, we performed a one-tailed t-test assuming unequal variances. To test our *a priori* hypothesis that the sunnier site would have lower nectar production per plant, we conducted a one-tailed t-test. Due to low sample sizes for nectar production per plant, we also conducted a power analysis to determine how many samples we would have needed to determine a significant difference in nectar production per plant between the two sites.

RESULTS

We found an overall difference in nectar measurements between the four treatments ($F_{3,178}$=15.52, P < 0.001, Fig. 1). Specifically, we did not find a difference in nectar production between the sunnier and shadier site. We found a significant difference in morning pollinator nectar consumption between the two sites. At the sunnier site, we found that bagged flowers had 27% more nectar than the un-bagged flowers. At the shadier site, bagged flowers had 58% more nectar than the un-bagged flowers.

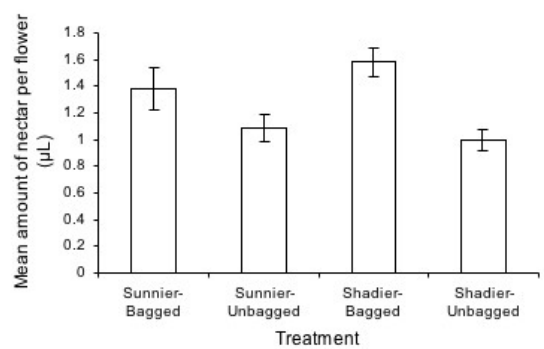

Figure 1. Mean amount of nectar in bagged (B) and un-bagged (U) flowers at the sunnier (Su) and the shadier (S) site with error bars representing ± SE. The amount of nectar represents nectar production throughout the morning for four hours in the bagged flowers and the standing nectar crop in the unbagged flowers.

The sunnier site had 300% more flowers per plant than the shadier site (t_{15}=2.28, P=0.04; Fig. 2). We found a marginally significant difference in approximate nectar production per plant between the sunnier site (mean ± SE=18.06 ± 5.87 µL) and the shadier site (mean ± SE=6.18 ± 2.29 µL) (t_{15}=1.97, P=0.07). We determined that a sample size of

71 plants would have given a statistically significant difference in approximate nectar production per plant between the two sites.

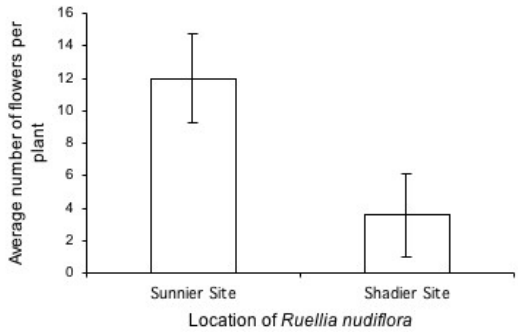

Figure 2. Mean number of flowers per plant between the two sites with error bars representing ± SE. Number of plants sampled for the sunnier and shadier site are N = 9 plants and N = 8 plants, respectively.

DISCUSSION

In our exploration of production and consumption of *R. nudiflora* nectar, we did not find variation in nectar production but did find differences in nectar consumption between the sunnier and shadier sites. Our first prediction, that nectar production would not vary between the sites, was supported. The similarity in per-flower nectar production between the two sites supports a previous study showing that environmental variables are not important in determining per-flower nectar production in neotropical plants (McDade and Weeks 2004).

Our second prediction, that the shadier site would have lower per flower nectar consumption, was also supported. Although we predicted nectar consumption would be higher at the sunnier site because the nectar would be warmer and therefore more attractive to pollinators, we did not test the temperature of the nectar between the two sites. In the hot Palo Verde environment, temperature may not vary greatly between sunlit and shaded site. Instead, it is possible that there is preferential selection by pollinators for certain environments for reasons other than that which we examined.

We found that there were more flowers per plant in the sunnier site than in the shadier one, refuting our third prediction that the sunnier site would have a lower number of flowers per plant, potentially due to lower soil moisture and extensive cattle disturbance. Soil moisture and disturbance might not be the determining factor in per plant flower production. The number of flowers per plant may be more biologically significant than per flower nectar production in determining the reproductive success of a plant because floral display may be more important for pollinator attraction in this system. While nectar per flower is important in encouraging pollinator revisitation, floral display may be more important in attracting pollinators initially. The higher nectar consumption at the sunnier site may have been driven by

pollinator preference for the site's large and attractive floral display.

We found a marginally significant difference in mean nectar production per plant between the two sites, a trend driven by the higher number of flowers per plant at the sunnier site. The floral display of a plant is influential in attracting pollinators, and pollinators will likely visit these plants and seek out the nectar rewards within each flower. However, plants with higher total nectar production may be able to serve a higher number of pollinators; therefore, there may be an increase in the plant's reproductive success at sunnier sites. Future studies might explore how total nectar production and reproduction per plant varies with experimental changes in environmental conditions.

Many other factors besides sunlight could have contributed to the differences in abiotic conditions that contribute to nectar production and consumption between the two sites. For example, one attribute we failed to explore was the temperature of the nectar at the two sites, which may have given more information about pollinator preference. Nectar temperature, however, may not be an important factor in pollinator preference in neotropical dry forests, as it is in temperate forests, because the ambient temperature is high in both shaded and sunny tropical sites. Future studies exploring plant-pollinator interactions should integrate pollinator preference across a variety of scales, from floral traits and rewards to environmental conditions.

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DIEL FORAGING BEHAVIOR OF *DENDROCYGNA AUTUMNALIS* AND *JACANA SPINOSA*

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Abstract: Diel foraging behavior plays an important role in the community structure of wetland habitats. Specifically, foraging patterns may help explain niche differentiation between Northern jacanas, *Jacana spinosa*, and Black-bellied whistling ducks, *Dendrocygna autumnalis*, both of which are found in the wetlands of Palo Verde National Park, Costa Rica. We aimed to quantify the differences in diel behaviors between jacanas and ducks. We focused on foraging behavior, predicting that the jacanas would spend more time foraging than the ducks, except at night, because ducks are mainly nocturnal feeders. We observed the two bird species throughout the day and night, noting their foraging behavior during five different time periods. Northern jacanas fed significantly more often during the day than ducks, with a peak of foraging activity midday. Black-bellied whistling duck foraging remained constant throughout the study period and at an overall lower level than jacanas. These foraging patterns may help to explain the bird community structure of the marsh.

Key Words: jacana, black-bellied whistling duck, foraging behavior, diel activity

INTRODUCTION

Diel behaviors of foraging animals have important implications for inter-specific interactions and food-web dynamics (Armbruster and McCormick 1990, De Meester *et al.* 1995, Koperski 1997). Marsh avian communities consist of a diversity of coexisting species with many different life-history traits and a variety of prey items and foraging behaviors (Kemp *et al.* 2004). The goal of our study was to characterize and compare the diel feeding patterns of two coexisting species, *Jacana spinosa* (hereafter referred to as jacanas) and *Dendrocygna autumnalis* (hereafter referred to as ducks).

Northern jacanas' main food source is surface insects, but they also feed on the ovules of water lilies (Jenni 1983). Jacanas have two different methods of foraging. They either skim insects off the surface while moving (known as gleaning) or remain stationary and peck at the water (Bourne and Osborne 1977). Jacanas may spend up to 80% of daylight hours foraging, unless they are protecting eggs or chicks (Jenni and Mace 1999). In contrast, black-bellied whistling ducks are mostly nocturnal feeders, and fly from roosting waters to foraging waters at

sunset (James and Thompson 2001). They feed mainly on wetland seeds, leaves, and shoots, and can be a nuisance to rice farmers (Stiles and Skutch 1989). Based on these differences in the birds' natural histories, we predicted that jacanas would spend more time feeding than ducks, except during the night, when we expected ducks to be feeding more.

METHODS

This study took place in the wetlands in front of the Organization for Tropical Studies (OTS) field station in Palo Verde National Park, located in the Guanacaste province of Costa Rica.

We observed mature jacanas and ducks at five times during the day on 9-10 January, 2007: early morning (0530-0630; n=10 birds/species), morning (0830-0930; n=25 birds/species), midday (1030-1130; n=20 birds/species), afternoon (1630-1730; n=10 birds/species), and night (2130-2230; n=5 birds/species). Unequal bird sample sizes among observation periods were due to study time constraints; we were only able to observe jacanas and ducks once during the afternoon, evening and early morning, while we had two days of observation for the morning and mid-morning time frames. We monitored individual birds for 2 min periods, during which we recorded their behavior at 10 sec intervals. During night observations, we used night vision binoculars to view jacanas and ducks. We divided behaviors into foraging (eating or looking for food) or not foraging. To avoid sampling the same individuals repeatedly, we observed birds at three locations that were approximately 50 m apart: the boardwalk, the bird tower area, and the main marsh area immediately in front of the OTS site. Night observations were only conducted at the boardwalk.

We tested the effects of bird species and time of day on the percent of time spent foraging with two separate analyses. Because mean percent time spent foraging was binomially distributed, we arcsine square-root-transformed our data. To compare foraging rate in ducks versus jacanas, we used a t-test assuming unequal variance because duck foraging was more variable than jacana foraging. To test for species effects, we analyzed only the day-time data because of the difficulty inherent in identifying birds at night. We tested the effect of time of day on foraging rates with separate ANOVAs for each species. We used the Tukey-Kramer Honestly Significant Difference (HSD) test to compare mean foraging time between each time category if the ANOVA was significant. Finally, we tested for a time of day*species interaction with a two-way ANOVA to test whether

time of day affected the degree to which species differed in time spent foraging. Data were analyzed using JMP 6.0.

RESULTS

Jacanas fed significantly more than ducks during the day ($t_{127.11}$=9.20, P<0.0001). On average, jacanas spent 53% more of their daytime foraging than ducks (means ± SE, jacanas: 76 ± 34%, ducks: 22 ± 32%,). In addition, there was a significant interaction between time of day and species ($F_{4,131}$=5.30, P=0.0006). In the morning, ducks and jacanas have more similar feeding behaviors than at midday.

Jacana feeding ranged from 0 ± 0% at night to 95 ± 8% at midday (means per time period ± 1 SE; Fig. 1). Additionally, during daytime observations, time of day had a significant effect on the percent of time jacanas spent foraging ($F_{4,66}$=16.14, P < 0.0001). Jacana feeding was moderate early in the morning (43 ± 43%), peaked midday (95 ± 8%), and declined to 72 ± 39% late in the afternoon. The two lowest feeding times (early morning and night) were each significantly different from all other times at α=0.05, while feeding did not vary significantly from morning through afternoon. Although the absolute maximum foraging at midday was not significantly different from either morning or afternoon, it showed lower variance (SD=8%) relative to early morning (SD=43%), morning (SD=32%), and afternoon (SD=39%; Fig. 1).

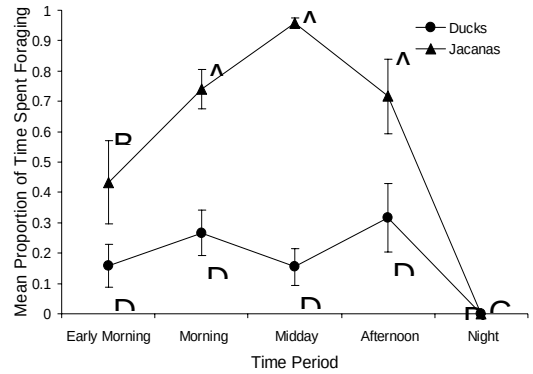

Figure 1. The mean proportion of time that northern jacanas and ducks spent foraging for each of five time periods ranging from early morning (0530) to night (2200). Error bars represent ± 1 SE. For each species, means with the same letters are not significantly different from each other.

The mean percent time spent foraging for the ducks ranged from 0% during the night to 31% in the afternoon (Fig. 1). However, because duck foraging was so variable within each time period, there was no significant effect of time of day on duck foraging ($F_{4, 65}$=1.34, P=0.26).

DISCUSSION

We found that Northern jacanas spent more time feeding during the day than black-bellied whistling ducks. This pattern may have been slightly biased toward finding a difference between species because we lumped all of our samples in the analysis and more of

our individual bird observations occurred midday, where the difference between bird feeding patterns was greatest (Fig. 1). The variation in feeding throughout the day occurred for jacanas but not for ducks. Specifically, jacana foraging peaked mid-day, compared to early morning and at night. In addition, variance was much lower at mid-day for jacanas than at any other time, suggesting that this is a biologically significant maximum in foraging. However, it is unclear whether behaviors were actually more constant mid-day or whether the lower variance was a result of unequal sampling effort throughout the day. Duck foraging behavior varied greatly within each time period, but average duck foraging fluctuated minimally throughout the day. Taken together, theses results suggest that there are species-specific differences in both the magnitude and consistency of diel foraging behaviors in Northern jacanas and black-bellied whistling ducks.

In contrast to past studies, which observed nocturnal feeding patterns in ducks (James and Thompson 2001), we did not find evidence that ducks were nocturnal feeders. We attribute this discrepancy to our night-time sampling protocol, which occurred in the same locations as our day-time sampling. Because ducks forage in different locations within the marsh than where they roost, we may not have been observing them at their nocturnal foraging site. With only night vision binoculars to observe feeding, our observations may also have been less accurate than during the day. We also had difficulty locating birds at night, thereby constraining our sample size. Finally, we also noticed a crocodile near the birds at night, which may have altered their foraging behaviors. Following ducks and jacanas to their respective nocturnal habitats could further help understand inter-specific variation in nighttime behaviors.

Finally, comparisons of diel behavior of the migratory versus resident duck individuals may help explain some of the large variation in foraging and resting patterns. Resident and migratory individuals of the same species are known to have different territorial behaviors that may also affect foraging behaviors (Forsyth and Miyata 1984). Lastly, jacana adults could be categorized by sex and current nesting status. These factors would help explain the variation seen in feeding behavior because different sizes and sexes may require different amounts of food and may have different responsibilities. The observed diel foraging behavior patterns in this study help demonstrate how different community members utilize resources throughout the day.

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INTRASPECIFIC AGGRESSION IN *JACANA SPINOSA* AS A FUNCTION OF AGE CLASS AND HABITAT TYPE

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Faculty Editor: Rebecca E. Irwin

Abstract: Aggressive behavior in foraging birds often results in dominance hierarchies based on age, territoriality, and distributions of food and reproductive resources. We examined how age-based (juvenile and adult) dominance hierarchies in foraging northern jacanas varied between two marsh habitats: floating vegetation mats (their preferred habitat) and grassy mudflats. We hypothesized that the frequencies of aggressive behaviors would vary with age class and habitat type. We predicted that adults would be more aggressive overall, and would dominate over juveniles. We also predicted that aggressive interactions would be more common in floating vegetation mats than on grassy mudflats because floating vegetation mats are their preferred habitat type. We recorded aggressive and submissive behaviors of juvenile and adult jacanas in the two habitat types. Adults were equally aggressive to adults and juveniles. In contrast, juveniles were more aggressive toward each other than to adults. Jacanas of both age classes were more aggressive on floating vegetation mats than grassy mudflats. While we observed more total interactions on the floating vegetation mats than the grassy mudflats, the frequency of interactions within and between age classes remained the same across habitats.

Key Words: Jacana spinosa, northern jacana, social aggression, habitat conditionality, dominance hierarchies

INTRODUCTION

Animals often aggressively defend limited resources, such as food and access to mates. Older and stronger individuals are often able to monopolize resources, establishing age–based hierarchies to secure these resources for themselves (Krebs and Davies 1993). Jacanas are suitable organisms to use when studying intra-specific aggression because they are conspicuous territorial birds that inhabit open marsh areas (Stiles and Skutch 1989).

Aggressive behavior may vary as a result of an individual's location with respect to available resources and other competing individuals. Different patterns of aggressive behavior in northern jacanas may be employed depending on these two factors. Aggressive jacana behaviors include ground charges, flying charges, and wing raises. These movements cause the victim of the behavior to run or fly away (Janzen and Carroll 1983). Some studies have found that aggression is correlated with jacana density (Dickinson and Poulin 1998,

Jackson and Kopplin 1996), while others have found no such correlation (Wright et al. 1998). Other studies have demonstrated that aggression is highest in floating vegetation mats, the jacanas' preferred habitat (Mahar *et al.* 2000). Further studies have shown that adults generally win aggressive encounters with juveniles (Theoharides *et al.* 2003). Finally, aggressive behaviors tend to be more common in morning and early evening when the birds congregate, and lowest in the middle of the day when the birds disperse throughout the marsh (Hourdequin *et al.* 1996, Jackson and Kopplin 1996).

While these and other studies have examined either the effects of age or habitat on intra-specific aggression in jacanas, no studies have tested the combined effects of age and habitat to understand how age-based patterns of aggression might vary across habitats. Therefore, we examined how different habitats affected age-based hierarchies of aggression in northern jacanas. We predicted that (1) aggressive interactions would be more common in the preferred floating vegetation mats than in grassy mudflats, (2) adults would dominate over juveniles in all habitat types, and (3) there would be more adult aggression than juvenile aggression in the preferred habitat.

METHODS

Study organism.

The northern jacana, *Jacana spinosa*, is a polyandrous territorial water bird ranging from southern Texas and northern Mexico, to western Panama and the Greater Antilles (Stiles and Skutch 1989). Jacanas are diurnal feeders and prefer to forage for macroinvertebrates in floating mats of aquatic vegetation (Mahar *et al.* 2000). Their breeding season coincides with the rainy season (April-November). Female jacanas are larger than males, socially dominant, and aggressively defend breeding territories on the marshes they inhabit (Goldfarb *et al.* 1991). Male-male aggressive interactions are rare (Goldfarb *et al.* 1991). While we observed both male and female adults in our study, we assumed that the majority of aggressive interactions would be initiated by territorial females (Stiles and Skutch 1989).

Field sites.

Our observations were made between 0800 and 1130 on 9 and 10 January, 2007 at four sites on the open marsh near the Organization for Tropical Studies field station in Palo Verde National Park, Costa Rica. We observed jacanas at four locations, each at least 50 m apart, in a 500 m stretch of marsh. We observed birds at two areas with

floating mat vegetation (immediately east of the bird tower and in the vicinity of the boardwalk west of the tower) and two areas with grassy mudflats (approximately 100 m east of the bird tower and at the eastward-most edge of the bird tower). We sampled two areas twice (immediately east and 100 m from the bird tower) and the remaining areas once.

Field methods.

Observation teams consisted of two observers and one recorder. An observer haphazardly selected a juvenile or an adult jacana and observed the individual for 10 min, or until the bird was lost from sight. For each individual bird, the observer noted the number of ground charges, flying charges, and wing raises, and the age class of the jacana(s) that each aggressive behavior(s) was directed against. The observer also noted the number of times individuals flew or ran away from another bird's aggressions, and the age class (juvenile or adult) of the aggressing bird. Juveniles can be differentiated from adults based on their physical characteristics; adults are red-brown, while juveniles are slightly smaller with grey and white coloration (Stiles and Skutch 1989). To prevent the observers from removing their eyes from their individuals, the recorder tallied the incidences. We minimized observer bias by shuffling group members between observation sites and by alternating observing and recording roles. Over the two-day observation period, we observed 22 adults and 17 juveniles in the floating vegetation mat habitats, and 23 adults and 19 juveniles in the grassy mudflat habitats.

Statistical analyses.

To test whether there were differences in the frequency of aggressive interactions between floating mats and grassy mudflats, we used a non-parametric van der Waerden test. We used a non-parametric chi-squared test because our data were left-skewed and no transformation would normalize our data. To understand the distribution of aggressive behavior within and between age classes, we performed a 2x2 chi-squared test with two factors: aggressor (juvenile or adult) and victim (juvenile or adult). To understand the difference in the relative rates of different interaction types between habitat types, we also performed a 2x2 chi-squared test with two factors: interaction type [aggressor-victim: adult-adult (AA), adult-juvenile (AJ), juvenile-adult (JA), and juvenile-juvenile (JJ)] and location (floating vegetation mats or grassy mud flats). After removing a single juvenile jacana that had 20 more aggressive interactions than any other juvenile, we performed the same analysis to test the strength of the observed relationship between

habitat type and the distribution of aggressive interactions.

RESULTS

On average, jacanas displayed more aggressive interactions per minute on the open mat vegetation than on the grassy mudflats (X^2_1=8.06, P=0.0032; Fig. 1).

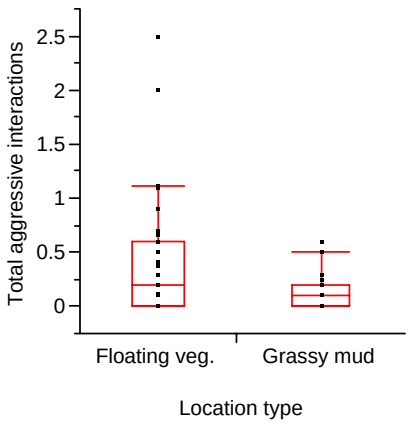

Figure 1. More aggressive interactions occur between jacanas on the floating vegetation mats (preferred habitat) than on the grassy mud flats (less preferred habitat). Each dot represents an individual jacana.

The frequency of interactions between aggressors and victims of different age classes also varied. When an adult was a victim of aggression, there was a 91% chance the aggressor was an adult and a 9% chance that the aggressor was a juvenile. When a juvenile was the victim of aggression, the aggressor was an adult 57% of the time and a juvenile 43% of the time (Fig. 2).

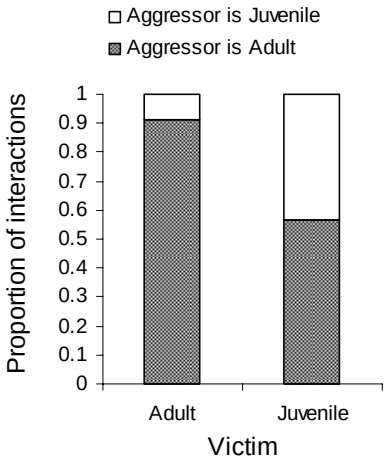

Figure 2. Aggressor age class by victim age class in northern jacanas. Adults were attacked in higher frequencies by other adults, while juveniles were attacked in higher frequencies by other juveniles.

When an adult was the aggressor, an adult was the victim 54% of the time, while a juvenile was the victim 46% of the time. When a juvenile was the aggressor, an adult was the victim 13% of the time, while a juvenile was the victim 87% of the time (X^2_1=29.45, P<.0001; Fig. 3).

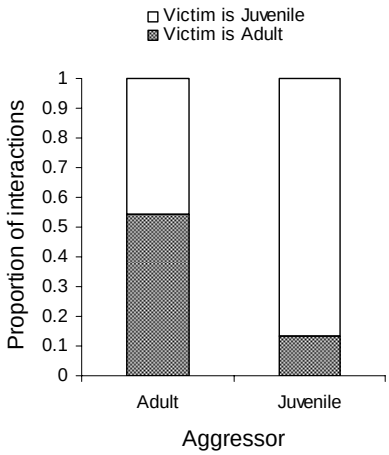

Figure 3. Victim age class by aggressor age class in northern jacanas. Adults attacked adults and

juveniles with equal frequencies, while juveniles usually attacked other juveniles.

There was a significant difference in the distribution of aggressive interactions between habitat types (X^2_1=9.76 P=0.0207; Fig. 4). However, one outlier drove this relationship. With the removal of one particularly aggressive juvenile, the distribution of aggressive interactions between habitat types was no longer statistically significant (X^2_1=3.60 P=0.31).

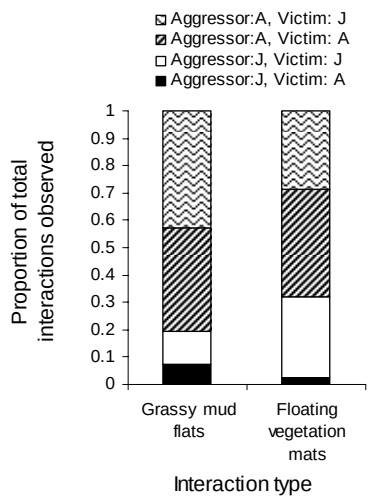

Figure 4. Interaction type by aggressor, victim, and age class in grassy mud flats and floating vegetation mats. There is a significant difference between the distributions of aggressive interactions between habitat types; however, this interaction is entirely driven by one especially aggressive juvenile.

DISCUSSION

In theory, aggressive interactions should be more common when more valuable resources are at stake (Krebs and Davies 1993). Previous studies have found that jacanas are more aggressive on their preferred habitat type and that territorial adult females are more aggressive overall (Stiles and Skutch 1986). However, no studies, to our knowledge, have looked at the interaction between adult female aggression and habitat type. We hypothesized that jacana aggression would vary with age class and that adult aggression would be greater in preferred habitat, perhaps due to female territoriality.

Our data revealed that while jacanas were more aggressive in their preferred, floating-vegetation mat habitat, adults typically dominated juveniles across both habitat types, which supports our hypothesis that jacana aggression varies across habitat types and with age class. This study adds to the growing body of literature documenting how age-based hierarchies structure marsh bird communities (Mahar *et al.* 2000).

Our results also support our prediction that adult jacanas are more likely to be aggressors than victims, complementing past studies that show that adult jacanas are more likely to win confrontations than are juveniles (Theoharides *et al.* 2003). Therefore, our study supports our hypotheses that frequencies of aggressive behavior will vary with habitat type and age class. Our work builds on previous studies of jacana aggression by identifying not only which age classes were more likely to be aggressors or victims, but also which age classes the jacanas were

more likely to be aggressive towards or victimized by. When adults were victims, their aggressor was more likely to be another adult than a juvenile (Fig. 2). When adults were aggressors, they were equally aggressive to adults and juveniles (Fig. 2). When juveniles were aggressors, they were rarely aggressive toward adults (Fig. 3).

The data do not support our prediction that adults will initiate a greater percentage of aggressive interactions. In fact, we found that a greater percentage of aggressive interactions on the preferred habitat type were initiated by juveniles (Fig. 4). However, this result was driven by one particularly aggressive juvenile, without which there would be no significant difference between the percentages of the four different interaction classes across habitat types. More observation hours are needed to understand whether this result is simply driven by one aggressive individual or whether juveniles do indeed initiate more aggressive interactions.

Finally, we noted in our field observations that larger individuals seem to be more aggressive than smaller ones. Because size is correlated with gender in jacanas, it has been hypothesized that this difference is due to the territoriality of the larger, female jacanas (Janzen and Carroll 1983). A next step in this system would be to test whether the greater number of aggressive interactions we observed on the preferred habitat could be due to a higher density of jacanas, which would create more interactions with larger, territorial females. Such a study should take into account both the age and gender of the jacanas. Further studies combining age-based hierarchies and habitat differences could prove important for understanding territoriality in birds.

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THE EFFECT OF ANT SIZE AND TRAP DIAMETER ON *MYRMELEON CRUDELIS* PREY CAPTURE SUCCESS

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Abstract: The outcomes of predator-prey interactions are affected by characteristics of both the predator and the prey. For example, ant lions, their traps, and their arthropod prey all vary in size and could all therefore affect predation rates. In this study, we examined how ant lion trap size and ant prey size combine to affect the overall probability of prey capture. We predicted that ant lions in larger pits would successfully capture both small and large ants, while ant lions in smaller pits would successfully capture only small ants. We placed four species of ants (two large and two small species) into ant lion pits that ranged from small to large and recorded whether the ants escaped or were captured. Our hypothesis and predictions were supported by the data. The likelihood that an ant prey would be captured by an ant lion increased with ant lion pit diameter and overall prey capture probabilities were lower for the larger ants than for the smaller ants.

Key Words: tropical dry forest, predation, ant lion

INTRODUCTION

A predator's foraging success determines its energy gain and survival, as well as the mortality and population dynamics of its prey (Begon et al. 1990). The body size of both predator and prey are important factors in determining the result of their interaction (Osenberg and Mittelbach 1989, Allan et al. 1987). Insects are ubiquitous study subjects that are ideal for examining the relationship between organisms' body sizes and their ability to successfully capture or escape.

Ant lions (*Myrmeleon crudelis*) are small, predatory insects. As flightless larvae, they build pitfall traps for prey capture in loose dry soil. Although ant lions eat a variety of small insects, ants comprise the majority of their diet (Crowley and Linton, 1999). Ant lions are abundant in areas with low rainfall, such as the tropical dry forest of Palo Verde, Costa Rica, where we conducted this study. The ant lion larvae spend up to three years maturing in the soil. As soon as they hatch, the larvae immediately build their first pit. The funnel-shaped pits vary in both diameter and depth. Once an ant or other small arthropod falls into the pit, the ant lion attempts to seize its prey and pull it down into the sand. Previous studies found that the diameter of the ant lion pit is directly related to the size of the larva (McClure 1983, Perlroth et al. 1995).

Thus, we used pit diameter as a surrogate for ant lion size. Finally, previous studies have also shown that the ant lion residents of larger pits are more successful at capturing prey (Gorman and Grabowsky 1991) and that capture time increases with prey size (Guidi 2003).

While several studies have addressed the effects of ant lion size and ant prey size on capture rates independently, no studies have simultaneously tested these two factors. Testing these two factors together is important because the effect of trap and ant lion size on prey capture may depend on prey size. We predicted that pits with larger diameters, and thus larger ant lions, would successfully capture both big and small ant species. Additionally, we predicted that pits with smaller diameters, and thus smaller ant lions, would successfully capture small but not large ant species. However, the effect of pit diameter may be less important when prey are very small. For example, small ants might always be captured regardless of pit diameter.

METHODS

The study took place on 12 and 13 January, 2007 at Palo Verde National Park in the Guanacaste region of Costa Rica. We conducted our study in two disturbed locations: along the field station access road and along the perimeter of the field station buildings. In both locations, pits were found in fine, dry soil. To ensure that our sample pits were representative of the Palo Verde ant lion population, we also measured 132 haphazardly chosen ant lion pit diameters from all over the station.

We chose four species of ants grouped into two size classes: large and small. Using an aspirator, we collected ants from multiple *Acacia collinsii* trees, along the ground, and on building walls. Ten ants from each location were measured, and mean ant lengths determined. Large ants were *Pseudomyrmex spinicola* (mean ± 1 SE: 6.21 ± 0.08 mm) and *P. flavicornis* (5.97 ± 0.15 mm) and small ants were *Crematogaster brevispinosa* (2.83 ± 0.23 mm) and another unknown black ant species (2.24 ± 0.18 mm). We used two species for each size class to ensure that the size effect was actually due to ant size and not differences in ant species.

We placed ten ants of each species into separate ant lion pits. For each, we recorded whether the ant escaped or was captured. An ant was considered captured if it was pulled completely into the hole and disappeared from the surface for at least 15 seconds. An ant was considered escaped if it successfully walked out of the pit. If there was no sign of struggle by the ant, we assumed that the pit was vacant and we discarded that trial. We measured the diameter of each ant lion pit to the nearest 0.01 mm using

calipers. We used an indicator variable multiple logistic regression to test the effects of pit diameter, ant size, and their interaction on the probability of capture with JMP 6.0 (Quinn and Keough 2002).

RESULTS

Large ants were 59% bigger than the small ants (t_{37} = 37.31, P < 0.0001). Additionally, ant species sizes were also significantly different from each other ($F_{3, 35}$= 1172.84, P < 0.001). Ant lion pit diameters were normally distributed for the population sample and for each of the sites. Pit diameters for the population sample and the two study sites combined varied six-fold, ranging from 9.8 mm to 60.6 mm. The mean pit diameter for the two sampling locations and the entire population sample were not significantly different from each other ($F_{4,312}$ = 0.354, P = 0.841).

Ant lion trap diameter and ant prey size both helped to predict prey capture probabilities (χ^2_3 = 55.49, R^2 = 0.31, P < 0.0001). Small ants were captured significantly more often than large ants across all pit diameters, larger pits had higher capture rates, and the effect of pit diameter on capture rate was independent of ant size (Fig. 1, Table 1). For example, in an ant lion pit with a diameter of 15 mm, a large ant had a 30% chance of being captured, whereas a small ant had a 65% chance. At 25 mm, large ants were captured 66% and small ants had a 98% chance. At approximately a 50 mm diameter, large and small ants were captured in 99% and 100% of encounters, respectively (Fig. 1).

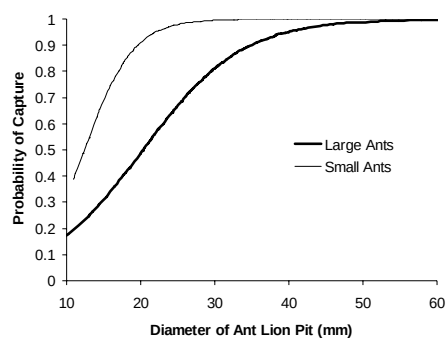

Figure 1. Probability of ant prey capture, based on our ant lion pit diameter and ant prey size according to the model: $g(x) = B_0 + B_1$*diameter + B_2*(size) + B_3*(size*diameter), where g(x) is the probability of being captured, expressed as the natural log of the odds ratio (Quinn and Keough 2002). Large ants were used as the size reference category. The interaction term was not statistically significant (Table 1).

TABLE 1. Model parameters from an indicator variable multiple logistic regression predict prey capture probability.

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	-3.4186112	0.9737082	12.33	0.0004
Diameter	0.22761577	0.05419	17.64	<.0001
Size[L]	-1.8611737	0.6998953	7.07	0.0078
(diameter-28.8762)*Size[L]	-0.0766807	0.05419	2.00	0.1571

DISCUSSION

Body size is an important factor in determining the result of predatory interactions (Osenberg and Mittelbach 1989, Allan et al. 1987). Ant lions and ants provide a good system to observe this aspect of predator-prey dynamics because both parties vary in size and interactions between them are easy to manipulate. The likelihood that a prey item will be captured by an ant lion depends both on the size of the ant lion's pit and the size of the prey.

We found that the larger diameter ant lion pits captured prey of both sizes. Specifically, ant lion pits that were greater than approximately 50 mm in diameter could capture even the largest ants used in the study. Although more large ants escaped from smaller pits, smaller ant lions still had significant success at capturing small ant species, suggesting that small and large ant lions have similar success at capturing prey comparable to their own size. Our hypothesis and predictions were supported; larger diameter ant lion pits successfully captured both large and small ants, while smaller diameter ant lion pits successfully captured small ants but fewer large ants. Additionally, the effect of pit diameter on prey capture rates was similar for both small and large ants.

Further experimentation is needed to evaluate the relationship between ant prey species and capture success. Our data cannot definitively demonstrate that size is the only determining factor in capture success. It is likely that some species ant species are more aggressive and naturally more resistant to capture than other species of comparable size. Using a greater number of ant species per size class, future studies could disentangle the effect of ant species (and their associated behaviors) and prey size. While this study used ants 3-7 mm in length, large ground ants in the Palo Verde National Park can be up to 9-10 mm in length *(personal observations).* We also noted that even some of the largest ant lion pits could not capture ants with a mean length equal to or greater than 9.3 mm. Therefore, there may be ant sizes that will always escape, regardless of ant lion size.

Our study takes into consideration a realistic spectrum of ant lion pit sizes, whereas previous studies have grouped ant lion pits into size classes (Goodale et al. 1991). Further studies could use a realistic range of ant lion pits combined with a full spectrum of ant sizes. A greater knowledge of the dynamic relationship between predator and prey characteristics can lead to a

better understanding of interaction outcomes and population dynamics.

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AN APPLICATION OF THE RESOURCE AVAILABILITY HYPOTHESIS TO EARLY AND LATE SUCCESSIONAL STANDS AT PALO VERDE NATIONAL PARK

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Abstract: The resource availability hypothesis (Coley *et al.* 1985) predicts that slow-growing, long-lived plants should invest in defenses with high initial costs but lower maintenance costs, while fast-growing, short-lived plants should invest in defenses with low initial costs. Because later successional species tend to be slow growing relative to earlier successional species (Begon *et al.* 1990), and because structural defenses such as thorns have high initial costs, the resource availability hypothesis led us to predict that a later successional forest stand at Palo Verde National Park would have a higher proportion of thorny plants than a nearby earlier successional forest stand. However, contrary to our predictions, we found that the earlier successional stand had a higher proportion of thorny plants than the later successional stand. We believe that our results were driven by the abundant thorns on the early successional acacia tree, *Acacia collinsii* (Fabaceae). The unique biology of the acacia, specifically its mutualistic interaction with *Pseudomyrmex* ant species, might explain why it allocates its resources to thorns contrary to predictions from the resource availability hypothesis.

Key Words: antiherbivory, structural defense, tropical dry forest

INTRODUCTION

Trade-offs central to the process of natural selection are exemplified by defense allocation in plants. The resource availability hypothesis describes trade-offs between inherent plant growth rates and plant investment in anti-herbivory defenses (Coley *et al.* 1985). The hypothesis predicts that long-lived species will invest in defenses with high initial costs, which reduce resources available for growth but lower maintenance costs over time. Conversely, short-lived species are predicted to invest in defenses with low initial costs in order to reserve resources for rapid growth and reproduction.

While the resource availability hypothesis mainly focuses on chemical defenses, we expanded on the theory by applying it to structural thorn defenses of plants in different successional stages. Later successional species tend to be slow-growing competition specialists, while early successional species tend to be fast-growing dispersal specialists (Begon *et al.* 1990). Because thorns have high initial production cost (Gomez and Zamora 2002, Rohner and Ward 1997), we used the resource availability hypothesis to predict that earlier

successional stands would have a lower proportion of plants with thorns than later successional stands.

METHODS

Study system.

We conducted this study on 13 January, 2007 in the tropical dry forest at Palo Verde National Park in the Guanacaste Province of Costa Rica. Prior to the establishment of the park in 1974, there was extensive logging and grazing in the forest (E. Gonzales, *personal communication*). Palo Verde's younger forests are dominated by earlier successional woody species, especially *Acacia collinsii* (Fabaceae), while older forests contain later successional trees, such as *Pseudobombax septenatum* (Bombaceae) and *Guaiacum sanctum* (Zygophyllaceae). We observed that many of the tropical dry forest plants in Palo Verde National Park invest in thorny structural defenses, which presumably protect them from the high herbivory that is characteristic of the neo-tropics (Coley and Barone 1996).

We examined one earlier succession stand located along the access road to the Organization for Tropical Studies field station in the park's lowland area and one later successional stand located along the Cerros Calizos Trail in the park's upland area. Due to its accessibility, the earlier successional stand has experienced more disturbances and is therefore at an earlier successional stage than higher elevation areas (E. Gonzales, *personal communication*).

Field methods.

We randomly selected locations for 5 m X 5 m sampling quadrats, spaced 15 – 30 m apart along both sides of the trail and access road (n=15 quadrats per stand). In each quadrat, we counted the total number of woody plants above knee-height, excluding all non-woody species. Then we counted the number of woody plants above knee-height with thorns on trunks, leaves, or stems. We used these numbers to calculate the percentage of plants with thorns per quadrat.

Statistical analysis. Because the distribution of the percent of plants with thorns was left-skewed, we arcsine-square root-transformed our data to achieve normality. We performed a two-tailed t-test to compare the percentage of woody plants with thorns in the two forest types using JMP 6.0. Although the late-successional stand had higher variance, this did not bias the inference statistics since our sample sizes were equal in the two sites (Box and Cox 1964).

RESULTS

In the earlier successional forest plots, 52.40 ± 7.82% of plants (mean ± 1 SE) in the quadrats had thorns. In the later successional forest plots, 5.56 ± 1.09 % of plants had thorns. Plots in the earlier successional stand contained a significantly higher proportion of thorny plants than plots in the later successional stand (t_{28}=6.41, P < 0.0001; Fig. 1).

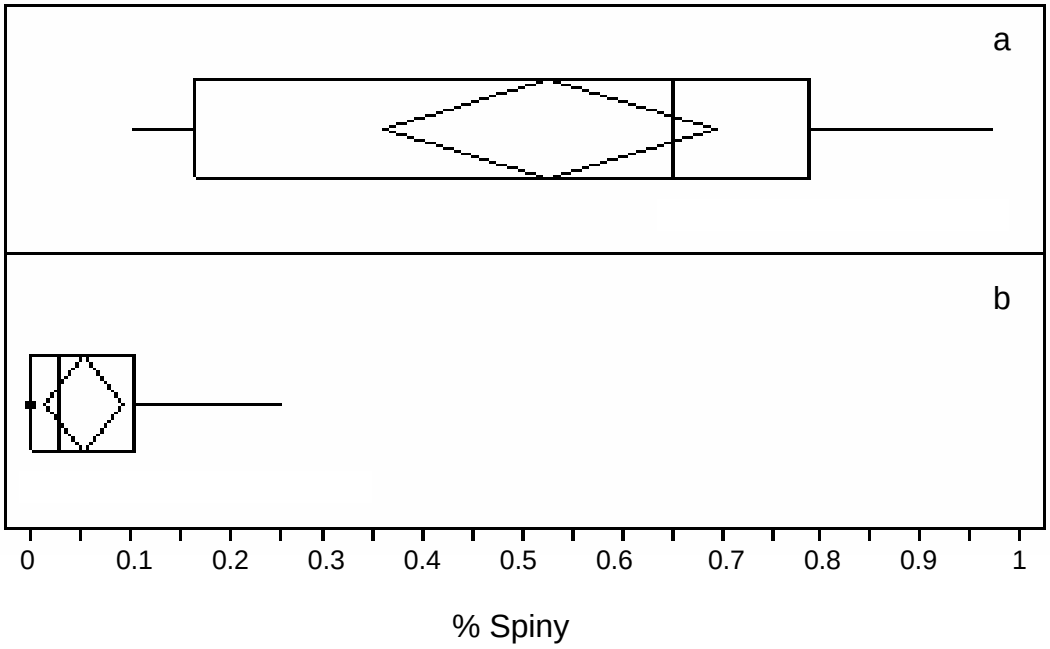

Figure 1. The percentage of woody plants with thorns was significantly greater in the earlier successional stand (a) than in the later successional stand (b) (n=15 quadrats). Vertical lines in the boxes represent medians and the ends of the rectangles represent inter-quartile ranges of untransformed data. Whiskers extend to the maximum and minimum of the percent thorniness range, and diamonds indicate means and standard deviations.

DISCUSSION

Our study revealed that the earlier successional stand that we studied in the tropical dry forest of Palo Verde contained a higher percentage of woody plants with thorns than the later successional stand. Our finding contradicted our prediction that an earlier successional stand would have a lower proportion of thorny plants than a later successional stand. Despite the high initial cost associated with producing structural defenses, such as thorns (Gomez and Zamora 2002), our results suggest that there may still be a net benefit for earlier successional plants of the Palo Verde tropical dry forest to invest in thorns.

The two stands we sampled differed topographically as well as in successional stage. The later

successional stand was situated on a limestone hill and the earlier successional stand on a lower, flatter dry forest close to the surrounding marshland. In order to disentangle the potentially confounding effects of site difference, future studies should sample multiple stands of each successional status.

From our observations in the field, it seems that our results were strongly driven by the high densities of acacia trees in the earlier successional stand. Although we only sampled one earlier successional stand, we believe that this trend would hold over larger areas as well, because acacia stands are very wide-spread in earlier successional forest throughout the park. Because acacia thorns are inhabited by ant mutualists who provide additional protection to the tree (Janzen 1966), it is possible that acacia investment in thorns has a double benefit to acacia fitness by reducing herbivory both directly and indirectly. Thus, the net cost of producing thorns may be lower, which could explain why acacias, an early successional species, allocates resources to structural defenses which potentially have high initial costs, contrary to the resource availability hypothesis.

While previous studies have applied the resource availability hypothesis to plant investment in secondary compounds (Coley *et al.* 1985), our study focuses on its application to thorns. The resource availability hypothesis failed to predict the distribution of thorn defenses in our system. Altogether, our study indicates that there are alternative selective pressures driving the use of structural defense in earlier successional plants of the tropical dry forest in Palo Verde National Park. Future studies should focus on understanding the mechanisms driving the increased thorniness in early successional plants.

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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).

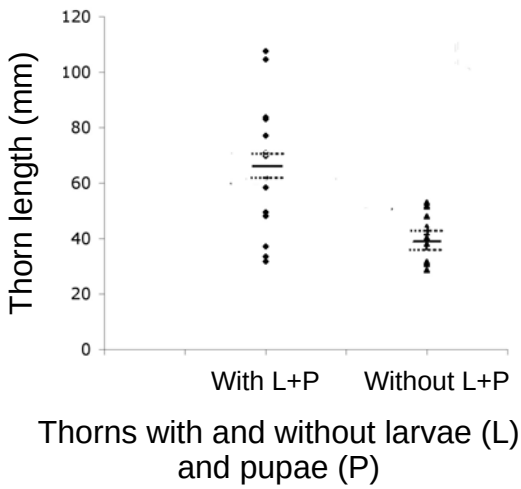

Figure 1. The mean length of thorns containing larvae (L) and pupae (P) was significantly higher than those without. Solid lines represent the mean, while dashed lines represent standard error. Sample size for thorns with larvae and pupae was N=14 thorns and for thorns without was N=16 thorns.

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).

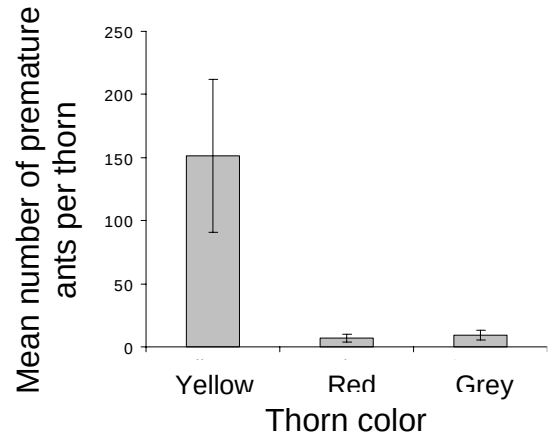

Figure 2. The mean number of premature ants (larvae and pupae) in yellow thorns was approximately 1200% higher than in either red or grey thorns. Sample size N = 16 for yellow thorns, N=5 for red thorns, and N=10 for grey thorns. Error bars represent ± SE.

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; $F_{2,51} = 9.07$, $P = 0.0004$).

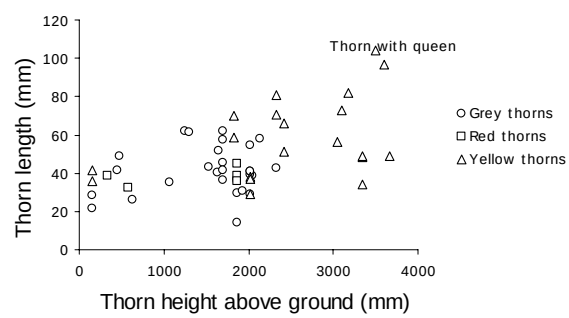

Figure 3. There was a positive correlation between thorn length (across all thorn colors) and thorn height above ground. Yellow thorns were found higher than red or grey thorns. Sample size was N = 54 thorns.

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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PATCH DYNAMICS OF THE BLACK-BELLIED WHISTLING DUCK

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Faculty Editor: Rebecca E. Irwin

Abstract: We observed the spatial distribution of patches of the black-bellied whistling duck, *Dendrocygnus autumnalis*, at Palo Verde National Wildlife Refuge, Guanacaste Province, Costa Rica. Over a period of two days, we measured the spatial dispersion of patches on the entire marsh at one-hour intervals. We also measured the group size and local immigration and emigration rates of individuals in particular patches during 15 minute observations. In general, patch size and location were stable for larger patches of *D autumnalis*. Smaller groups changed more in size than larger groups, sometimes going extinct during the observation while other times growing by up to 50%. Conversely, larger groups were more consistent in size than smaller groups, growing only slightly during the observation period. The stability of patch locations and the size (relative and absolute) of larger patches may be important for future study concerning the spatial patterns of *D. autumnalis* in relation to resource and microhabitat dispersion.

Key Words: Dendrocygnus autumnalis*, Population ecology, Palo Verde*

INTRODUCTION

The concept of the metapopulation is important for understanding the distribution of organisms in fragmented resources patches. While each individual patch is distinct and isolated from the others, constant flow of individuals between patches creates metapopulation dynamics that are greater than the sum of interactions within each individual patch. Any population that is spatially distributed in patches and that maintains flow between patches can be thought of as a metapopulation (Krebs and Davies 1993). Such patchiness can even be observed on relatively small spatial scales, such as in communities of migratory birds on open water wetlands.

Some species of migratory waterfowl, such as the Black-Bellied Whistling Duck, *Dendrocygna autumnalis*, congregate in flocks of over a thousand individuals on open water habitats (James and Thompson 2001). The spatial distribution of these patches has been linked to biotic resources in the marshland they inhabit (E. González, *personal communication*). At the Palo Verde National Wildlife Refuge, the majority of the birds wintering on the marsh are *D. autumnalis*. While many of these *D. autumnalis* are year-round residents, the majority are migratory individuals from North America (M. Gómez, *personal communication*). *Dendrocygna autumnalis* break into small groups at night and fly away from their daily resting sites to feed; however, during

the day, they spend the majority of their time resting in large groups in open-water wetlands (Stiles and Skutch, 1989). Because *D. autumnalis* make up a large portion of the bird biomass on the marsh (M. Gómez, *personal communication*), they likely play an important role in the large interspecies flocks, and their spatial patterns may affect aquatic plant communities and nutrient cycling in the wetland (Mueller and van der Valk 2002).

We hypothesized that the distribution of *Dendrocygna autumnalis* on the open-water marsh in Palo Verde was a stable metapopulation, where the size larger patches does not change significantly over long time periods and never go extinct. To test our hypothesis, we analyzed the patches of *D. autumnalis* on the marsh. We predicted that (1) the net local immigration of *D. autumnalis* to a given patch would approximately equal the local emigration of *D. autumnalis* from that patch, regardless of patch size, and that (2) the size and location of the larger patches of *D. autumnalis* would remain constant within and between days.

METHODS

We conducted our study on 13 and 14 January, 2007, on a stretch of actively managed marsh along the Rio Tempisque directly in front of the Organization for Tropical Studies (OTS) research station in the Palo Verde National Wildlife Refuge, Guanacaste Province, Costa Rica. The actively managed regions of the marsh that we observed are periodically cleared of cattails. The cattail clearing creates shallow regions of open water with floating mat vegetation, which is preferred habitat for a wide variety of waterfowl, including *D. autumnalis* (Stiles and Skutch, 1989). *Dendrocygna autumnalis* distribute themselves throughout the marsh in dense, clearly defined clusters or patches, with few or no *D. autumnalis* occupying the space between patches.

To address our first prediction, that the net local immigration of *D. autumnalis* to a patch would approximately equal the net local emigration of *D. autumnalis* from that patch, two observers and a recorder were positioned atop a bird tower in front of the OTS station for three hours each morning and afternoon. Each observer selected a patch of *D. autumnalis*, estimated the number of individuals in that patch, and recorded every duck that flew into or out of the patch over 15 min. For each patch and observation period, we also recorded the time of day, location, microhabitat type (mud, shore, or floating mat vegetation), and whether the patch was disturbed by the passage of livestock

through it. To determine the relationship between initial patch size and net flux of *D. autumnalis* per 15 min observation period, we used a correlation analysis.

To address our second prediction, that the size and location of the larger patches of *D. autumnalis* would remain constant within and between days, we climbed to the Mirador Guayacán lookout behind the OTS station approximately 400 feet above the marsh. On two mornings at 0745, we took three sets of photographs of the entire marsh, each an hour apart and used Jasc Paint Shop Pro 8 to generate time-lapse maps of the distribution of duck patches on the marsh for both mornings. We also photographed one large patch in 15 min intervals over 150 min, starting at 0755, to document the changes of one large patch over two days.

RESULTS

We found a significant positive correlation between the initial patch size (natural-log transformed) and net change in the number of *D. autumnalis* ($r = 0.57$, $n = 24$, $p = 0.0033$; Fig. 1). Larger patches of D. *autumnalis* tended to have a net local immigration of *D. autumnalis*, while smaller patches tended to have a smaller local immigration, or even a local emigration, of *D. autumnalis*.

To calculate how volatile patches were, we took the absolute value of the percent change in the number of *D. autumnalis* over 15 minutes. We found a strong, negative correlation between the initial patch size (natural-log transformed) and the absolute value of the percent change in number of individuals (natural-log transformed) over 15 minutes in that patch. ($r = -0.55$, $n = 24$, $p = 0.0055$; Fig. 2). As patch size increased, the patches were less likely to change significantly; the larger patches are more stable.

Over the course of two days, the distribution of large patches of *D. autumnalis* over the entire marsh did not noticeably change (Fig. 3). The largest, most visible patches of *D. autumnalis* were in the same locations on both days.

We also found that a single large patch of *D. autumnalis* did not go extinct after 150 minutes starting at 8 AM, even after disturbance by a horse (Fig. 4). After disturbance, the patch quickly regained its original shape, with a slightly lower density (Fig. 4, panels 1 and 10).

DISCUSSION

Fragmented populations connected by local immigration and emigration between patches can be thought of as one metapopulation (Krebs and Davies 1993). These patches can exist at equilibrium, where the immigration rate approximately equals the emigration

rate from each patch, or they can be sources or sinks, where either immigration or emigration

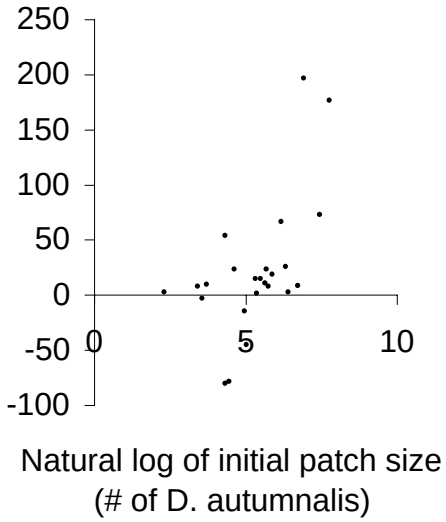

Figure 1. Relationship between initial patch size and net change in number of *Dendrocygnus autumnalis* individuals, as observed over 15 min intervals. Larger patches tended to have a larger local immigration of *D. autumnnalis* than smaller patches.

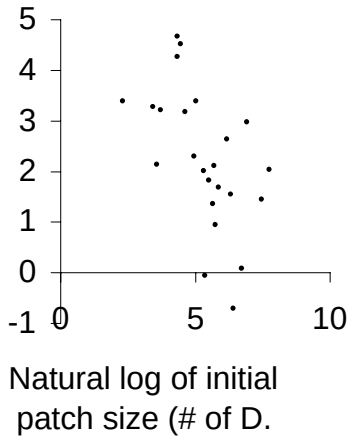

Figure 2. Relationship between initial patch size and percent change in number of *Dendrocygnus autumnalis* individuals, as observed over 15 min intervals. Smaller patches varied more in their percent changes, while larger patches consistently had a small, positive percent change.

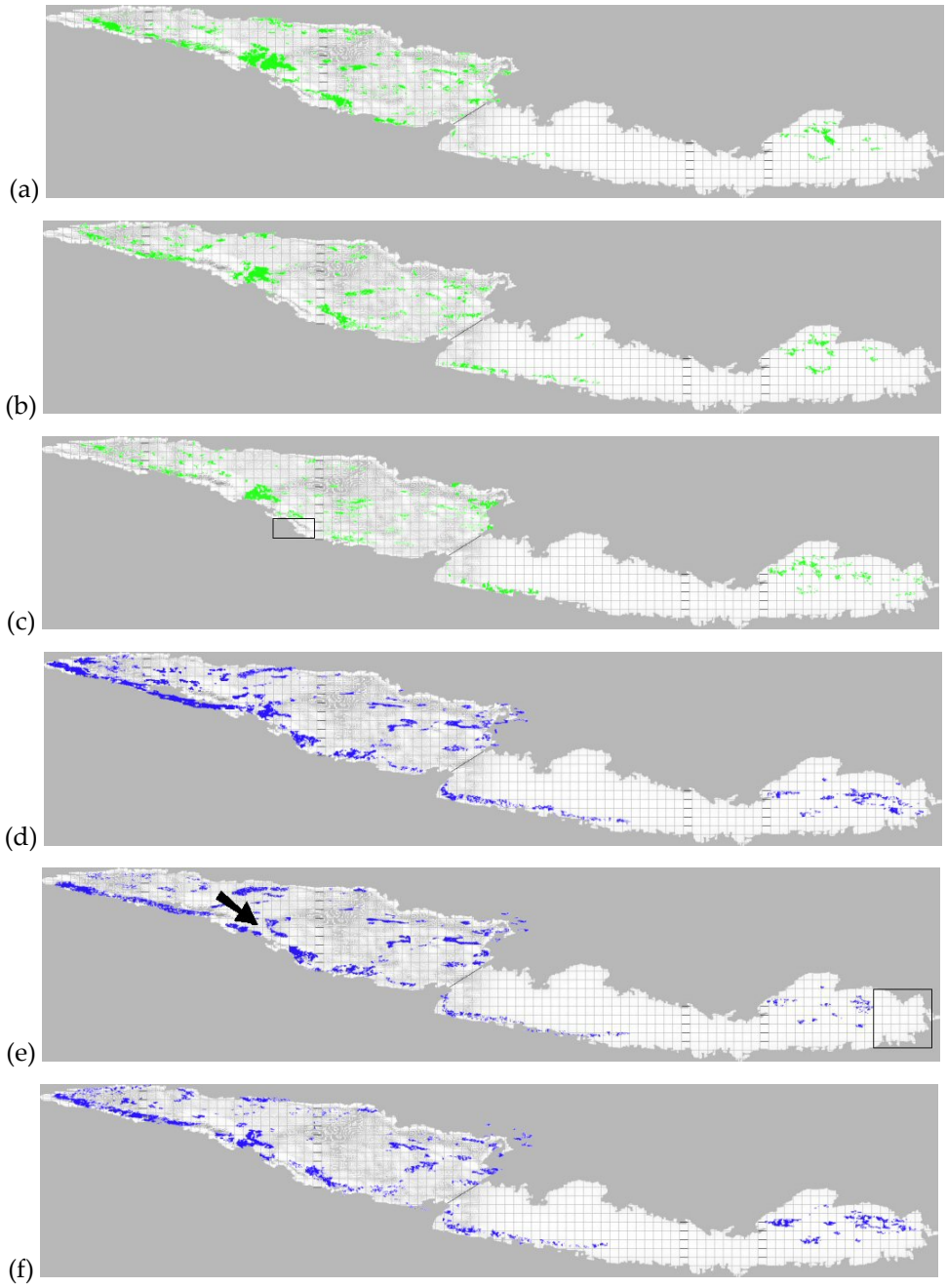

Figure 3. Maps of *Dendrocygnus autumnalis* patch distributions in the marsh on the mornings of 12 and 13 January, 2007. Green areas represent duck patches on Day 1, while blue areas represent patches on Day 2. The black boxes are areas for which there is no data. Maps were constructed from photos taken at (a) 0740 on 12 January, 2007, (b) 0840, (c) 0940, (d) 0745 on 13 January, 2007, (e) 0845, and (f) 0945. The black arrow in (e) shows the location of a disturbance caused by a grazing horse at 0840, which displaced a large number of *D. autumnalis*. Over the course of both days, the locations of the major patches of *D. autumnalis* did not change significantly.

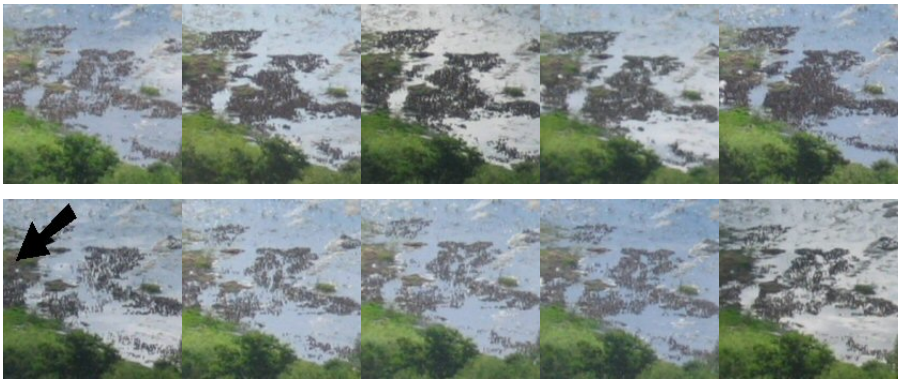

Figure 4. Time lapse photographs of a single large patch of black-bellied *Dendrocygnus autumnalis* on 13 January, 2007. Photographs were taken every 15 min and the first photograph was taken at 0755. The black arrow points to the source of a disturbance caused by a horse at 0840. Note that the shape of the patch does not change, while the size recovers after disturbance.

outweighs the other (Krebs and Davies 1993). Patches within a metapopulation often occur on patches of resources valuable to a specific species (1993).

A metapopulation can be found in any population that is spatially distributed in patches and that maintains some flow of individuals between patches. Such a distribution can be observed in the way *Dendrocygnus autumnalis* are spatially distributed across the marsh in Palo Verde, Costa Rica. This metapopulation is characterized by large, stable aggregations of *D. autumnalis*, interspersed with smaller, more volatile patches. The large patches of *D. autumnalis* are isolated by stretches of empty water, but there is frequent exchange of individuals between patches. Because the larger patches tend to grow in size, there appears to be a stable, long-term distribution of the larger patches of *D. autumnalis* in the marsh.

We found that larger patches of *D. autumnalis* tended to have a greater rate of immigration than emigration, while smaller patches had a net loss of individuals (Fig. 1). *Dendrocygnus autumnalis'* preference for larger patches is indirectly supported by the significant positive correlation we observed between initial patch size and the net change in number of individuals over a 15-min observation period, contradicting our first prediction, that the net inflow of ducks into a patch would equal the outflow of ducks from that patch, regardless of patch size. This behavior tends to create large, stable patches of *D. autumnalis* (Fig. 3); larger patches attract more individuals. While this prediction was disproven, it does not

necessarily mean that the ducks do not exist in a metapopulation. The larger patches may act as population sinks, but the percent change in patch size over the observation period averaged 1-2% of the total patch size.

Our results support our second prediction that the size and dispersion of major patches would not change greatly over time. We found that large patches of *D. autumnalis* tended to grow by a small amount over the observation period. The static location of these major patches as well as the observed immigration and emigration from patches provide partial support of our hypothesis that *D. autumnalis* exist in a metapopulation.

Large daily groups may also be important for predator defense, and larger groups may therefore be stable due to the dilution of individuals (Kornet et. al., 1992). Previous studies have suggested that large flocks of birds are better able to detect predators (Kornet et. al., 1992); *D. autumnalis* rapidly took flight in huge numbers in response to disturbance. Because larger flocks provide better protection against predators (Kornet et. al, 1992), it is logical that individual *D. autumnalis* would join larger patches on the marsh, which would tend to create large, stable patches of *D. autumnalis*. This hypothesis warrants further experimental investigation. Our observations of the flocks of *D. autumnalis* show that the largest, most visible patches of *D. autumnalis* are stable throughout the day, as well as between days, providing support for our second prediction, that the patches are stable throughout and between days. Our observations show very little change in the over-all distribution and size of large patches throughout the marsh. Even after significant disturbance, the largest patches of *D. autumnalis* quickly reformed, adding further evidence to support the idea that the *D. autumnalis* form large, stable flocks.

To further evaluate the patch dynamics of *D. autumnalis*, it would be informative to test whether an individual duck will pick a larger or smaller patch, when presented with the option. We would also like to further evaluate emigration from large patches, to test whether this behavior is due to random chance, or if individuals will leave dense patches in search of less dense ones. Finally, it would be informative to determine whether certain factors, such as the location of food resources, can be used to predict where a large patch of *D. autumnalis* is located. Because metapolulations are often distributed according to resource patches, correlation patches of *D. autumnalis* in the marsh at Palo Verde with the location of food resources would strongly support the hypothesis that the *D. autumnalis* community is a metapopulation.

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NEST TREE SELECTION BY RUFOUS-NAPED WRENS IN PALO VERDE NATIONAL PARK, COSTA RICA

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Abstract: Tree-nesting birds may enhance their fitness by nesting near colonies of aggressive social insects as a strategy to protect their offspring from predators; however, little is known about which tree characteristics might influence bird nest site selection. Nest placement by the rufous-naped wren, *Campylorhynchus rufinucha*, may be complicated by the fact that the wren shares its nest tree with acacia ants, which do not have visually conspicuous nests. To explore the question of what tree traits are associated with rufous-naped wren nests, we measured *Acacia collinsii* tree height, diameter at breast height (DBH), number of major branches from the main stem, and number of leaves, and thorns on a 30 cm segment of a canopy branch. We found that birds nested in taller, leafier trees. Because taller, leafier trees are likely to be well-defended by their ant mutualists, the wrens may receive the benefits of distancing their nests from ground predators and from the associations of an effective ant population that may defend the tree and their nests.

Key Words: Acacia collinsii, Campylorhybchus rufinucha, Pseudomyrmex spinicola, *mutualism*

INTRODUCTION

Many bird species nest near aggressive social insects as a fitness-enhancing strategy, utilizing the insects' swarming and stinging behaviors to protect their offspring from predators (Beier and Tungbani 2006). Even though the insects often do not receive any benefit from this interaction, they inadvertently protect the birds' nests (Wiley 1983). Some aggressive wasps and ants have conspicuous nests (Beier and Tungbani 2006), which would allow nesting bird species to choose trees that harbor these swarming insects. However, other aggressive insects. such as *Pseudomyrmex spinicola*, which nest inside the thorns of acacia trees, do not have conspicuous nests (Collins et al., *personal observation*).

Nevertheless, the rufous-naped wren, *Campylorhynchus rufinucha*, nests in acacias and receive protection from predators, such as monkeys and snakes, by the acacia ant *P. spinicola* (Janzen 1969, Young et al. 1990). It is unlikely that the birds are able to learn specific locations of aggressive ant colonies because the birds build new nests every year (Wiley 1983). Therefore, we hypothesized that wrens would use visual cues on the trees as a

proxy for healthy ant populations. We measured visually detectable traits of the acacia tree *Acacia collinsii* (Fabaceae; hereafter referred to simply as acacias) with intact nests of rufous-naped wrens and surrounding acacia trees without nests. We predicted that trees containing nests would be larger and healthier.

METHODS

We conducted our study on 8 January, 2007 along the access road to the east of the Organization for Tropical Studies field station in Palo Verde National Park, Costa Rica. We haphazardly chose twelve acacia trees containing intact rufous-naped wren nests. For each nest tree, we also sampled the three nearest acacia neighbors without nests. We chose near neighbors without nests with a minimum trunk diameter at breast height (DBH) of 1 cm because smaller trees would be incapable of supporting nests.

For each tree, we measured DBH, number of leaves and thorns per 30 cm of a randomly selected canopy branch, number of major branchings off the main trunk, and tree height using a clinometer and distance from the tree. We measured these variables because they are visual traits that a bird might use as indicators of ant activity when choosing a nest site. Because plants in tropical dry forests experience high rates of herbivory (Coley and Barone 1996), tree leafiness should be a reliable indicator of effective defense by ant mutualists. Thorniness is also a strong indicator of effective ant defense, according to the nest space limitation hypothesis (Fonesca 1999). The hypothesis states that living space is the most limiting resource in myrmecophytes, and therefore, the trees with more thorns are associated with larger ant populations.

We first performed pair-wise correlation analyses on the five indicators (DBH, tree height, thorniness, leafiness, and number of branchings) to prevent the use of multiple t-tests on correlated response variables, reducing Type I error rate. We excluded DBH and major branchings because they were correlated with height (height and DBH: $r = 0.85$, $N = 24$, $P < 0.0001$; branching and DBH: $r = 0.67$, $N = 24$, $P = 0.0003$; height and branching: $r = 0.49$, $N = 24$, $P = 0.0003$). For the three trees we measured without a nest per tree with a nest, we averaged the response variables across those trees. We performed paired two-sample t-tests on tree height, leafiness, and thorniness between trees with and without nests using JMP 6.0. We paired nest and no nest samples in identical habitats to eliminate biases among microhabitats.

RESULTS

Nest trees had higher thorn density than no nest trees (mean ± SE, nest: 12.83±0.94, no nest: 11.33±0.75, paired two-sample $t_{11} = -1.76$, $P = 0.053$). Nest trees were 43.7% leafier than no-nest trees (paired two-sample $t_{11} = -2.98$, $P = 0.0062$, Figure 1A). Moreover, nest trees were 70.3% taller than no-nest trees (paired two-sample $t_{11} = -5.55$, $P < 0.0001$, Figure 1B).

DISCUSSION

The location of rufous-naped wren nests suggests the birds use indirect and direct associations of certain physical parameters in their selection of acacia trees. Directly, wrens may select nest sites using height and leafiness as indicators of good tree health and stability. Greater height might also be important for distancing the nest from terrestrial predators, such as rats and coatis.

In addition to these direct physical benefits, the wrens' selectivity among acacia trees may indirectly involve the unique mutualism between these trees and resident ants. Nest trees had a higher thorn density, which may be associated with a larger ant population because myrmecophyte-inhabiting ant colonies are generally space limited (Fonesca 1999). The strength of a resident ant population's protection against grazing herbivores and competing plants has also been shown to promote the tree's overall health (Janzen 1969); therefore, an effective ant colony may indirectly increase the probability that a wren nests in that given tree. Moreover, the ant population may enter into a mutualistic relationship with the wrens (Janzen 1969, Young et al 1990), thereby causing wrens to seek out trees with strong health indicators both for their inherent benefits and for their association with effective ants. Acacia ants may adjust to the nests' presence and actually defend the nests from predators, such as white faced capuchins (Joyce 1992), while the wrens may benefit these ants by consuming or deterring ant predators (Janzen 1969).

Further observation is required to link resident ant activity, nest site selectivity, and the physical parameters of a given acacia tree emphasized in this study. Future work should address the fitness benefits and costs of nesting wrens, cohabitating ant species, and acacia trees as they coexist in the lowlands of Costa Rica's Pacific plain.

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