INSIGHTS

Mutualistic acacia ants exhibit reduced aggression and more frequent off-tree movements near termite mounds

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ABSTRACT

In many ant–plant mutualisms, ants establish colonies in hollow thorns, leaf pouches, or other specialized structures on their host plants, which they then defend from herbivores. Resource heterogeneity could affect the maintenance of these mutualisms if it leads to one or both partners altering their investment in the interaction. Such a phenomenon may be especially pertinent to the *Acacia–ant* mutualism found in East African savannas, where termite mounds have a profound effect on the spatial structuring of resources used by both plants and ants. Here, we examined whether the proximity to termite mounds of *Acacia drepanolobium* trees is associated with variation in the behavior of one of their ant associates, *Crematogaster nigriceps*. We found that ant colonies near termite mounds had decreased aggressive responses to simulated herbivory as well as increased off-tree movement. We hypothesize that these changes are the result of resident ant colonies near termite mounds shifting investment from defense of their host plant to foraging for nearby resources.

Many tropical plant species have specialized structures known as domatia (*e.g.*, leaf pouches, hollow thorns) in which mutualistic ant species can establish their colonies. Ant colonies protect their host plants against herbivores and may also feed from extrafloral nectaries or tend insects for their exudates (Bronstein 1998). Not all ants form equally good mutualistic associations with plants, with some partners better at protecting their host plants against herbivory than others (Bronstein 1998, Lapola *et al.* 2003, Bruna *et al.* 2004, Frederickson 2005, Palmer & Brody 2007, Martins 2010, Stanton & Palmer 2011). However, the behavior of even high-quality partners could potentially vary as a function of local abiotic and biotic conditions. For example, during the dry season, acacia trees in Mexico invest more in costly extrafloral nectaries to retain protective *Pseudomyrmex* ants, and in return, ants respond more aggressively to simulated herbivory (González-Teuber *et al.* 2012). Such context dependence in partner responses is central to understanding the dynamics of interspecific mutualisms (Bronstein 1994), but has been poorly explored for ant–plant systems.

In the East African savanna, four different species of ants—*Crematogaster sjostedti*, *C. mimosae*, *C. nigriceps*, and *Tetraponera penzigi*—form mutualistic associations with *Acacia drepanolobium* trees (Palmer *et al.* 2000). These savannas are also home to termites (*Odontotermes* species), whose mounds create spatial heterogeneity in the distribution of resources (Fox-Dobbs *et al.* 2010, Pringle *et al.* 2010, Bonachela *et al.* 2015). The heterogeneity in available resources may lead to differences in the ant–acacia mutualism.

For example, ant colonies on trees near termite mounds tend to be larger (Palmer 2003). Additionally, competitively dominant *C. sjostedti* more frequently overtakes subdominant species on trees near termite mounds, suggesting that trees near termite mounds offer high-quality habitats (Palmer 2003). Finally, the nitrogen isotope levels in ants from colonies near termite mounds suggest they spend more time foraging for food off trees (Palmer 2003), perhaps because of a greater abundance of potential prey items in these locations (Pringle *et al.* 2010). If so, then this may limit their capability to defend the tree from herbivores. The above findings suggest that proximity to termite mounds influences the mutualistic behaviors of ants. If the defensive behavior of ants varies with proximity to termite mounds, it could have consequences for the dynamics of the ant–acacia mutualism at the landscape scale. Here, we present the results of experiments assessing how the mutualistic behavior of *C. nigriceps* varies with host plant proximity to termite mounds.

METHODS AND RESULTS

We conducted our study at the Mpala Research Center in Laikipia, Kenya (Fig. S1) from 13–20 January 2017. For each of 15 termite mounds, we randomly chose six *Acacia drepanolobium* trees inhabited by *C. nigriceps*: three trees within 5 m of the mound edge (*i.e.*, "near trees") and three trees 20–25 m from the mound edge (*i.e.*, "far trees"). All trees were between 1 and 2 m in height to control for the potentially confounding effects of tree age, colony age, or colony size.

To assess whether additional food sources for ants were more abundant near mounds, we counted the number of spider webs in each of the selected trees. We used spider webs as bio-

indicators of increased prey availability; we reasoned that because spiders are carnivorous, more spider webs are associated with increased prey abundance and, potentially, high-quality tree location for ants. Indeed, we found more spider webs near termite mounds (Fig. S2). We also rated the condition of all trees as either "good" (>50% leaf cover), "poor" (<50% leaf cover), or dead; we found more trees in good health closer to mounds (Fig. S3). These results are in agreement with others of prior studies, suggesting that proximity to termite mounds is associated with spatial heterogeneity in resources (Fox-Dobbs *et al.* 2010, Pringle *et al.* 2010, Bonachela *et al.* 2015).

We then examined the defensive and foraging behaviors of ants residing in our focal trees. We examined the defensive responses using an established bioassay for simulating herbivory in this system (Palmer & Brody 2007); such behavioral bioassays are a common technique for quantifying ant aggression in ant–plant mutualisms (Agrawal 1998, Lapola *et al.* 2003, Christianini & Machado 2004, Heil *et al.* 2004, Romero & Izzo 2004). For each focal tree, we selected two branches of equivalent height and health—one on the north side and one on the south side—at ~1.5 m height. We then simultaneously stroked the selected branches three times in rapid succession while wearing leather gloves, after which we held the branches for 30 seconds. We then counted the number of ants on each glove and calculated the average number of ants per glove. Trees near termite mounds had 47% fewer ants responding to simulated herbivory than trees far from mounds (18.4 ± 1.57 SE vs. 27.1 ± 2.45 SE, Mann–Whitney U-test, $P = 0.025$, $U = 733.5$, Fig. 1A).

As a proxy for foraging behavior, we estimated the number of ants moving off trees. We did so by counting the number of ants moving up and down a portion of each tree's trunk that was located near the ground and below all branches. We always selected the side of the trunk with the most ants for our counts and only counted ants visible on that side of the trunk. We counted ants for 60 seconds before and after each simulated herbivory trial. Although there was no significant difference in the ant counts before and after simulated herbivory (Mann–Whitney U-test, $P = 0.8867$, $U = 157$, $N = 36$), we present only the foraging counts recorder prior to simulated herbivory because this was the most natural, undisturbed observation. Trees near termite mounds had significantly more off-tree movement—near mounds we counted an average of 35.8 ± 3.73 SE ants moving up or down the base of each tree, far from mounds we counted an average of 16.8 ± 2.71 SE ants (Mann–Whitney U-test, $P < 0.001$, $U = 733.5$, Fig. 1B).

DISCUSSION

We hypothesize that the ant responses we observed are driven by elevated resource levels near termite mounds leading to reduced investment in colony defense. Although ants could be moving off trees to search for new ones to colonize or to defend the colony from other ants (Carroll & Janzen 1973), our observations of ants returning to the tree with prey or scavenged items are consistent with the idea that *C. nigriceps* likely forage off trees to
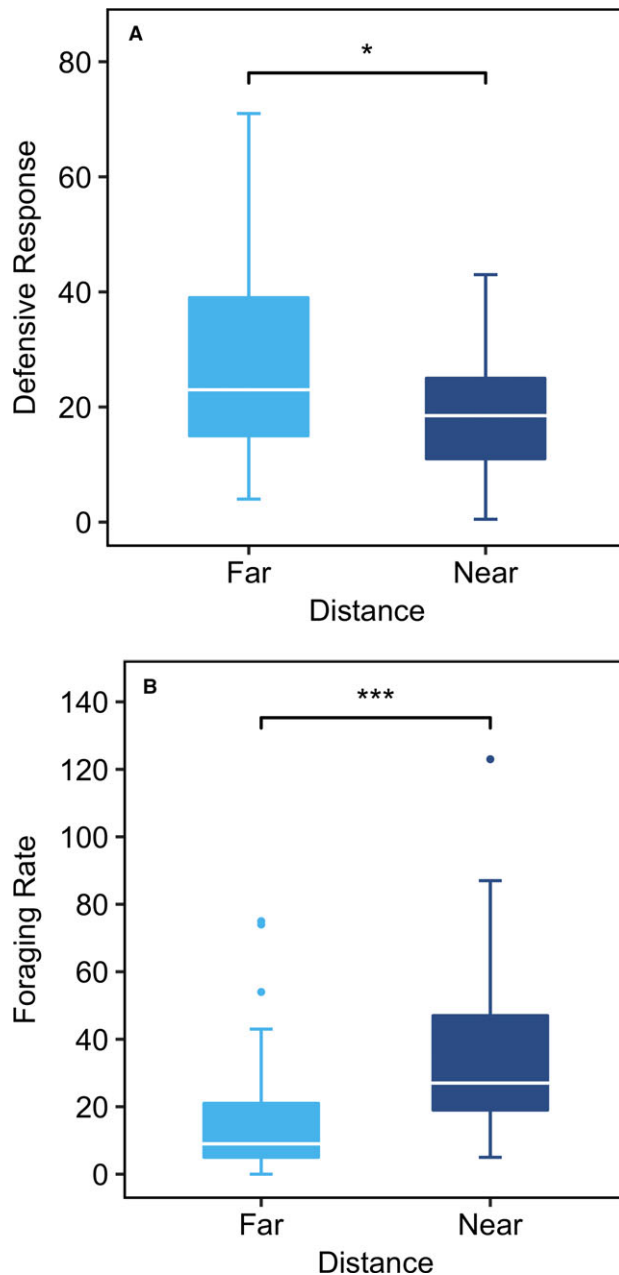

FIGURE 1. (A) The defensive response of colonies near and far from termite mounds to simulated herbivory. Ants in trees near termite mounds had reduced defensive responses to simulated herbivory (Mann–Whitney U-test, $P = 0.025$, $U = 733.5$). The defensive response of a colony was calculated as the average number of ants counted on two gloves after 30 sec simulated herbivory. (B) Off-tree movement, as a proxy for foraging rate, of colonies by distance from termite mounds. Ants in trees near termite mounds had increased off-tree movement (Mann–Whitney U-test, $P < 0.001$, $U = 733.5$). Foraging rate was measured as ants moving up or down the base of a tree trunk in 60 sec.

acquire the protein necessary for reproduction, as extrafloral nectar from their host-tree provides primarily carbohydrates (Palmer *et al.* 2000, Palmer 2003, Rudolph & Palmer 2013). Other ant

mutualists have been observed foraging off of their host plants for nutrients, but the ecological mechanisms underlying the variation in this behavior and the consequences for the mutualism are not well characterized in many systems (Carroll & Janzen 1973, Heil & McKey 2003, Mayer *et al.* 2014). Additionally, we also observed more spider webs in trees near termite mounds (Fig. S2), suggesting, like Pringle *et al.* (2010), that termite mounds harbor a higher abundance of arthropods that can be consumed by spiders and ants alike.

If more ants are foraging off trees, this could mean fewer ants are available for the primary benefit ants provide their hosts —defense against herbivores. *Crematogaster nigriceps,* like New World acacia ants, exhibit distinct, age, and/or morphology-based division of labor, including defense—some workers ("defenders") readily leave the swollen thorns following a disturbance, while other individuals ("domestics") remain in domatia at all times tending to the brood (Stapley 1999, Amador-Vargas 2012). To examine this possibility, we collected five domatia from each tree sampled and characterized the contents of each domatium. Despite notable differences in defensive responses, we found no difference between the numbers of adult workers in the domatia in trees at different distances from mounds (Mann–Whitney U-test, $P = 0.705$, $U = 1060$). This is consistent with our hypothesis that the decreased defensive response near termite mounds is due to a spatial reallocation of a colony's "defender" workforce to foraging. In free-living Argentine ant species, a single colony is spread across multiple nests and allocates more workers to foraging when food resources are spatially heterogeneous, potentially at the detriment to defense of the queen (Holway & Case 2000). These free-living ants changed their behavior in response to spatially aggregated resources, and this may also apply to *C. nigriceps* colonies spread across trees. The drivers and consequences for division of labor in maintaining ant–plant protective mutualisms are not well understood (Mayer *et al.* 2014).

Three important caveats to our conclusions merit further investigation. First, we focused on ant behavior, but ant behavior in ant–plant mutualisms often depends on reciprocal investment by the plant (Bronstein 1998, Heil & McKey 2003, Heil *et al.* 2009). While we examined leaf cover as a proxy for tree health (Fig. S3), we did not examine any traits to measure tree investment in the mutualism. Host plants provide greater rewards for higher quality defense from ants, and in return, ants invest more in defense when rewards are greater (Heil *et al.* 2009). Our finding that ants spend more time foraging off of the tree near termite mounds might also be explained by trees near termite mounds providing fewer rewards for their ants. Second, we did not study if or how ant colonies spread and distribute resources across multiple trees (Palmer *et al.* 2000, Palmer 2003). Colonies of *C. nigriceps*, as well as the other *Crematogaster* species in this ant symbiont guild, are frequently spread across multiple trees (Palmer *et al.* 2000, Palmer 2003). Larger colonies of ants typically occupy more trees (Palmer 2004). We did not find any difference in ant colony size near or far from termite mounds as measured by counting workers in the domatia of the selected tree (Wilcoxon Rank Sum $W = 1060$, P-value $= 0.7045$), but, again, we did not test for spread across trees. If colonies were spread across trees, our results would imply ants on trees far from mounds have a different strategy—investing more in defense because they occupy fewer trees, and each tree is more important. Alternatively, the off-tree movement may represent ants moving within colony, across trees, with ants in colonies near termite mounds spending more time moving between trees. However, the role of off-tree movement for colonies spread across trees is not well understood in protective ant–plant mutualisms (Debout *et al.* 2007). Finally, another limitation was that our study occurred during a severe drought. Ant behavior changes seasonally in response to precipitation, with a reduction in defensive behaviors during the dry season (Rudolph & Palmer 2013). This suggests that environmental stressors lead to different ant behaviors, like less aggressive defense that may reduce the benefits that maintain the mutualism. Future research will be necessary to understand how context dependence of different stressors shapes variation in mutualistic behaviors in ant–plant systems.

In conclusion, termite mounds spatially structure resources in ways that can influence the behavior of mutualist ants and ultimately the dynamics of the *C. nigriceps*–acacia mutualism. Reduced investment in rewards to mutualistic partners could destabilize the mutualism. Consequently, destabilized mutualisms may lead to turnover to different ant symbionts, facilitating coexistence between ant species colonizing acacia trees (Palmer 2003, Palmer *et al.* 2010). Future work should examine whether the variation in defensive and off-tree movement we observed is unique to *C. nigriceps* or is also observed in other partners, and how this variation contributes to coexistence between members of the ant guild.

ACKNOWLEDGMENTS

DATA AVAILABILITY

Data are available from Zenodo: https://doi.org/10.5281/zenodo.1226746 (Henry *et al.* 2018).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

FIGURE S1. Map showing the location of tested termite mounds within the Mpala Research Center.

FIGURE S2. Proximity to termite mounds is associated with more spider webs (as a proxy for prey abundance for ants).

FIGURE S3. Proximity to termite mounds is associated with better health of trees.

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