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Economy of scale: third partner strengthens a keystone ant-plant mutualism

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Abstract. While foundation species can stabilize ecosystems at landscape scales, their ability to persist is often underlain by keystone interactions occurring at smaller scales. *Acacia drepanolobium* is a foundation tree, comprising >95% of woody cover in East African black-cotton savanna ecosystems. Its dominance is underlain by a keystone mutualistic interaction with several symbiotic ant species in which it provides housing (swollen thorns) and carbohydrate-rich nectar from extra-floral nectaries (EFN). In return, it gains protection from catastrophic damage from mega-herbivores. *Crematogaster mimosae* is the ecologically dominant symbiotic ant in this system, also providing the highest protection services. In addition to tending EFN, *C. mimosae* tend scale insects for carbohydrate-rich honeydew. We investigated the role of scale insects in this specialized ant-plant interaction. Specifically, does this putatively redundant third partner strengthen the ant-plant mutualism by making the ant a better protector of the tree? Or does it weaken the mutualism by being costly to the tree while providing no additional benefit to the ant-plant mutualism? We coupled observational surveys with two scale-manipulation experiments and found evidence that this third partner strengthens the ant-plant mutualism. Trees with scale insects experimentally removed experienced a 2.5X increase in elephant damage compared to trees with scale insects present over 10 months. Reduced protection was driven by scale removal causing a decrease in ant colony size and per capita baseline activity and defensive behavior. We also found that ants increased scale-tending and the density of scale insects on trees when EFN were experimentally reduced. Thus, in this system, scale insects and EFN are likely complementary, rather than redundant, resources with scale insects benefitting ants when EFN production is low (such as during annual dry periods in this semi-arid ecosystem). This study reveals that a third-partner strengthens an ant-plant mutualism that serves to stabilize a whole ecosystem.

Key words: ant-hemipteran mutualism; ant-plant mutualism; foundation species; keystone interaction; myrmecophyte; tripartite mutualism.

Introduction

Deep sea vents, coral reefs, mangroves, seagrasses, and dominant trees in forests and savannas are examples of "foundation species" that play key roles by forming the basis of food webs, and influencing biodiversity and ecosystem processes (sensu Dayton 1972, Ellison et al. 1996, Stachowicz 2001, Carpenter et al. 2008, Goheen and Palmer 2010). While foundation species can stabilize ecosystems at landscape scales, their ability to persist are often underlain by keystone species interactions occurring at much smaller scales (Stachowicz 2001, Palmer et al. 2015). Mutualisms can drive high population growth rates of partners and facilitate ecological dominance (May 1982, Bronstein 1994, Simberloff and VonHolle 1999), and as a result often underlie dominant, foundation species (Dayton 1972, Stachowicz 2001). Identifying key players in mutualisms and determining how their interactions facilitate the dominance of foundation species is critical to uncover how species interactions contribute to ecosystem stability.

Mutualistic interactions between ants and other organisms can play key roles in structuring communities and ecosystems (Farji-Brener and Illes 2000, Christian 2001, O'Dowd et al. 2003, Frederickson et al. 2005, Palmer et al. 2008, Prior et al. 2015). Some of these interactions are highly specialized, such as those between symbiotic ants and plants that house ants in specialized structures ("myrmecophytes"). Myrmecophytes occur in over 100 genera of tropical angiosperms, with several species being conspicuous or ecologically important components of tropical communities (Davidson and McKey 1993, Heil and McKey 2003). *Acacia (Vachellia) drepanolobium* is a myrmecophytic foundation species that occurs in "black cotton" savannas in many areas of East Africa (Ahn and Geiger 1987, Young et al. 1997, Khaemba et al. 2001). In these savannas, *A. drepanolobium* comprises >95% of the woody cover, forming an integral component of the food web and playing a critical role in several ecosystem processes (Pringle and Fox-Dobbs 2008, Riginos 2009,

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Fox-Dobbs et al. 2010). Its dominance is underlain by a keystone interaction with several symbiotic species of ants for which it provides housing and other resources, in return gaining protection from herbivores including large mammals such as elephants, *Loxodonta africana* (Palmer et al. 2008, Goheen and Palmer 2010, Palmer and Brody 2013, Pringle et al. 2016).

Myrmecophytes make several investments in ant partners. In addition to housing, many species provide nutritional resources to ants, including carbohydrates. Carbohydrates play essential roles in ant colony energetics, providing fuel necessary for activities such as foraging for protein, nest protection, and colony and population growth (Davidson 1997, Grover et al. 2007, Ness et al. 2009, Kay et al. 2012). Plants provide carbohydrates to ants directly via nectar from extrafloral nectaries (EFN) or indirectly, via a common third partner, hemipteran trophobionts that feed on plant phloem and produce carbohydrate-rich waste ("honeydew") (Davidson and McKey 1993). Carbohydrate investments to ants can be costly because carbon is shunted away from other physiological needs of plants (Pringle 2016). As a result, plants may have reduced growth or reproduction when housing ants (e.g., Stanton and Palmer 2011, Frederickson et al. 2012, Palmer and Brody 2013, but see Rudgers and Gardener 2004, Rutter and Rausher 2004). However, if losses to herbivory reduce reproduction or survivorship, carbon allocation to effective defenders can be adaptive (Bronstein et al. 2006).

The benefits of ant-tended hemipterans to plants can vary between antagonistic and mutualistic, given that they add a cost not incurred by plants that provide only direct resources to ants (Heil and McKey 2003, Styrsky and Eubanks 2007). When ants tend hemipterans, plants likely have limited control over carbohydrate flow because scale insects tap directly into plant phloem. As a result, ants can easily act as parasites if they tend scale insects at levels above what is optimal for the host plant (Becerra and Venable 1989, Davidson and McKey 1993, Gaume et al. 1998, Yu and Pierce 1998). Thus, the role of scale insects as tripartite mutualists, in which they provide net benefits to plants, is uncertain. This is especially the case for specialized myrmecophyte associations in which the costs of maintaining ant associates are particularly high (Heil and McKey 2003, Styrsky and Eubanks 2007, Pringle et al. 2011).

Ants tend hemipterans in a large proportion of myrmecophytes ($\approx$ 67%), of which $\approx$ 19% also have EFN (Davidson and McKey 1993). Ants readily shift between foraging for nectar and honeydew (McKey 1991, Davidson and McKey 1993, Katayama and Suzuki 2003). As a result, the importance of hemipterans as third partners varies among plant species (Davidson and McKey 1993, Heil and McKey 2003). They play more essential roles for myrmecophytes that do not directly provide carbohydrate resources to ants (i.e., species that lack EFN; e.g., Fiala and Maschwitz 1991, Fonseca 1993, Pringle et al. 2011), and less essential or more putatively redundant roles in cases in which plants directly provide carbohydrates via EFN (e.g., Young et al. 1997, Gaume et al. 1998). EFN are thought to be a derived trait in plants (Ward 1991, Davidson and McKey 1993), having evolved to give plants control over carbohydrate flow to ants (Becerra and Venable 1989, except see Buckley 1983, Delclaro and Oliveira 1993). However, ants tend hemipterans in most myrmecophyte species, including several species that also produce EFN (Davidson and McKey 1993). The role of hemipterans in ant-plant systems in which ants tend EFN is especially interesting given that, in these cases, hemipterans may play a more putatively redundant and potentially costly role.

Crematogaster mimosae, the ecologically dominant symbiotic ant associated with *A. drepanolobium*, tends EFN and hemipteran scale insects (Family: Coccidae). Although partnering with *C. mimosae* is costly to host plants, this ant species provides a net benefit to plants in the form of protection (Palmer et al. 2010, Stanton and Palmer 2011, Palmer and Brody 2013). We examined the role of hemipteran partners in this ant-plant mutualism, particularly in weakening or strengthening the interaction between *A. drepanolobium* and *C. mimosae*. Specifically, we ask if scale insects strengthen the mutualism by making the ant a better protector of the host plant or weaken the mutualism by providing no additional benefits to ants and plants, while being an extra cost to plants. We predict that scale insects benefit ants and host plants by playing a complementary rather than a redundant role to EFN in the provisioning of carbohydrate resources to ants. To examine the role of scale insects in this ant-plant mutualism, we coupled observational surveys with a longer-term experiment in which we measured ant and plant responses to the manipulation of direct and indirect resources to ants, and a shorter-term in situ colony behavior experiment to uncover mechanisms by which this third partner benefits its ant and plant partners.

Several other observational studies have examined if hemipterans function as third partners in obligate myrmecophyte mutualisms, generally finding that scale insects benefit plants (Gaume et al. 1998, Itino et al. 2001, Pringle et al. 2011). Our manipulative study provides a particularly robust test of the role of scale insects in the functioning of a specialized ant-plant mutualism. Additionally, most research on mutualisms is strongly phytocentric, examining how ants influence plant traits or fitness (Ness et al. 2010 except see e.g., Itino et al. 2001, Frederickson et al. 2012). By measuring how ant colonies respond to manipulations of indirect and direct plant-provided resources, we are measuring the effect of host plants on ants, along with how this interaction feeds back to affect host plants.

Materials and Methods

Study system

We conducted this research in a semiarid (annual rainfall = 550 mm/yr), savanna-bushland in the Laikipia region in Kenya at Mpala Research Centre (MRC)

(0°17′ N, 37°52′ E). Rainfall in this region follows a weakly tri-modal annual pattern, with a dry season from December to March. *Acacia drepanolobium* is the monodominant overstory tree in heavy clay "black cotton" soils in this ecosystem (Ahn and Geiger 1987, Young et al. 1997). This plant is well defended, with long, sharp stipular spines, a subset of which are swollen and hollow ("domatia"), providing living space for symbiotic ants. Ants forage on nectar produced from 1 to 3 EFN on bipinnate leaves (Young et al. 1997). These two plant-provided resources for ants are phenotypically plastic, costly to the tree, and are reduced following browser exclusion (Huntzinger et al. 2004, Palmer et al. 2008, Stanton and Palmer 2011).

Acacia drepanolobium houses four species of mutually exclusive symbiotic ants that co-occur at fine spatial scales in this study area (Hocking 1970, Young et al. 1997, Palmer et al. 2000). Three species, *Tetraponera penzigi, Crematogaster nigriceps,* and *C. mimosae*, nest exclusively in domatia, while *C. sjostedi* nests in trunk and stem cavities (Palmer et al. 2008). *Crematogaster mimosae* is the dominant ant, occupying ≈ 50% of the trees in our study area (Young et al. 1997), with multi-queen colonies spanning 1–4 trees (Palmer 2004). Ant species compete for host plants, resulting in a high frequency of turnover in species occupancy (Palmer et al. 2000, 2010, Palmer 2004). *Crematogaster mimosae* is the most aggressive defender of the tree from herbivores (Stanton and Palmer 2011), along with being dominant over all other species, except *C. sjostedi* (Palmer 2004). Both *C. mimosae* and *C. sjostedi* tend hemipteran scale insects (Family: Coccidae), with the former tending scale insects to higher degree than the latter (Young et al. 1997). Scale insects tended by *C. mimosae* are putatively of two species, the first occurring inside domatia along the main stem, and the second on the underside of stems outside of domatia. These scale insects are morphologically distinct, and have been tentatively described as *Hockiana* sp. (inside domatia) (Baker 2015, Baker et al. 2016) and *Ceroplastes* sp. (outside domatia) (Young et al. 1997). In our study area, the scale species occurring outside the domatia is more common than the species occurring inside the domatia. Young et al. (1997) found scale insects outside domatia present on 45% of 122 surveyed trees occupied by *C. mimosae*, and scale insects present inside domatia on 15% of trees. In our survey of 130 trees (see below), we found scale insects outside domatia on 95% of *C. mimosae*-occupied trees (Appendix S1: Fig. S1). In our experimental trees (see below), we found scale insects inside domatia on 47% of trees (N = 164), all of which had scale insects present outside of domatia.

Surveys of scale density and ant behavior

To examine the relationship between scale insects and ant behavior, we surveyed 130 trees in 13 locations, with each location separated by at least 200 m (10–15 trees per location). We surveyed trees that were occupied by *C. mimosae* along 20 m wide belt transects perpendicular to a dirt access road that were 2–2.5 m in height and were at least 20 m apart. On each tree, we counted all visible scale insects (outside domatia), and calculated scale density as the number of scales per elliptical canopy area that we estimated by measuring the length and width of the canopy. We also counted all intact domatia, and estimated active EFN by choosing two branches each at four polar coordinates and counted the number of active EFN out of five on the most distal leaves on each branch. Active EFN are red, green, or yellow while inactive EFN are dark (Palmer et al. 2008). We measured baseline ant activity, by assessing ant behavior prior to disturbing the tree. On three, 30 cm branch segments, we counted individual ants within and moving into segments for 30 s. To examine ants behavioral response to attack by a vertebrate herbivore, we simulated an attack by holding onto new growth with a leather glove, performing a chewing motion with our hand three times, and counting the number of ants swarming onto the glove after 10 s (Palmer and Brody 2013). Baseline activity and defensive behavior were correlated ($r = 0.236$).

We performed a linear model with three-predictor variables: scale density (no. scales/canopy cm^2), the number of active EFN, and the number of domatia on trees, and the response variable, baseline ant activity. We performed models with each predictor variable alone, in all two-way combinations, and with all three predictor variables. Predictor variables were not correlated ($r < 0.13$). Final models were chosen based on models with the lowest Akaike Information Criterion (AIC) score and if the $\Delta AIC \leq 2$ (Burnham and Anderson 2002; see Appendix S2). We performed a generalized linear model (GLM) with a negative binomial distribution (to account for overdispersion) for the number of ants swarming onto a glove and all predictor variables, choosing the final models as described above. All statistical analyses were conducted in R version 3.1.3 (2015) (R Foundation for Statistical Computing, Vienna, Austria). The MASS package was used to run negative binomial models (Venables and Ripley 2002) and the bbmle package was used to compare AIC scores and calculate AIC weights (Bolker 2014).

Longer-term scale manipulation experiment: effects on ant colony size and herbivory

We manipulated scale insects and plant-provided resources to ants (domatia and EFN) for 10 months to examine how these resources to ants affected tree growth and reproduction, ant colony size, and the ability of colonies to protect trees from herbivores. First, we located trees occupied by *C. mimosae* in which scale insects were present. *Crematogaster mimosae* colonies can occupy multiple trees (typically 1–4) (Palmer 2004), and we chose colonies that spanned two trees to standardize colony size and to determine changes in colony size by assessing if a tree was taken over by another ant colony (of the same or different ant species) over the 10-month experiment.

Colonies were delineated by reciprocally transplanting workers in domatia among all trees within a 5 m radius from a chosen focal tree (see Appendix S1). Ants were deemed to be from separate colonies if they engaged in aggressive interactions (see Palmer 2004 for details). Each colony contained a larger "focal" tree and a smaller "satellite" tree.

We randomly assigned one of eight treatments blocked by tree size (sum of the two tree canopy areas) to each colony: domatia reduced, EFN reduced, scale insects reduced, and all combinations in a fully factorial design, including controls in which no resources were manipulated (N = 11 colonies per treatment, 88 in total). Domatia were reduced by cutting open every other domatia on focal and satellite trees, EFN were reduced by dotting out ~50% of active EFN with tree sealant (Tanglefoot®, Asphalt Pruning Sealer, Contech Enterprises, Victoria, British Columbia, Canada), and all visible scale insects (i.e., outside domatia) were removed by hand. We reduced direct plant resources, but removed all visible scale insects on branches to reflect the variation in these resources in nature; all trees produce domatia and EFN, albeit at varying levels (Huntzinger et al. 2004, Palmer et al. 2008), while scale presence and abundance is heterogeneous (Young et al. 1997), with individual trees ranging from 0 to 224 visible scales (Appendix S1: Fig. S1). However, since we could not remove the scale insects inside the domatia, the scale treatment was effectively a reduction treatment. Given that scale insects occurring outside domatia are more common than those occurring inside the domatia at our study site (Young et al. 1997), we were likely removing the majority of scale insects. We reapplied treatments every 2 months for the duration of the experiment, since scale insects returned and both EFN and swollen thorn domatia are produced on new growth (Appendix S1: Fig. S2). We performed procedural controls by cutting one stipular (non-swollen) thorn on every other domatia and dotting leaves with tree pruning sealer beside every other active EFN.

Pre-treatment and every other month prior to reapplying treatments, we measured new growth as the total length of green shoots and new domatia (i.e., red or green in color), and we calculated average new growth and new domatia production over the 10 months. Trees often fruit after the long-rain season in April, and we counted the number of fruit pods produced in the two surveys after the rainy season. In our final survey, we assessed the total damage on trees by vertebrate browsers other than elephants (e.g., giraffe, eland, Grant's gazelle, and steinbuck) by counting signs of browsing, including bite marks, and nipped branch tips. We assessed elephant damage by counting the number of elephant damage events on a tree, defined as: large broken branches, bark stripping, or top-browsing (Stanton and Palmer 2011). A handful of trees were catastrophically damaged in that they were completely knocked over. When this was the case, we assigned these trees a damage score that was +1 greater than the tree with the highest number of elephant damage events. We assessed leaf damage by invertebrate herbivores by haphazardly choosing five leaves on new growth and assigning each leaf to one of four leaf damage categories (1, 0–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%). Finally, we counted scale insects and assessed active EFN by haphazardly choosing three branches with new growth and counting the number of active EFN on five leaves on each branch.

We estimated colony size before treatments and 10 months after treatments by collecting three intact domatia each from three different sizes classes: small (<2 cm in diameter), medium (2–3 cm), and large (>3 cm) from each tree. Workers, reproductive ants, and brood were counted in each collected domatia. We counted the number of intact domatia in each size class on each tree and extrapolated to total colony size from the ants counted in the collected domatia. Given the number of ants and size of trees, we were not able to count all ants on trees. Counting the number of ants in a subset of domatia and extrapolating to the tree has been previously used in studies in this system as a way to estimate colony size (Palmer 2004) (see Appendix S1 for further discussion). Pre-treatment all colonies spanned two trees, but some trees were either lost or gained by intraspecific or interspecific colony take-overs over the course of 10 months. We assessed tree takeover dynamics by re-delineating colony boundaries post-treatment (see Appendix S1). Colony size was determined by the density of worker ants inside domatia, the number of intact domatia on trees, and the number of trees occupied by a colony.

We ran linear models with each treatment effect separately (domatia, EFN, and scale reduction), along with all two-way interactions, and the three-way interaction to test for effects on host plant growth and reproduction: average total new growth, average number of new domatia (log-transformed), and fruit production after the rainy season (log-transformed). We ran linear models with all treatment combinations on post-treatment colony size (there was no difference in pre-treatment colony size [$P > 0.05$]). We also used a linear model to test for relationships between colony size and baseline ant activity. We ran GLM with negative binomial error distributions (to account for overdispersion) to test the effects of treatments on herbivore damage: vertebrate browser damage events, elephant damage events, and insect damage (category number). Last, we used a linear model on final scale density only in the non-scale removal treatments, and a binomial GLM on the proportion of active EFN only for the non-EFN removal treatments. In all cases, we compared AIC scores of all treatment models and reported statistics and P-values for the models with the lowest AIC scores, and $\Delta AIC \leq 2$ (see Appendix S2: Tables S1–S3).

Shorter-term scale removal experiment: effects on colony behavior

To examine how plant-provided (nectar) and scale-provided (honeydew) carbohydrates influenced colony

behavior of *C. mimosae*, we assessed behavior under alternative carbohydrate removal scenarios over a short period (4 weeks). Forty *C. mimosae* trees that were 2–2.5 m tall, at least, 20 m apart, and had at least 75 scale insects were chosen. One of four treatments were randomly assigned to trees: EFN completely removed, visible scale insects completely removed, both EFNs and scale insects removed, and controls. All EFN were occluded (as described above), and visible scale insects were removed by hand. In this case, we maintained treatments every 2 weeks to ensure consistent and significant removal of carbohydrate resources. We placed a Tanglefoot® barrier on the trunk of the tree to prevent ants from leaving the tree or the colony to be taken over (that we also re-applied). Given that ants could not abandon the tree or be taken over by other colonies, and were forced to forage on the tree (limiting the intake of the protein necessary for colony growth), along with the short duration of the experiment, we assume that ant responses in this experiment were primarily due to changes in behavior, rather than in colony size.

Pre- and post-treatment (after 30 d), we monitored several ant behaviors three times on each tree pre-treatment, and six times on each tree post-treatment. We conducted all trials between the hours of 10:00 and 16:00 on non-windy days. We measured baseline ant activity, and the number of ants swarming onto a glove (see above for descriptions for details). We also monitored scale and EFN tending behaviors by locating at least five scale insects or five active EFN in a 10 cm segment of a haphazardly chosen branch on each tree, and counting the number of ants visiting EFN or scales for 30 s. We did not keep track of individual ants so multiple visits by the same ant to resources were possible. The number of scale insects or EFN were counted, and we calculated the number of ants per EFN or scale insect present.

We found no differences in behavior prior to the imposition of our treatments ($P > 0.05$). We conducted linear models on baseline ant activity, and on the number of ants responding to simulated herbivore attack with scale removal, EFN removal, and their interaction post-treatment. The AIC scores of the models were compared, and statistics for the final models can be found in Appendix S2. Last, we conducted linear models on the number of ants tending EFN (per EFN) in the presence and absence of scales, and on the number of ants tending scales (per scales) in the presence and absence of EFN.

Results

In our observational surveys, for baseline activity, the model with the lowest AIC score contained EFN and scale density, with a significant positive relationship between scale density and baseline activity ($F_{1,125} = 51.23$, $P \ll 0.001$) and EFN and baseline activity ($F_{1,125} = 19.97$, $P < 0.001$; Fig. 1a; see Appendix S2: Table S1). For the number of ants responding to simulated herbivore attack, the model with the lowest AIC score also included EFN and scale density, with a positive relationship between scale density (GLM: $\chi^2_{1,126} = 144.86$, $P \ll 0.001$) and response to simulated attack, but not with EFN and response to simulated attack ($\chi^2_{1,126} = 175.15$, $P = 0.589$; Fig. 1b; Appendix S2: Table S1).

In our longer-term scale reduction experiment, we found no effect of treatments on average new growth (Appendix S2: Table S2). However, EFN reduction had a positive effect on the average number of fruits ($F_{1,83} = 4.2$, $P = 0.044$; Appendix S2: Fig. S3) and scale reduction had a marginally positive effect on the average number of new domatia ($F_{1,84} = 3.7$, $P = 0.056$; see full statistical results in Appendix S2: Table S2). Elephant damage events increased by 2.5X when scale insects were removed. Scale, domatia × scale, and domatia all had $\Delta AIC \leq 2$ with only

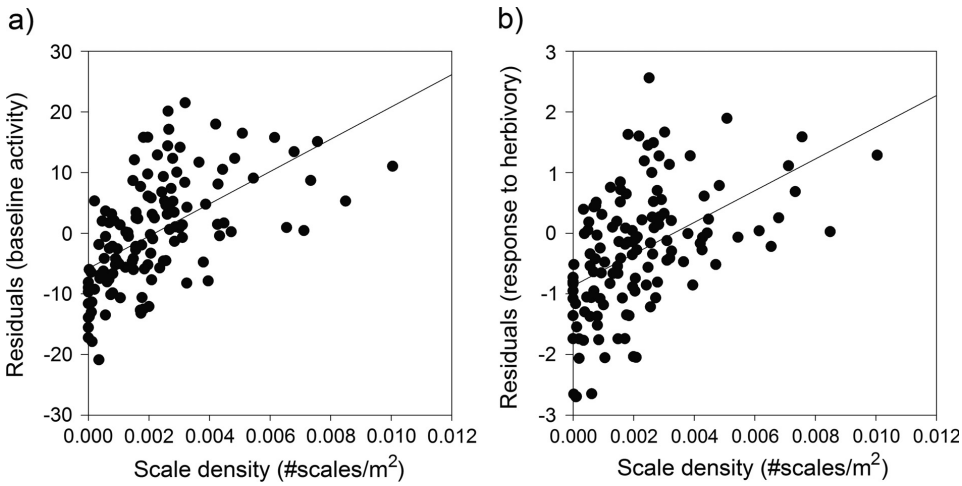

Fig 1. Partial regression plots between scale density (no. scales/canopy area m^2) and the residuals of regressions between (a) baseline activity and EFN, and (b) ants responding to simulated herbivory and EFN (N = 130). Baseline activity ($P < 0.001$, $R^2 = 0.32$), and the number of ants responding to simulated herbivory ($P < 0.001$, $R^2 = 0.23$) had positive relationships with scale density and EFN.

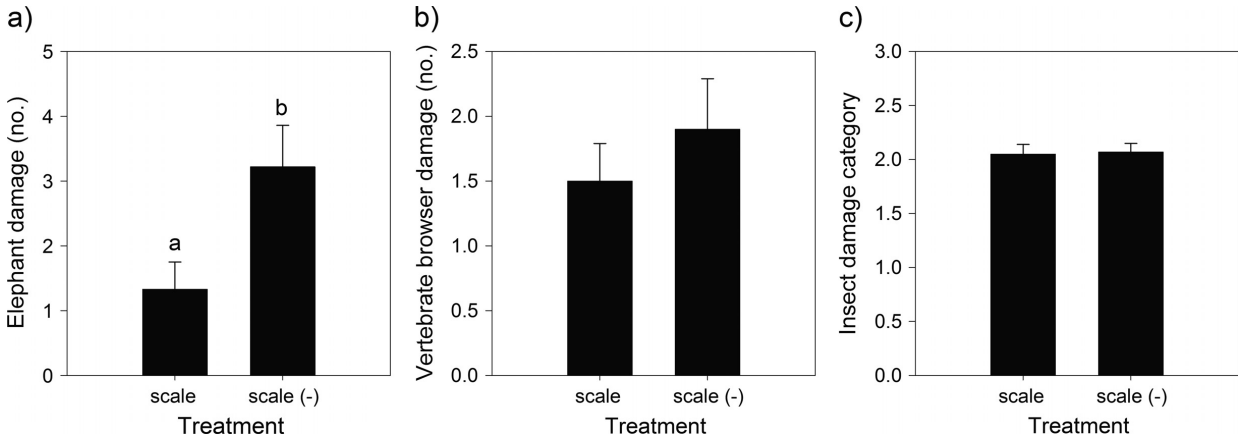

FIG 2. Mean (±SE) number of (a) elephant damage events, (b) other vertebrate browser damage events, and, (c) insect damage (category 1–4) in scale insect control and reduction treatments after 10 months. Means are calculated by pooling domatia and EFN removal treatments that did not have significant effects on any of the damage types. Different letters represent significant differences between treatments.

scale removal having a significant effect on elephant damage ($\chi^2_{1,84}$ = 58.3, P = 0.0374; Fig. 2a; Appendix S2: Table S2). There were no treatment effects on damage due to vertebrate browsers (Fig. 2b), or invertebrate herbivores (Fig. 2c; Appendix S2: Table S2). Colony size was smaller in the scale reduction treatment, but only when domatia was also reduced (domatia × scale, $F_{1,78}$ = 5.3, P = 0.023; Fig. 3a; Appendix S2: Table S2). This reduction in colony size was driven by a higher proportion of trees being taken over in the domatia x scale reduction treatments (Fig. 3b). There was a weak positive relationship between colony size and baseline ant activity on the focal trees in the last survey ($F_{1,79}$ = 3.82, P = 0.054, R^2 = 0.046).

In our shorter-term experiment, scale removal had stronger effects on ant behavior than EFN removal, with no interaction between the resource types (Appendix S2: Table S3). When scale insects were removed, baseline ant activity decreased (scale, $F_{1,38}$ = 18.46, P < 0.001; Fig. 4a); and fewer ants responded to simulated herbivore attack (scale, $F_{1,38}$ = 6.09, P = 0.018; Fig. 4b). When EFN were removed, ants spent more time tending scales ($F_{1,19}$ = 4.62, P = 0.045; Fig. 5a). However, when scales were removed there was only a trend for increased EFN-tending ($F_{1,19}$ = 3.37, P = 0.083; Fig. 5c). Additionally, in our longer-term experiment, we found a significant increase in scale density post-treatment in the EFN removal

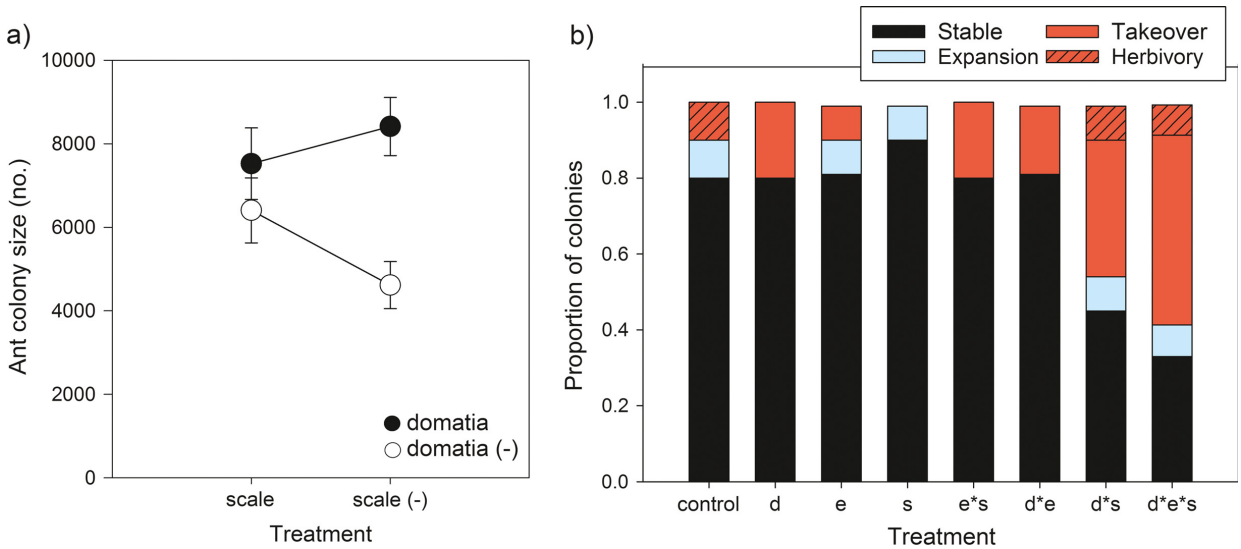

FIG 3. (a) Mean (±SE) estimates of ant colony size in scale insect control and reduction treatments, and domatia control (solid circles) and reduction (open circles) treatments in the 10-month experiment. Means are calculated over the EFN treatment that did not have a significant effect. The interaction between domatia reduction and scale reduction (P = 0.001) influenced ant colony size. (b) The proportion of *C. mimosae* colonies that remained stable (i.e., on two trees) after 10 months, experienced a colony expansions (i.e., expanded to one or more trees), or a colony reduction by losing a tree to a catastrophic herbivory event or a takeover by another ant colony.

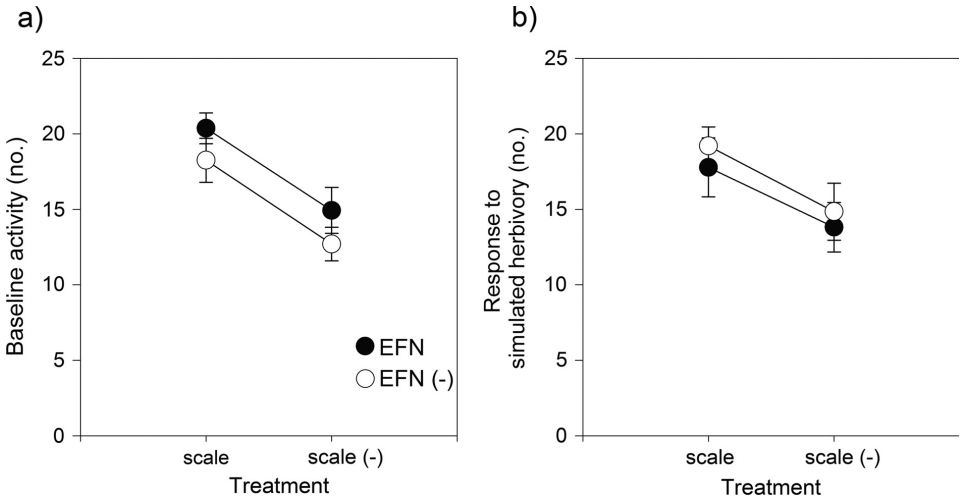

FIG 4. Means (±SE) of (a) baseline ant activity, and (b) ants responding to simulated herbivory attack in scale control and scale reduction treatments and EFN control (dark) and EFN reduction (light) treatments in the 1-month behavior experiment (n = 10). Scale reduction was significant in reducing ant activity (P = 0.001) and response to simulated herbivore attack (P = 0.020).

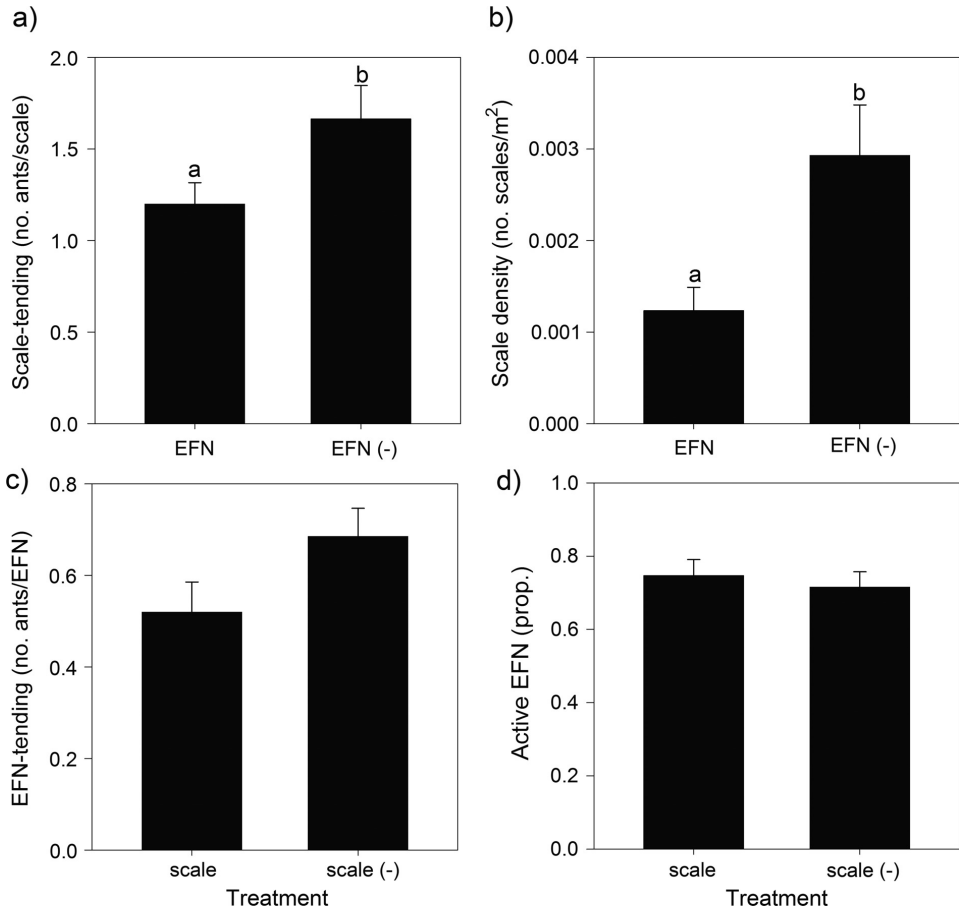

FIG 5. Mean (±SE) (a) number of ants tending scale insects (per scale), and (b) density of scale insects on trees in EFN control and reduction treatments. Mean (±SE) (c) number of ants tending EFN (per EFN), and (d) proportion of active EFN on trees in scale control and reduction treatments. (a) and (c) are from the 1-month colony-level behavior experiment. (b) and (d) are from the 10-month scale reduction experiment. Different letters indicate significant differences between treatments.

treatments (EFN, $F_{1,40} = 5.04$, $P = 0.031$; Fig. 5b), but not in the proportion of active EFN on trees that had scales removed (scales, $\chi^2_{1,40} = 132.09$, $P = 0.3730$; Fig. 5d; see Appendix S2: Table S3).

Discussion

Our results suggest that a third partner strengthens the specialized mutualism between *C. mimosae* and *A. drepanolobium* by increasing the defensive efficacy of ants against elephants. *Crematogaster mimosae* colonies were more active and displayed greater defensive behavior on trees with naturally high scale insect densities. Experimentally manipulating scale insects revealed that both ants and host plants benefited from the ant-scale interaction. Removing scale insects caused a 2.5X increase in elephant damage that was likely driven by a reduction in ant colony size and per capita baseline activity and defensive behavior. The mutualism between *A. drepanolobium* and its symbiotic ants stabilizes tree cover in this savanna ecosystem by protecting trees from catastrophic destruction by elephants (Goheen and Palmer 2010, Stanton and Palmer 2011). Our study shows that a third partner plays an important role in strengthening this keystone ant-plant mutualism.

Our results suggest that EFN and scale insects provide complementary, rather than redundant, carbohydrate resources to ants that influence their role as mutualists. A benefit of EFN to ants is that they provide a consistent resource in space; all trees have EFN, while scale insects are only present on a subset of trees occupied by *C. mimosae* (Young et al. 1997; Appendix S1: Fig. S1). Here we show that one benefit that scale insects provide is that they are a more consistent resource than EFN in time. During the dry season and periods of more prolonged drought, *A. drepanolobium* produces little or none of the new growth on which EFN are produced and the most active EFN occur (Palmer et al. 2008). On the other hand, we found that scale insect density did not decline during the dry season, suggesting that ants can continue to gain carbohydrate resources from tending scale insects for honeydew when trees are not producing new growth that support active EFN (Appendix S1: Fig. S2). Thus, supplemental carbohydrates obtained from scale tending might be particularly important for *C. mimosae* during annual dry periods.

Our results also suggest that another benefit of scale insects to ants is that ants are likely able to regulate their carbohydrate intake from scale insects more than from EFN. We found that reducing EFN increased scale tending and the number of scale insects on trees over 10 months, while reducing scale insects had little effect on the number of active EFN on trees. Ants likely have more control over the amount of resources they acquire from tending scale insects that than from tending EFN (Becerra and Venable 1989, Davidson and McKey 1993, Gaume et al. 1998, Yu and Pierce 1998). One way that ants may manipulate resource intake from EFN and scale insects is by influencing the secretion or exude rates of nectar and honeydew (Agrawal and Rutter 1998, Heil et al. 2000, Katayama et al. 2013). We did not test if ants influence secretion or exude rates in our system; however, our results suggest the possibility that ants have higher control over the number of scale insects on host plants than the number of active EFN. Several other studies have also found that scale insect abundance can be controlled by ants (Buckley and Gullan 1991, Abbott and Green 2007). In contrast, the production of new EFN is largely under control of the host plant (reviewed in Heil 2015). In our system, while ants may be able to keep EFN active on *A. drepanolobium* via tending, EFN eventually become inactive, with new EFN produced with new leaves and shoots.

We do not know if nectar or honeydew differs in quality or quantity in our system. Nectar and honeydew contain mostly water and sugars with some amino acids (Baker et al. 1978). Variation between nectar and honeydew content is highly variable both within and among host plant species, but generally nectar contains higher concentrations of simple sugars such as glucose and fructose, and honeydew contains more polysaccharides. One of these polysaccharides, melezitose, has been shown to be particularly attractive to some ant species (Beattie 1985, Bluthgen et al. 2004, Katayama et al. 2013). Other than resource quality, variation in the quantity of resources that ants can gain from tending EFN or scale insects could differentially benefit ants. Katayama et al. (2013) found that while the volume of nectar per unit provided more sugars to ants than honeydew, the quantity of honeydew produced per hemipteran per day was higher than that of nectar per EFN and colonies of just two hemipterans provided greater total sugar to ants than the host plant (that had 8–10 EFN).

While scale insects may act as a buffer when EFN is reduced over short time periods, such as during annual dry cycles, previous work suggests that scale insects cannot fully compensate for EFN loss over a prolonged period. In a 10-yr large herbivore exclusion experiment Palmer et al. (2008) found that domatia and EFN were reduced in the absence of large herbivores, but that scale insects over this longer period did not completely buffer loss of EFN (and domatia). Similar to results from our present study, they found more scale insects on trees with fewer active EFN (in the large herbivore removal treatment). However, this increase in scale insects did not compensate for the reduction in direct resources to ants. Ant colony size and activity were still reduced when herbivores were excluded. EFN (and domatia) likely set the minimum limit for ant colony function, whereas scale insects might provide a boost to *C. mimosae* colonies.

We found that ant colonies' ability to protect trees from elephants were compromised when scale insects were removed, but not when EFN or domatia were removed. This reduction in protective efficiency could be due to scale insects affecting ant colony size or per capita

ant defensive behavior. Palmer and Brody (2013) manipulated ant colony size and found an increase in elephant damage; so, ant colony size clearly influences ants' ability to protect trees. We found that ant colony size decreased when domatia and scale insects were removed together (scale × domatia interaction), but not when scale insects were removed alone. Thus, our results show that ant colony size is influenced by scale insects and domatia together. Given this finding, the effect of scale insects on ants' ability to protect trees is only partially mediated by its influence on ant colony size. In our shorter-term experiment, we controlled for ant colony size by forcing ants on trees, and we found that removing scale insects decreased baseline activity and defensive behavior. This result suggests that scale insects also influence the ants' efficacy against herbivores by increasing their per-capita activity level and energy for defense. Taken together, the effect of scale insects on the efficacy of ants to protect host plants is likely driven by a combination of the effect of these supplemental resources on ant colony size, and the energy ant colonies allocate to defensive behavior, which has been shown to increase in other systems where there are surplus carbohydrates (e.g., Ness et al. 2009).

Crematogaster mimosae ants are costly for *A. drepanolobium* to host (Stanton and Palmer 2011, Palmer and Brody 2013). Here, we link the cost of maintaining ants to mutualism-related resources. We found an increase in new domatia production when scale insects were reduced, and an increase in fruit set when EFN were reduced, suggesting that both scale and EFN are costly. However, we only manipulated resources over a short period and these long-lived plants could take years for costs to be fully realized (Stanton and Palmer 2011). Despite not fully knowing the long-term costs of hosting scales to the host plant, we show that the putative cost is likely outweighed because scale presence reduces elephant damage on trees. Given that elephants affect tree survival (a high cost), the contribution that scale insects make to enhancing the protective service of ants can translate into a large fitness benefit to the host plant. If the cost of hosting scales to host plants were high, we would expect that there may be selective pressure for plants to evolve defenses against scale insects (e.g., increased woody biomass, or chemical defenses in phloem) (Frederickson et al. 2012). However, our study shows that the net benefit of scale insects to ants and host plants are likely high enough that there should be little selection for defense against scales. Given that EFN and scale insects provide complementary resources that benefit both ant and host plant fitness, this tripartite mutualism might be adaptive. Studies in several other systems have revealed that mutualisms often involve multiple mutualistic partners that provide complementary rewards (Gaume et al. 1998, Currie et al. 2003, Palmer et al. 2010, Afkhami et al. 2014, Afkhami and Stinchcombe 2016).

We were only able to remove and assess the abundance of the scale insect occurring outside of the domatia, but not the scale insect occurring inside of the domatia that is putatively of a different species (Young et al. 1997, Baker 2015, Baker et al. 2016). Domatia removal likely also reduced the number of scale insects inside the domatia, potentially confounding the effect of domatia removal with scale insect removal. Recall that we found that colony size was affected only when domatia and visible scale insects were removed together, but not when domatia or scale insects were removed alone. In addition to reduced living space, reduction in scale abundance inside the domatia could be one mechanism by which the number of domatia on a tree can influence ant colony size. Different scale insect species tended by the same ant species can play varying roles in ant-plant mutualisms (Gaume et al. 1998), and uncovering the role of the scale insect inside the domatia would provide us with a more complete understanding of this rich multispecies mutualism.

The strength of mutualisms is predicted to be higher in low-productivity environments where resources are limited (Bronstein 1994, Thrall et al. 2007), and several studies have found evidence for this expectation (Kersh and Fonseca 2005, Pringle et al. 2013). We suggest that scale insects benefit ants in this water-stressed system by increasing access to carbohydrates during times in which the ants may have limited access directly from the plant. Uncovering the direct link between plant stress, EFN production and scale insects would be an interesting avenue for future research. Also, *A. drepanolobium* is widespread throughout the East African tropics (Dharani 2006), and there is some evidence that there is variation in the identity and presence of scale insects (Baker 2015). It would be interesting to examine these interactions throughout the trees broader distribution, in which we might expect this tripartite mutualism to be more prevalent in areas with higher water stress.

Ants that tend hemipterans are often ecologically dominant in communities (Bluthgen et al. 2004). *Crematogaster mimosae* is the numerically dominant symbiotic ant in our study area (Young et al. 1997). There is high turnover of ant occupancy on trees, and competition-colonization tradeoffs among ant species support coexistence (Stanton et al. 2002, Palmer 2004). The relative dominance of each ant species and the order in which they colonize the host plant over its lifetime drives the population demographics and stability *of A. drepanolobium* cover (Palmer et al. 2008, 2010). We found that more colonies were taken over by neighboring *C. mimosae* and *C. nigriceps* colonies when scale insects were removed, and we also discovered that ants are more willing to engage in interspecific fighting with *C. nigriceps* in trees with high scale insect densities (K. M. Prior, *unpublished data*). We do not know how scale insects disperse, or how scale insects are spatially distributed, other than that a subset of *C. mimosae* trees have scale insects, and that scale insects range from 0 to 224 (median = 48) (Appendix S1: Fig. S1). Future work to uncover the role of scale insects in facilitating the ecological

dominance of *C. mimosae* in this community is an important next step in understanding the role of third partners in the broader context of this multispecies mutualism.

Our experiments enabled us to uncover the important function of a third partner, demonstrating a link between its presence and abundance and the ability of symbiotic ants to protect host plants from damage by large herbivores. *Acacia drepanolobium* is a foundation tree in this ecosystem, forming the base of the food web and structuring the broader plant and animal community (Pringle and Fox-Dobbs 2008, Riginos 2009). At the landscape scale, tree cover for this foundation species is underlain by a keystone interaction between symbiotic ants and the host plant at much smaller scales (Goheen and Palmer 2010, Stanton and Palmer 2011, Pringle et al. 2016). Our study reinforces the idea that multispecies mutualisms at small scales can facilitate the persistence of foundation species that shape entire ecosystems.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2104/suppinfo

Data Availability

Data associated with this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.sh1p2.