

1 **Mutualistic Acacia Ants exhibit reduced aggression and more frequent off-**  
2 **tree movements near termite mounds**

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5 Lucas P. Henry<sup>1,3,\*</sup>, Christopher K. Tokita<sup>1,3,\*</sup>, Mayank Misra<sup>1,2</sup>, Avery B. Forrow<sup>1</sup>, Daniel I. Rubenstein<sup>1</sup>

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7 <sup>1</sup>Department of Ecology and Evolutionary Biology, Princeton University

8 <sup>2</sup>Woodrow Wilson School of Public and International Affairs, Princeton University

9 <sup>3</sup>Corresponding author; emails: [lhenry@princeton.edu](mailto:lhenry@princeton.edu), [ctokita@princeton.edu](mailto:ctokita@princeton.edu)

10 \*Contributed equally

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26 **ABSTRACT**

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

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40 **Key Words:** ant-acacia mutualism; termite mounds; spatial heterogeneity; Kenya;

41 *Crematogaster nigriceps*; *Acacia drepanolobium*

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

61           In the East African savanna, four different species of ants -- *Crematogaster sjostedti*, *C.*  
62 *mimosae*, *C. nigriceps*, and *Tetraponera penzigi* -- form mutualistic associations with *Acacia*  
63 *drepanolobium* trees (Palmer *et al.* 2000). These savannas are also home to termites  
64 (*Odontotermes* species), whose mounds create spatial heterogeneity in the distribution of  
65 resources (Fox-Dobbs *et al.* 2010, Pringle *et al.* 2010, Bonachela *et al.* 2015). The heterogeneity  
66 in available resources may lead to differences in the ant-acacia mutualism. For example, ant  
67 colonies on trees near termite mounds tend to be larger (Palmer 2003). Additionally,  
68 competitively dominant *C. sjostedti* more frequently overtakes subdominant species on trees near  
69 termite mounds, suggesting that trees near termite mounds offer high quality habitats (Palmer  
70 2003). Finally, the nitrogen isotope levels in ants from colonies near termite mounds suggest

71 they spend more time foraging for food off trees (Palmer 2003), perhaps because of a greater  
72 abundance of potential prey items in these locations (Pringle *et al.* 2010). If so, then this may  
73 limit their capability to defend the tree from herbivores. The above findings suggest that  
74 proximity to termite mounds influences the mutualistic behaviors of ants. If the defensive  
75 behavior of ants varies with proximity to termite mounds, it could have consequences for the  
76 dynamics of the ant-acacia mutualism at the landscape scale. Here we present the results of  
77 experiments assessing how the mutualistic behavior of *C. nigriceps* varies with host-plant  
78 proximity to termite mounds.

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## 80 **METHODS AND RESULTS**

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

87 To assess if additional food sources for ants were more abundant near mounds, we  
88 counted the number of spider webs in each of the selected trees. We used spider webs as bio-  
89 indicators of increased prey availability; we reasoned that because spiders are carnivorous, more  
90 spider webs are associated with increased prey abundance and, potentially, high quality tree  
91 location for ants. Indeed, we found more spider webs near termite mounds (Figure S2). We also  
92 rated the condition of all trees as either ‘good’ (>50% leaf cover), ‘poor’ (<50% leaf cover), or  
93 dead; we found more trees in good health closer to mounds (Figure S3). These results are in

94 agreement with others of prior studies suggesting that proximity to termite mounds are  
95 associated with spatial heterogeneity in resources (Fox-Dobbs *et al.* 2010, Pringle *et al.* 2010,  
96 Bonachela *et al.* 2015).

97 We then examined the defensive and foraging behaviors of ants residing in our focal  
98 trees. We examined the defensive responses using an established bioassay for simulating  
99 herbivory in this system (Palmer & Brody 2007); such behavioral bioassays are a common  
100 technique for quantifying ant aggression in ant-plant mutualisms (Agrawal 1998, Lapola *et al.*  
101 2003, Christianini & Machado 2004, Heil *et al.* 2004, Romero & Izzo 2004). For each focal tree  
102 we selected two branches of equivalent height and health—one on the north side and one on the  
103 south side—at ~1.5m height. We then simultaneously stroked the selected branches three times  
104 in rapid succession while wearing leather gloves, after which we held the branches for 30  
105 seconds. We then counted the number of ants on each glove and calculated the average number  
106 of ants per glove. Trees near termite mounds had 47% fewer ants responding to simulated  
107 herbivory than trees far from mounds ( $18.4 \pm 1.57$  SE vs.  $27.1 \pm 2.45$  SE, Mann-Whitney U,  $p =$   
108  $0.025$ ,  $U = 733.5$ , Fig. 1A).

109 As a proxy for foraging behavior we estimated the number of ants moving off trees. We  
110 did so by counting the number of ants moving up and down a portion of each tree's trunk that  
111 was located near the ground and below all branches. We always selected the side of the trunk  
112 with the most ants for our counts, and only counted ants visible on that side of the trunk. We  
113 counted ants for 60 seconds before and after each simulated herbivory trial. Though there was no  
114 significant difference in the ant counts before and after simulated herbivory (Mann-Whitney U,  $p$   
115  $= 0.8867$ ,  $U = 157$ ,  $n = 36$ ), we present only the foraging counts recorder prior to simulated  
116 herbivory because this was the most natural, undisturbed observation. Trees near termite mounds

117 had significantly more off-tree movement – near mounds we counted an average of  $35.8 \pm 3.73$   
118 SE ants moving up or down the base of each tree, far from mounds we counted an average of  
119  $16.8 \pm 2.71$  SE ants (Mann-Whitney U,  $p < 0.001$ ,  $U = 733.5$ , Fig. 1B).

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## 121 **DISCUSSION**

122 We hypothesize that the ant responses we observed are driven by elevated resource levels  
123 near termite mounds leading to reduced investment in colony defense. Although ants could be  
124 moving off trees to search for new ones to colonize or to defend the colony from other ants  
125 (Carroll & Janzen 1973), our observations of ants returning to the tree with prey or scavenged  
126 items are consistent with the idea that *C. nigriceps* likely forage off trees to acquire the protein  
127 necessary for reproduction, as extra-floral nectar from their host-tree provides primarily  
128 carbohydrates (Palmer *et al.* 2000, Palmer 2003, Rudolph & Palmer 2013). Other ant mutualists  
129 have been observed foraging off of their host plants for nutrients, but the ecological mechanisms  
130 underlying the variation in this behavior and the consequences for the mutualism are not well  
131 characterized in many systems (Carroll & Janzen 1973, Heil & McKey 2003, Mayer *et al.* 2014).  
132 Additionally, we also observed more spider webs in trees near termite mounds (Figure S2),  
133 suggesting, like Pringle *et al.* (2010), that termite mounds harbor a higher abundance of  
134 arthropods that can be consumed by spiders and ants alike.

135 If more ants are foraging off trees, this could mean fewer ants are available for the  
136 primary benefit ants provide their hosts – defense against herbivores. *C. nigriceps*, like New  
137 World acacia ants, exhibit distinct, age and/or morphology-based division of labor, including  
138 defense -- some workers (“defenders”) readily leave the swollen thorns following a disturbance,  
139 while other individuals (“domestics”) remain in domatia at all times tending to the brood

140 (Stapley 1999, Amador-Vargas 2012). To examine this possibility, we collected five domatia  
141 from each tree sampled and characterized the contents of each domatium. Despite notable  
142 differences in defensive responses, we found no difference between the numbers of adult  
143 workers in the domatia in trees at different distances from mounds (Mann-Whitney U,  $p = 0.705$ ,  
144  $U = 1060$ ). This is consistent with our hypothesis that the decreased defensive response near  
145 termite mounds is due to a spatial reallocation of a colony's "defender" workforce to foraging. In  
146 free-living Argentine ant species, a single colony is spread across multiple nests, and allocates  
147 more workers to foraging when food resources are spatially heterogeneous, potentially at the  
148 detriment to defense of the queen (Holway & Case 2000). These free-living ants changed their  
149 behavior in response to spatially aggregated resources, and this may also apply to *C. nigriceps*  
150 colonies spread across trees. The drivers and consequences for division of labor in maintaining  
151 ant-plant protective mutualisms are not well understood (Mayer *et al.* 2014).

152         Three important caveats to our conclusions merit further investigation. First, we focused  
153 on ant behavior, but ant behavior in ant-plant mutualisms often depends on reciprocal investment  
154 by the plant (Bronstein 1998, Heil & McKey 2003, Heil *et al.* 2009). While we examined leaf  
155 cover as a proxy for tree health (Fig. S3), we did not examine any traits to measure tree  
156 investment in the mutualism. Host plants provide greater rewards for higher quality defense from  
157 ants, and in return ants invest more in defense when rewards are greater (Heil *et al.* 2009). Our  
158 finding that ants spend more time foraging off of the tree near termite mounds might also be  
159 explained by trees near termite mounds providing fewer rewards for their ants. Second, we did  
160 not study if or how ant colonies spread and distribute resources across multiple trees (Palmer *et*  
161 *al.* 2000, Palmer 2003). Colonies of *C. nigriceps*, as well as the other *Crematogaster* species in  
162 this ant symbiont guild, are frequently spread across multiple trees (Palmer *et al.* 2000, Palmer

163 2003). Larger colonies of ants typically occupy more trees (Palmer 2004). We did not find any  
164 difference in ant colony size near or far from termite mounds as measured by counting workers  
165 in the domatia of the selected tree (Wilcoxon Rank Sum  $W = 1060$ ,  $p\text{-value} = 0.7045$ ), but,  
166 again, we did not test for spread across trees. If colonies were spread across trees, our results  
167 would imply ants on trees far from mounds have a different strategy--investing more in defense  
168 because they occupy fewer trees, and each tree is more important. Alternatively, the off-tree  
169 movement may represent ants moving within colony, across trees, with ants in colonies near  
170 termite mounds spending more time moving between trees. However, the role of off-tree  
171 movement for colonies spread across trees is not well understood in protective ant-plant  
172 mutualisms (Debout *et al.* 2007). Finally, another limitation was that our study occurred during a  
173 severe drought. Ant behavior changes seasonally in response to precipitation, with a reduction in  
174 defensive behaviors during the dry season (Rudolph & Palmer 2013). This suggests that  
175 environmental stressors lead to different ant behaviors, like less aggressive defense that may  
176 reduce the benefits that maintain the mutualism. Future research will be necessary to understand  
177 how context-dependence of different stressors shapes variation in mutualistic behaviors in ant-  
178 plant systems.

179         In conclusion, termite mounds spatially structure resources in ways that can influence the  
180 behavior of mutualist ants and ultimately the dynamics of the *C. nigriceps*-acacia mutualism.  
181 Reduced investment in rewards to mutualistic partners could destabilize the mutualism.  
182 Consequently, destabilized mutualisms may lead to turnover to different ant symbionts,  
183 facilitating coexistence between ant species colonizing acacia trees (Palmer 2003, Palmer *et al.*  
184 2010). Future work should examine if the variation in defensive and off-tree movement we



185 observed is unique to *C. nigriceps* or is also observed in other partners, and how this variation  
186 contributes to coexistence between members of the ant guild.

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## 199 **DATA AVAILABILITY**

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

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## 202 **SUPPORTING INFORMATION**

203 Additional Supporting Information may be found in the online version of this article:

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

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

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

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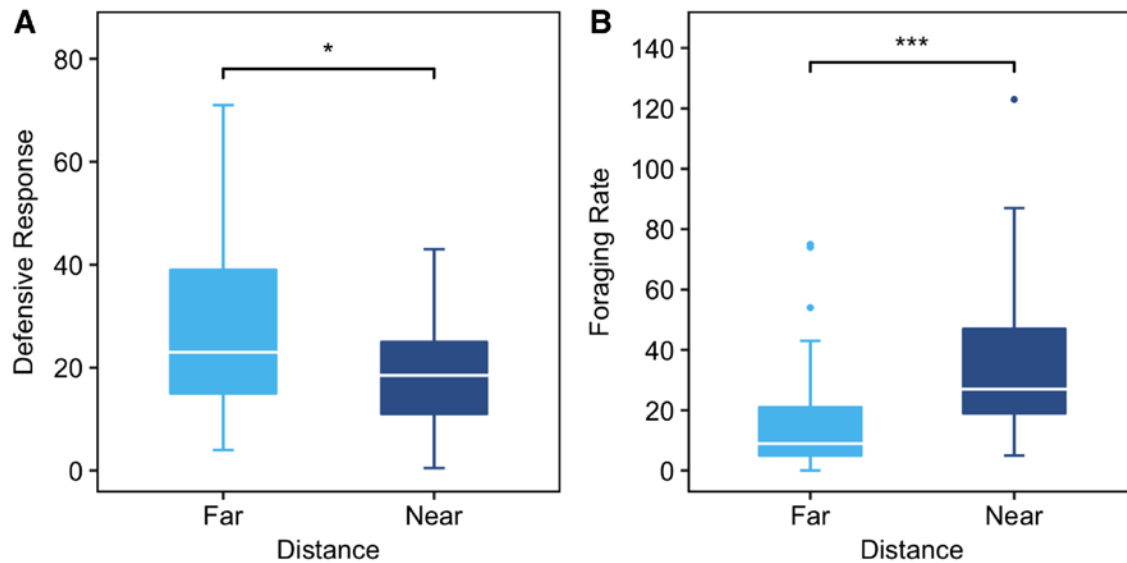
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287 **FIGURE 1.** (A) The defensive response of colonies near and far from termite mounds to  
288 simulated herbivory. Ants in trees near termite mounds had reduced defensive responses to  
289 simulated herbivory (Mann-Whitney U,  $p=0.025$ ,  $U=733.5$ ). The defensive response of a colony  
290 was calculated as the average number of ants counted on 2 gloves after 30 seconds simulated  
291 herbivory. (B) Off-tree movement, as a proxy for foraging rate, of colonies by distance from  
292 termite mounds. Ants in trees near termite mounds had increased off-tree movement (Mann-  
293 Whitney U,  $p<0.001$ ,  $U=733.5$ ). Foraging rate was measured as ants moving up or down the  
294 base of a tree trunk in 60 seconds.

295