Trait-mediated indirect interactions in invasions: unique behavioral responses of an invasive ant to plant nectar

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Abstract. Exotic species often form beneficial, facultative associations with indigenous species. However, we still have a limited understanding of the influences that these positive associations may have on the dynamics and impacts of species invasions. Highly invasive species may respond differently than less invasive species to resources that are exchanged in mutualisms, leading to trait-mediated indirect interactions between native species via invaders that may reshape native communities. In this study, we tested the hypothesis that the highly invasive ant species, Anoplolepis gracilipes, exhibits stronger trait changes in response to increasing levels of nectar than co-occurring, less invasive ant species. Across two islands in the Samoan Archipelago, we located multiple sites dominated by A. gracilipes and multiple sites dominated by other, less invasive species. At each site, we manipulated nectar levels on a common extrafloral nectary-bearing shrub and assessed short-term changes in ant worker recruitment and aggression. We found that the recruitment response of the highly invasive ant species A. gracilipes was not unique: other dominant ant species also increased recruitment in response to increasing nectar levels. However, A. gracilipes did show unique changes in aggressive behaviors: as nectar levels increased, the proportion of prey discovered, attacked and removed by A. gracilipes workers and the speed at which they performed these aggressive behaviors all increased strongly. Other ant species showed no such responses. In addition, fewer subordinate ants persisted on plants at sites invaded by A. gracilipes. Finally, plot-level, simultaneous manipulations of ant access to the plants and nectar availability demonstrated that Morinda citrifolia-ant mutualisms influenced the β-diversity of local arthropod communities differently when A. gracilipes dominated local ant assemblages. These results suggest that mutualisms between invasive ants and native plants can modify interactions between invaders and co-occurring arthropods, possibly leading to more negative consequences for native communities. They also underscore the importance of incorporating both positive species interactions and indirect pathways into our studies of both community ecology and invasion biology.

Key words: aggression; Anoplolepis gracilipes; ant-plant mutualisms; forager recruitment; invasive species; Morinda citrifolia; nectar; Samoa; trait-mediated indirect interactions.

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INTRODUCTION

Although traditionally studied as pairwise associations, interspecific mutualisms involve interactions among diverse assemblages in complex, multispecies communities. The outcome of mutualisms can depend strongly upon the identity of the interacting partners (Bronstein 1994). For example, Acacia drepanolobium trees associate with several species of ant bodyguards;
these ant species not only differ in the costs and benefits provided to host trees, but also influence plant host fitness most strongly at different phenological stages. Consequently, the fitness of *A. drepanolobium* plants is highest when the plants associate with multiple ant species throughout their lifetime (Palmer et al. 2010). Significant variation in the costs and benefits associated with different mutualist partners has been reported for other arthropod-plant protection mutualisms (Rudgers and Strauss 2004, Whitney 2004, Miller 2007), mycorrhizal mutualisms (Maherali and Klironomos 2007, Mangan et al. 2010), pollination mutualisms (Gomez et al. 2010), and seed dispersal mutualisms (Whitney 2005, Manzaneda and Rey 2008).

Because mutualisms are embedded in complex communities, variation in partner identity may have significant consequences for community structure and function. The community-wide consequences of mutualisms are still poorly understood. However, recent studies have demonstrated that the abundance, diversity, and composition of local communities can be strongly influenced by the presence of mutualistic interactions (Stachowicz et al. 2001, Bruno et al. 2003, Hay et al. 2004, Rudgers et al. 2007, Lach 2008, Matthews et al. 2009). Furthermore, variation in the degree and type of benefits exchanged in mutualisms can have cascading effects on the structure and dynamics of communities. For example, Rudgers et al. (2010) demonstrated that geographic variation in the benefits provided to plants by ant guards was associated with significant differences in the composition and abundance of plant-associated arthropods that were not directly involved in the mutualism.

We have a limited understanding of the mechanisms that underlie these community-level responses to variation in mutualist partner identity. However, interaction modifications via trait-mediated indirect pathways likely influence these differential effects of mutualist partners on ecological communities. These effects occur when the presence of one species modifies one or more traits of another species, with consequent effects on other community members through interaction modification (Werner and Peacor 2003). For example, Bernot and Turner (2001) demonstrated that periphyton-consuming *Physa integra* snails sought refuge in the presence of predacious fish, but not predacious crayfish. Consequently, periphyton abundance was as much as 110% higher in mesocosms with fish than those with crayfish. Similar trait-mediated community-level shifts could occur when different species are involved in mutualistic associations.

Species invasions provide ideal systems for investigating the importance of species identity in ecological interactions. Invasive species often display exaggerated traits compared to native species (e.g., more rapid growth rates; Pysek and Richardson 2007, van Kleunen et al. 2010), and these traits may influence co-occurring community members. Additionally, novel interactions between invaders and native species provide a contrast to the same associations involving native species that share a long history of interaction. When exotic species invade novel habitats, they may respond differently than native species to the resources or services exchanged in mutualisms. Moreover, it is likely that different exotic species differ in their responses to mutualist-derived resources, although such a pattern has never been explicitly described, to our knowledge. If a highly invasive species displays different traits (e.g., responses to carbohydrate availability, see below) when it displaces native or less invasive species in facultative mutualisms and this trait change results in community-level effects, then trait-mediated pathways may be important mechanisms underlying the detrimental impacts of species invasions. However, surprisingly few studies have investigated the role of trait-mediated indirect interactions in invasions. White et al. (2006) recently surveyed the ecological literature and found only two studies that experimentally demonstrated that (1) novel positive interactions between an invader and a native species influenced co-occurring community members via interaction modification and (2) these effects were trait-mediated. In both cases, the presence of an invasive plant altered pollinator visits to native plant species (Grabas and Laverty 1999, Chittka and Schurkens 2001). Since non-native species frequently form positive associations with native species (Bruno et al. 2003), we could be underestimating the importance of indirect, trait-mediated mechanisms in driving the dynamics and impacts of species invasions.

In this study, we investigated positive interac-
tions between ants and extrafloral nectary (EFN)-
bearing plants in Samoa. Like many other island
ecosystems, Samoa is dominated by non-native
ant species (Wetterer and Vargo 2003), in
particular by the highly invasive species Anoplo-
lepis gracilipes. A pan-tropical ‘tramp ant,’ A.
gracilipes has strong negative impacts on native
island flora and fauna in the Pacific and Indian
Oceans (Holway et al. 2002, Hill et al. 2003,
2009). Anoplolepis gracilipes invasions are hypoth-
esized to be driven by subsidies from carbohy-
drate-excreting plants and insects (Holway et al.
2002, Davidson et al. 2003, Lach 2003). In support
of this hypothesis, we observed that variation in
the abundance of EFN-bearing plants was posi-
tively associated with the abundances of A.
gracilipes across the Samoan Archipelago, but
was unrelated to the abundances of other exotic
ant species (Savage et al. 2009). However, the
precise mechanisms underlying the invasive
impacts of A. gracilipes are poorly understood.
In a recent experiment, we demonstrated that A.
gracilipes responded to increasing levels of plant
nectar by both recruiting strongly to EFN-
bearing plants and decreasing tending behaviors
of honeydew excreting insects (Savage et al.
2011). These results, together with our earlier
surveys across the Samoan Archipelago, suggest-
ed the hypothesis that unique responses of A.
gracilipes to nectar might underlie the particularly
strong negative impact of A. gracilipes on
arthropod communities.

Carbohydrate subsidies may facilitate ant
invasions through both density and trait-mediat-
ed indirect pathways. Here, we focus on the
prediction that carbohydrate-rich, mutualist-de-
ferred resources (such as plant nectar) influence
the behaviors of highly invasive ants more
strongly than the behaviors of co-occurring less
invasive ants. Nectar availability in the Pacific
varies both naturally (Savage et al. 2009) and in
response to human activities, e.g., the creation of
plantations of Morinda citrifolia, an economically
important EFN-bearing plant (Potterat and Hamb-
gerer 2007). Therefore, we examined predictions
of the carbohydrate subsidy hypothesis across a gradient of nectar availability. Specifi-
cally, we hypothesized two separate, but non-
mutually exclusive, trait-mediated indirect path-
ways whereby these interactions could influence
arthropod community structure. First, we hy-
pothesized that (1) as nectar resources were
experimentally increased, the highly invasive
ant species A. gracilipes would recruit more
workers to EFN-bearing plants than other non-
native ant species. We expected that increased A.
gracilipes recruitment would be associated with
increased activity outside the nest and increased
negative impacts on co-occurring arthropods (via
predation and/or the threat of predation) relative
to less invasive ant species (dotted lines, Fig. 1A).
Second, we hypothesized that (2) as nectar
resources were increased, highly invasive A.
gracilipes workers would become more aggres-
sive toward co-occurring arthropods than less
invasive ants. Increased levels of aggression
could also lead to stronger negative communi-
ity-wide impacts of A. gracilipes relative to less
invasive ants (dashed lines, Fig. 1A). Importantly,
both of these pathways are not dependent upon
changes in the density of invasive ants through
increased colony growth (Oliver et al. 2008),
because they require only a change in the
allocation of workers to tasks (nectar foraging
vs. other behaviors) or a change in worker
aggression, respectively. Thus, we consider both
of these hypothesized indirect interactions be-
tween nectar-producing plants and the arthro-
pod community to be trait-mediated. Finally, we
predicted (3) that changes in partner identity
within M. citrifolia-ant mutualisms (A. gracilipes
vs. all other ant species) would cascade to affect
co-occurring arthropods, leading to reductions in
community diversity when A. gracilipes is present
(Fig. 1A, solid arrows).

METHODS

Study sites

We conducted nectar manipulation experi-
ments from 16 June 2009 to 26 September 2009
on the islands of Tutuila, American Samoa and
Savaii, Independent Samoa (Table 1). In a
previous survey spanning six islands of the
Samoan archipelago, we found that A. gracilipes
were least abundant and widespread in Tutuila
and most abundant and widespread in Savaii
(Savage et al. 2009). We selected eight sites
dominated by A. gracilipes and six sites dominat-
ed by other ant species (with A. gracilipes absent).
Data on foraging recruitment from three of the 14
sites were used in a previous study of an *A. gracilipes* invasion front in northeastern Savaii (Savage et al. 2011). On Savaii, sites were separated by 150 m–66.7 km (mean 27.12 ± 9.46 km); sites on Tutuila were separated by 150 m–35.1 km (mean 16.11 ± 3.90 km). We do not have data on average foraging distances for *A. gracilipes* or co-occurring ant species in Samoa. However, average foraging distances for *A. gracilipes* in Australia are ~30 m (Ben Hoffmann, personal communication) indicating that foragers observed in different sites were likely from different colonies.

Because it would be logistically difficult and unethical to experimentally manipulate the identity of the dominant ant species at a site (e.g., adding a highly invasive ant species to a previously uninvaded site), there is the risk that observed differences in ant species responses to nectar manipulations are confounded by different environmental conditions at sites dominated by different species. To partially address this concern, we examined spatial autocorrelation of ant species responses (forager recruitment and per-worker aggressive responses to experimentally manipulated nectar levels, see below). We constructed Euclidean distance matrices of (1) geographic distances between sites and (2) dissimilarities between relevant test statistics per site per response variable. We used betas from regressions for the forager recruitment responses and chi square values for aggressive responses. We then conducted Mantel tests using the RELATE function in Primer v. 6.1.10 (Clarke

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Fig. 1. Interaction web diagrams depicting (A) hypothesized and (B) actual trait-mediated indirect interactions (TMII) between plant nectar and herbivores. Solid lines represent direct interactions and dotted lines represent TMII. Dotted lines on the left side of the diagrams are associated with *Anoplolepis gracilipes* and those on the right side are associated with other dominant ant species. Thicker lines represent stronger effects. (A) We predicted that nectar would affect traits of both highly invasive and less-invasive dominant ant species similarly, but with stronger effects for highly invasive ant species. (B) Actual patterns detected in our experiments. Both *A. gracilipes* and other dominant ant species responded to plant nectar by increasing forager recruitment, but *A. gracilipes* was the only species to increase aggression in response to increasing nectar levels.
Table 1. Descriptions of ant assemblages at sites on the islands of Savaii (S) in Independent Samoa and Tutuila (T) in American Samoa in 2009. Invasiveness scores (see Table 2 for detailed description) are reported as percentages of the total possible score. Densities are reported from surveys of ants on the ground and on Morinda citrifolia plants (mean ± SE). Dominant species were defined as the species at each site with the highest numerical abundance. Subordinate species included all species except the numerically dominant species.

<table>
<thead>
<tr>
<th>Site</th>
<th>Dates of experiments</th>
<th>Dominant species</th>
<th>Invasiveness score (%)</th>
<th>Density of dominant species</th>
<th>Density of all subordinate species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ground</td>
<td>M. citrifolia</td>
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<tr>
<td>Falealupo (S)</td>
<td>September</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>23.5 ± 6.36</td>
<td>46.50 ± 8.66</td>
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<td></td>
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<td></td>
<td>1.00 ± 0.58</td>
<td>2.00 ± 2.16</td>
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<td>Mauga_West (S)</td>
<td>June</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>21.25 ± 2.72</td>
<td>46.50 ± 4.80</td>
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<td></td>
<td>2.00 ± 0.91</td>
<td>4.75 ± 1.65</td>
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<tr>
<td>Mauga_South (S)</td>
<td>September</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>38.75 ± 1.32</td>
<td>53.21 ± 4.65</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Saleaula_East (S)</td>
<td>June</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>28.0 ± 1.47</td>
<td>39.25 ± 2.14</td>
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<td>2.50 ± 1.04</td>
<td>1.75 ± 0.63</td>
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<tr>
<td>Saleaula_North (S)</td>
<td>September</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>17.5 ± 2.96</td>
<td>33.00 ± 4.14</td>
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<td>0</td>
<td>1.00 ± 0.41</td>
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<td>Saleaula_South (S)</td>
<td>September</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>15.38 ± 3.99</td>
<td>29.44 ± 4.37</td>
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<td>0.22 ± 0.22</td>
<td>2.33 ± 1.0</td>
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<tr>
<td>Futiga (T)</td>
<td>August</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>12.50 ± 3.23</td>
<td>15.13 ± 3.59</td>
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<td></td>
<td>3.23 ± 0.48</td>
<td>1.38 ± 0.63</td>
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<tr>
<td>Masausi_East (T)</td>
<td>August</td>
<td>Anoplolepis gracilipes</td>
<td>100</td>
<td>8.13 ± 1.03</td>
<td>36.50 ± 5.17</td>
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<td>0</td>
<td>2.00 ± 0.71</td>
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<tr>
<td>Mauga_North (S)</td>
<td>September</td>
<td>Pheidole megacephala</td>
<td>83</td>
<td>9.40 ± 1.60</td>
<td>11.32 ± 0.98</td>
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<td>2.31 ± 0.86</td>
<td>5.61 ± 2.15</td>
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<tr>
<td>Fagatogo (T)</td>
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<td>Pheidole megacephala</td>
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<td>6.75 ± 3.90</td>
<td>8.25 ± 2.78</td>
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<td>0.25 ± 0.25</td>
<td>2.78 ± 4.42</td>
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<td>Tafuna (T)</td>
<td>July</td>
<td>Solenopsis geninata</td>
<td>61</td>
<td>12.25 ± 1.75</td>
<td>10.50 ± 3.07</td>
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<td>1.50 ± 0.65</td>
<td>2.50 ± 1.19</td>
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<tr>
<td>Gatai (S)</td>
<td>September</td>
<td>Paratrechina longicornis</td>
<td>33</td>
<td>15.75 ± 5.11</td>
<td>21.75 ± 4.53</td>
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<tr>
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<td></td>
<td>8.75 ± 5.91</td>
<td>10.0 ± 4.08</td>
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<tr>
<td>Masau (E)</td>
<td>August</td>
<td>Tapinoma melanoccephalum</td>
<td>22</td>
<td>11.25 ± 3.01</td>
<td>56.25 ± 4.00</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3.25 ± 2.36</td>
<td>3.40 ± 1.68</td>
</tr>
<tr>
<td>Illilli (T)</td>
<td>July</td>
<td>Tetramorium bicarinatum</td>
<td>11</td>
<td>2.25 ± 0.63</td>
<td>0.75 ± 0.48</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>7.00 ± 1.58</td>
<td>4.50 ± 2.53</td>
</tr>
</tbody>
</table>

and Gorley 2007) with Spearman rank correlations and 9,999 iterations. We conducted these analyses both across all sites and for sites within each island (for a total of three tests per response variable). We interpret a lack of spatial autocorrelation in these responses as an indication that we are measuring differences in ant species biology as opposed to differences in environmental conditions.

Additionally, due to natural abundance patterns, 75% of the sites (six of eight) dominated by Anoplolepis gracilipes were located on Savaii and 67% of the sites (four of six) dominated by other ant species were located on Tutuila (Table 1), leading to potential island effects. Therefore, we conducted additional tests to examine whether ant responses to our experimental manipulations of nectar availability (described below) differed between islands. For forager recruitment data, we used ANOVAR; for proportional aggression data, we used logistic regression; and for data on the time it took ants to perform different aggressive responses, we used survival analyses. All tests were conducted for both A. gracilipes and for other dominant ant species; models included island and treatment as factors. Anoplolepis gracilipes responses did not significantly differ on Savaii versus Tutuila (all $P > 0.25$), nor did the responses of other ant species differ between the islands (all $P > 0.10$). These patterns suggest that, while island and dominant ant identity are partially confounded, island effects are not sufficiently large to bias our findings.

During the time of the experiments, average monthly rainfall was 0.1325 mm ($\pm 0.19$ mm) and average daily temperatures ranged between 27.08°C and 29.6°C in Tutuila (Pago Pago Weather station); temperatures ranged between 25.83°C and 29.17°C with no measurable rainfall in Savaii (Avao Weather Station).
Study organisms

Numerically dominant ant species at our study sites were all exotic and varied their invasiveness (both on EFN-bearing plants and the ground; Table 1). Using the methods of Ward et al. (2008), we calculated invasiveness scores based upon literature accounts of the biological traits associated with invasiveness and the ecological impacts of the invaders. We report each species' score as a percentage (species score/maximum possible score × 100). Scores ranged from 11% (Tetramorium bicarinatum) to 100% (A. gracilipes) (Table 2).

Across the Samoan Archipelago, ants have access to multiple resources for carbohydrates, the most common and abundant being the extrafloral nectary (EFN)-bearing plant, Morinda citrifolia. While the timing and nature of arrival of M. citrifolia to islands throughout the Pacific are unclear (i.e., natural vs. Polynesian-mediated dispersal ~3000 years ago; Whistler 1993, Nelson 2006), we consider it to be native. Morinda citrifolia possesses annular disk nectaries clustered on inflorescences (hereafter ‘nectary body’; Waki et al. 2007, Fig. 2). At our sites in Samoa, nectary bodies contained 2 to >50 nectaries and reached a maximum size of 80 cm³. Morinda citrifolia plants produce nectary bodies year-round. Ants frequently visit nectaries of this plant, which are often dominated by the highly invasive ant species, A. gracilipes (Savage et al. 2009).

Characterization of the ant assemblage at each site

At each site, we first assessed local ant assemblages by haphazardly selecting three M. citrifolia plants and counting the number and identity of ants on each plant. We used a comprehensive count of all ants per plant completed in ~5 minutes/plant. We also counted ants on the ground ~1 m from each plant, using a 10 × 10 cm white paper card and counting the number of ants that crossed the card in 30 sec (following methods in Abbott 2005). The cards were laminated with plastic and these assessments were conducted on different days (at least 2 days between counts). Therefore, it is unlikely that any pheromones were absorbed by the cards. However, even if this occurred, any pheromones likely dissipated by the time that cards were reused. We used the card approach because ~60% of all sites were located on lava fields, making it impossible to use pitfall traps. We also collected foragers at tuna and honey baits at all sites. However, these responses were not substantially different from card counts. We opted to use card counts here, because they are less likely to be influenced by dominance hierarchies than resources.

Experimental design

We established 5 m × 5 m plots of plants at each site, focusing on areas dominated by M. citrifolia. Depending upon the size of the site and the naturally-occurring densities of M. citrifolia plants, we established 3–5 plots per site. Plots were separated from each other by ~8 m to increase the independence of observations. Within each plot, we haphazardly selected five M. citrifolia plants that were similar in size and within 1 m of each other to be used for nectar manipulations. We first counted the number of nectary bodies, aphids (Aphididae), scale insects (Coccididae), and mealybugs (Pseudococcidae) on each plant and measured the height of the main stem and the diameter of the base of each plant to the nearest cm. Each plant was then randomly assigned to a nectar availability treatment (see below).

Hypothesis 1: As nectar levels increase, highly invasive ants will recruit more workers to EFN-bearing plants than less invasive ants

We manipulated nectar availability at the plant scale: 0, 50, 100, 150, and 200% of ambient levels per plant. To reduce access to nectar, we secured bags constructed of lightweight organza material to the base of nectary bodies with a plastic cable tie. Bags were ~1.5–2× larger than nectary bodies to minimize contact between nectaries and bagging material. We bagged all nectary bodies, regardless of treatment assignment, to control for any effects of the bags. However, in all treatments except 0%, we cut holes (~3–6 cm) in bags to allow ants to access actual M. citrifolia nectar. Thus, for plants in the 50% treatment, we cut holes in half of the bags, and all bags had holes in the 100%, 150%, and 200% treatments.

We used artificial nectaries (Seal-Rite micro-centrifuge vials, USA Scientific 1605-0000, Ocala, Florida, USA, filled with 500 μL of a 30% sucrose solution) to supplement nectar levels. In a
previous study, Freeman et al. (1991) demonstrated that *M. citrifolia* nectar is dominated by sucrose, with sucrose contributing an average of 72.6–88.9% to total nectar carbohydrates. To inform the design of our nectar availability treatments, we assessed natural nectar production and concentration for *M. citrifolia*. We excluded insect visitors from nectary bodies for 24 h and collected nectar in microcapillary tubes. Average nectar production per plant per day was 2249 $\mu$L ± 642 SE (range = 645–5226, $n = 6$ plants). The concentration of *M. citrifolia* nectar, using a field refractometer (EZ-Red B1, EZ Red Co., Deposit, New York), was 28.06% ± 1.04% SE.
(n = 8 plants). Therefore, we considered the 30% sucrose solution in our artificial nectaries to be a reasonable approximation of naturally-occurring *M. citrifolia* nectar. We inserted a 5 μL microcapillary tube into the center of each microcentrifuge vial to allow ants to access sucrose in the artificial nectaries. Air bubbles occasionally formed inside the microcapillary tubes. Therefore, all microcapillary tubes were cleared (removed and then re-inserted) ~15 minutes before each census. Vials were affixed to plants using twist ties, and all plants received 10 vials. The 0, 50, 100, 150 and 200% treatment levels contained 0, 0, 0, 5 and 10 filled vials, respectively. Thus in each experiment, we provided local ant assemblages with 0, 0, 0, 2500 or 5000 μL of artificial nectar per plant over the course of 48hrs. Short-term pulses of nectar availability allowed us to disentangle trait- from density-mediated effects of nectar on local arthropod assemblages, since ant population growth responses to nectar would have required much more time.

**Response variables.**—We conducted six ant censuses per plant: morning (~6:00–8:00), mid-afternoon (~12:00–14:00), and evening (~1600–18:00) over two consecutive days. All censuses occurred during daylight hours due to Samoan cultural restrictions. While this sampling scheme provided a good estimate of the relative foraging rates for diurnal and crepuscular workers, it did not account for the nocturnal activities of local ant assemblages. Counts took ~5 minutes per plant. We collected specimens of each ant species from nearby non-treatment plants and identified them using Wilson and Taylor (1967) and Shattuck (1999).

**Data analyses.**—We compared ant responses at sites invaded by *A. gracilipes* versus sites where *A. gracilipes* was absent. At each site, we assigned ants to one of two dominance categories: dominant (the most abundant ant species per site) or subordinate (all other co-occurring ants; see Table 1). Although multiple metrics are used to describe ant dominance (e.g., behavioral, numerical), we focused on numerical dominance in this study (hereafter, we refer to numerical dominance simply as dominance). Subordinate ants at sites invaded by *A. gracilipes* included species that were dominant at *A. gracilipes*-uninvaded sites and other species that were subordinate both in the presence and absence of *A. gracilipes*. We then conducted repeated measures general linear models on ant density (number of workers per plant) following recommendations in von Ende (2001), with separate tests for sites invaded by *A. gracilipes* vs. not invaded. Models included the following independent factors: ant dominance status (dominant or subordinate), nectar treatment (0, 50, 100, 150 or 200% ambient levels), site, plot (nested in site), and all interactions with time (Proc GLM; SAS Institute 2003). Ant dominance status, site and plot were treated as categorical factors, and the nectar availability level was treated as a continuous factor. Plant size (height × diameter at base), the number of nectary bodies per plant, and the abundance of honeydew excreting insects per plant were used as covariates. Because the repeated factor (elapsed time) had no significant influence on ant responses to our treatments, we pooled data across time to simplify data presentation. Statistical analyses met assumptions of normality of residuals and
homogeneity of variances at each time following square-root transformation of ant density.

Finally, previous findings suggested that A. gracilipes invasions were associated with significant declines in co-occurring, plant-associated ant species (Savage et al. 2009). Therefore, we also used t-tests to determine if the abundance of subordinate ants on M. citrifolia plants differed between sites invaded or not invaded by A. gracilipes.

Hypothesis 2: As nectar resources increase, highly invasive ants will become more aggressive toward co-occurring arthropods than less invasive ants

To examine the relative effects of nectar levels on ant aggression, we followed the recruitment trials (above) with aggression trials ~12 h after the last recruitment survey. We replenished all filled tubes, so that each plant’s nectar availability was at the same level at the start of the aggression trials as it was at the start of the recruitment trials (0, 50, 100, 150 or 200%). Approximately 6 h later, we placed an M. citrifolia-feeding larva within 3 cm of a nectary body (1 larva per plant, 3–5 replicates per nectar treatment, resulting in 15–25 aggression trials per site). In Tutuila, we collected nitidulid (Coleoptera) larvae from fallen M. citrifolia fruits ~24 hours before aggression trials were conducted. These larvae were weighed to the nearest 0.1mg and randomly assigned to nectar availability treatments. Although nitidulid larvae were common in Tutuila, they were rare in Savaii, so we instead used nectary-body-feeding pyralid (Lepidoptera) larvae there. We used digital calipers to measure the length of pyralid larvae (collected from non-treatment plants) to the nearest 0.01 mm prior to aggression trials. For both test prey, there were no significant differences among the nectar treatments in the size of the larvae that were presented to ants (Nitidulidae: F = 0.01, P = 0.9210; Pyralidae F = 0.08, P = 0.7773). To assess potential differences in ant responses to prey types, we conducted an additional test using both nitidulids and pyralids which co-occurred at one site in Savaii (Saleaula_North). We found no significant differences in the responses of ants to the two different larvae at that site (F = 0.71, P = 0.4027). Therefore, we pooled ant responses across both target prey types in the analyses (below).

Response variables.—Based upon prior observations of ant behaviors (Savage, unpublished data), we determined that the interaction between ants and herbivorous larvae occurs quickly; therefore trial length was 150 seconds (unless the larva was removed from the plant by ants before the elapsed time). For each trial, we recorded the time it took ants to discover, attack, and/or remove each larva from treatment plants. Discovery occurred when ants approached the larva and tapped it with their antennae. Attack occurred when ants bit, stung, or sprayed formic acid at the larva. Removal occurred when ants forcibly ejected the larva from the plant, either by throwing it off the plant or by carrying it away from the plant.

Data analyses.—We examined aggressive behaviors both in terms of the proportion of prey larvae that were subject to ant aggression and in terms of the time required for the aggressive behaviors to be initiated. We used logistic regression (Proc LOGISTIC; SAS Institute 2003) with a binomial distribution and a chi square test to evaluate proportional data. To examine the time it took ants to perform aggressive behaviors, we conducted survival analyses (Proc LIFEREG, SAS Inc. 2003, version 9.1, Cary, NC) with a Weibull distribution and Wald Chi square test statistics. This type of analysis allowed us to account for right-censored data.

We first examined the relative aggression displayed by A. gracilipes vs. other dominant, exotic ant species in terms of the overall proportions of prey discovered, attacked, and removed and the time to perform these behaviors. To do this, we conducted logistic regressions and survival analyses (as described above) with A. gracilipes invasion status and site (nested in A. gracilipes invasion status) as categorical factors. For these tests we used the full dataset and pooled data across all nectar treatment levels.

Next, we assessed the influence of the nectar treatment on the relative aggression displayed by A. gracilipes and other dominant ant species towards prey larvae. Because we were interested in the independent effects of nectar on different stages of ant aggression, we examined prey discovery, attack and removal in terms of the proportion of all larvae that were discovered, the proportion of discovered larvae that were at-
tacked, and the proportion of attacked larvae that were removed from *M. citrifolia* plants. Similarly, we examined the time to discovery of all prey, the time to attack of discovered prey, and the time to removal of attacked prey. In all models (both logistic regressions and survival analyses), the factors included *A. gracilipes* invasion status, the nectar treatment, and their interaction. We also included site (nested in *A. gracilipes* invasion status) and the forager recruitment response of the dominant ant species for each plant (as estimated in the recruitment trials, above) as covariates. Inclusion of the latter covariate allowed us to isolate the effects of per-worker aggressiveness from the effects of worker number on the proportions of prey discovered, attacked, and removed and on the time to perform these behaviors.

Finally, when the interaction between *A. gracilipes* invasion status and the nectar treatment was significant, we conducted post-hoc linear and quadratic regressions, with the nectar treatment as the independent variable and the average proportion of prey discovered, attacked, and removed and the time to perform these behaviors as dependent variables. When both linear and quadratic models were significant, we used F-tests to evaluate model fit and present only the best-fit model.

**Evidence for community-level indirect effects mediated by ants**

In order to demonstrate that TMII could have community-level effects in this system, we examined dynamics of *M. citrifolia*-ant mutualisms at sites dominated by *A. gracilipes* versus those dominated by other ant species. To do this, we conducted a plot-level, factorial experiment in which we simultaneously manipulated ant access to (permitted/excluded) and nectar levels of *M. citrifolia* plants (reduced/ambient). This experiment was replicated across sites dominated by *A. gracilipes* (*n* = 6) and those dominated by other ant species (*n* = 5) on the island of Savaii. Three and six months after treatment application, we sampled arthropod communities using both sweepnets and surveys on the plants. A detailed description of this experiment and responses of arthropod communities can be found in Savage et al. (*in preparation*). Here, we present data on β-diversity of plant-associated arthropods (excluding ants and honeydew-excreting insects). To assess these arthropod responses, we conducted a permuted dispersion test of among-plot dissimilarity (PERMDISP using Primer-E v. 6.1.13 with perMANOVA+ 1.0.3 extension, Clarke and Gorley 2007). The factors for this test were *A. gracilipes* invasion status, the ant access treatment and the nectar availability treatment. For the purposes of this study, we were particularly interested in determining if the presence of ants influenced interactions between the co-occurring arthropod community and plant nectar. Consequently, when the three way interaction between all three factors was significant, we conducted post-hoc pairwise tests comparing the effects of the ant access treatment within each *A. gracilipes* invasion status x nectar availability level.

**RESULTS**

**Lack of spatial autocorrelation in ant responses to nectar**

Analyses of spatial autocorrelation demonstrated that the ant responses to nectar described below are unlikely to be driven by spatial variation in environmental conditions, but are rather the result of species-specific differences in ant behaviors. Specifically, there was no significant spatial autocorrelation in forager recruitment responses (all tests: *P* > 0.21), the proportion of prey discovered (all tests: *P* > 0.48), the proportion of prey attacked (all tests: *P* > 0.44), the proportion of prey removed (all tests: *P* > 0.20), the time to prey discovery (all tests: *P* > 0.18), the time to prey attack (all tests: *P* > 0.11) or the time to prey removal (all tests: *P* > 0.13).

**Hypothesis 1: As nectar levels increase, highly invasive ants will recruit more workers to EFN-bearing plants than less-invasive ants**

We predicted that the highly invasive species *A. gracilipes* would demonstrate significantly stronger recruitment of workers to increasing nectar availability than other ant species (Fig. 1A). Within sites invaded by *A. gracilipes*, this prediction was supported. *Anoplolepis gracilipes* workers recruited strongly to *M. citrifolia* plants as experimentally manipulated nectar levels increased, with 281% more *A. gracilipes* workers observed on plants with the highest nectar levels.
(200%) compared to those with no nectar (Fig. 3a). In contrast, co-occurring subordinate ants were rarely observed on *M. citrifolia* plants, regardless of nectar availability level (Fig. 3a, Table 3: Dominance × Nectar treatment, *P* < 0.0001). The number of nectary bodies per plant also significantly influenced the abundance of ants per plant (Table 3). However, the response of ants to the nectar treatment was not significantly influenced by this covariate, as evidenced by a non-significant interaction between the number of nectary bodies and the nectar treatment. Similarly, ant responses to treatments did not vary over time (Table 3) at sites dominated by *A. gracilipes*.

Contrary to our predictions, other, less invasive ant species also recruited strongly to nectar resources in sites where they did not co-occur with *A. gracilipes* (Fig. 3b, Table 4). In fact, there was no significant difference between the responses of *A. gracilipes* and other ants to nectar availability (Invasion status × Nectar treatment: *F* = 0.30, *P* = 0.5857). Subordinate ants at sites lacking *A. gracilipes* did not display a significant response to experimentally elevated nectar levels, as evidenced by a significant Dominance × Nectar treatment interaction (Table 4, *P* = 0.0024). However, significantly more subordinate ants were observed foraging on *M. citrifolia* plants at sites without *A. gracilipes* than at sites with *A. gracilipes* (mean number of subordinate ants ± SE; *A. gracilipes* invaded sites = 0.90 ± 2.16; *A. gracilipes* uninvaded sites = 10.36 ± 2.50; t-test *t* = −7.2, *P* < 0.0001). At sites uninvaded by *A. gracilipes*, ant responses were not significantly influenced by any of the covariates, nor did they vary over time (Table 4).

**Hypothesis 2: As nectar resources increase, highly invasive ants will become more aggressive toward co-occurring arthropods than less invasive ants**

We predicted that highly invasive *A. gracilipes* workers would not only display higher overall levels of aggression, but also increase their aggressiveness more strongly in response to nectar than co-occurring less invasive ants (Fig. 1A). Overall aggression (pooling across nectar levels) was, in fact, higher for *A. gracilipes* than for other dominant ant species. Compared to sites where other ant species were dominant, the average proportion of prey larvae that were discovered (*χ²* = 6.53, *P* = 0.0106), attacked (*χ²* = 7.20, *P* = 0.0073), and removed (*χ²* = 6.42, *P* = 0.0113) was 27%, 203% and 460% higher, respectively, at sites where *A. gracilipes* was dominant (Fig. 4). On average, *A. gracilipes* workers also discovered prey 37% faster (*χ²* = 12.0, *P* = 0.0005), attacked prey 49% faster (*χ²* = 24.45, *P* < 0.0001), and removed prey 44% faster (*χ²* = 25.8, *P* < 0.0001) than the average time taken by other dominant ants (Fig. 5).

In support of our hypothesis, the amount of nectar strongly influenced aggressive behaviors of *A. gracilipes*, while aggression of other domi-
nant ant species was unresponsive to increasing nectar (Tables 5 and 6; Fig. 6). The number of foragers recruiting to nectar had a significant effect on the proportion of and time to prey discovery and the time it took ants to attack discovered prey (Table 4). However, the influence of nectar on ant aggression, particularly for *A. gracilipes*, was significant even when accounting for these numerical effects, indicating that nectar availability influenced per-capita worker aggression. There was a significant effect of the interaction between the nectar treatment and *A. gracilipes* invasion status for all responses with the exception of the proportion of prey removed (Tables 5 and 6). Specifically, at the highest nectar level (200%), *A. gracilipes* workers discovered 205% more prey in 89% less time (Fig. 6a, b), attacked 32% more discovered prey in 93% less

Table 3. Results from a repeated measures analysis of covariance of ant density per plant at sites invaded by *Anoplolepis gracilipes*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominance (Dominant/Subordinate)</td>
<td>1, 296</td>
<td>194.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nectar treatment</td>
<td>1, 296</td>
<td>35.74</td>
<td>&lt;0.0001</td>
</tr>
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<td>Dominance × Nectar treatment</td>
<td>1, 296</td>
<td>139.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>7, 296</td>
<td>4.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plot (Site)</td>
<td>28, 296</td>
<td>1.46</td>
<td>0.0730</td>
</tr>
<tr>
<td>Plant size</td>
<td>1, 296</td>
<td>1.74</td>
<td>0.1878</td>
</tr>
<tr>
<td>Abundance of honeydew-excreting insects (HEI)</td>
<td>1, 296</td>
<td>1.57</td>
<td>0.2113</td>
</tr>
<tr>
<td>Number of nectary bodies</td>
<td>1, 296</td>
<td>11.25</td>
<td>0.0009</td>
</tr>
<tr>
<td>Nectar treatment × Abundance of HEI</td>
<td>1, 296</td>
<td>0.00</td>
<td>0.9498</td>
</tr>
<tr>
<td>Nectar treatment × Number of nectary bodies</td>
<td>1, 296</td>
<td>4.65</td>
<td>0.0519</td>
</tr>
<tr>
<td>Time</td>
<td>5, 292</td>
<td>0.67</td>
<td>0.6459</td>
</tr>
<tr>
<td>Time × Dominance</td>
<td>5, 292</td>
<td>3.28</td>
<td>0.0068</td>
</tr>
<tr>
<td>Time × Nectar treatment</td>
<td>5, 292</td>
<td>1.23</td>
<td>0.2954</td>
</tr>
<tr>
<td>Time × Dominance × Nectar treatment</td>
<td>5, 292</td>
<td>0.76</td>
<td>0.5761</td>
</tr>
<tr>
<td>Time × Site</td>
<td>25, 168</td>
<td>6.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time × Plot (Site)</td>
<td>70, 168</td>
<td>1.34</td>
<td>0.0080</td>
</tr>
<tr>
<td>Time × Plant size</td>
<td>5, 292</td>
<td>0.29</td>
<td>0.9181</td>
</tr>
<tr>
<td>Time × Abundance of honeydew-excreting insects (HEI)</td>
<td>5, 292</td>
<td>0.50</td>
<td>0.7786</td>
</tr>
<tr>
<td>Time × Number of nectary bodies</td>
<td>5, 292</td>
<td>0.77</td>
<td>0.5744</td>
</tr>
<tr>
<td>Time × Nectar treatment × Abundance of HEI</td>
<td>5, 292</td>
<td>0.95</td>
<td>0.4465</td>
</tr>
<tr>
<td>Time × Nectar treatment × Number of nectary bodies</td>
<td>5, 292</td>
<td>0.63</td>
<td>0.6785</td>
</tr>
</tbody>
</table>

Table 4. Results from a repeated measures analysis of covariance of ant density per plant at sites uninvaded by *Anoplolepis gracilipes*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominance (Dominant/Subordinate)</td>
<td>1, 172</td>
<td>1.25</td>
<td>0.2647</td>
</tr>
<tr>
<td>Nectar treatment</td>
<td>1, 172</td>
<td>3.39</td>
<td>0.0671</td>
</tr>
<tr>
<td>Dominance × Nectar treatment</td>
<td>1, 172</td>
<td>9.47</td>
<td>0.0024</td>
</tr>
<tr>
<td>Site</td>
<td>5, 172</td>
<td>2.88</td>
<td>0.0159</td>
</tr>
<tr>
<td>Plot (Site)</td>
<td>14, 172</td>
<td>0.46</td>
<td>0.9521</td>
</tr>
<tr>
<td>Plant size</td>
<td>1, 172</td>
<td>0.00</td>
<td>0.9892</td>
</tr>
<tr>
<td>Abundance of honeydew-excreting insects (HEI)</td>
<td>1, 172</td>
<td>1.34</td>
<td>0.2485</td>
</tr>
<tr>
<td>Number of nectary bodies</td>
<td>1, 172</td>
<td>0.06</td>
<td>0.8015</td>
</tr>
<tr>
<td>Nectar treatment × Abundance of HEI</td>
<td>1, 172</td>
<td>2.95</td>
<td>0.0878</td>
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<tr>
<td>Nectar treatment × Number of nectary bodies</td>
<td>1, 172</td>
<td>1.31</td>
<td>0.2538</td>
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<tr>
<td>Time</td>
<td>5, 168</td>
<td>1.14</td>
<td>0.3387</td>
</tr>
<tr>
<td>Time × Dominance</td>
<td>5, 168</td>
<td>2.19</td>
<td>0.0573</td>
</tr>
<tr>
<td>Time × Nectar Treatment</td>
<td>5, 168</td>
<td>0.67</td>
<td>0.6455</td>
</tr>
<tr>
<td>Time × Dominance × Nectar Treatment</td>
<td>5, 168</td>
<td>1.05</td>
<td>0.3887</td>
</tr>
<tr>
<td>Time × Site</td>
<td>25, 76</td>
<td>6.16</td>
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</tr>
<tr>
<td>Time × Plot (Site)</td>
<td>17, 76</td>
<td>0.98</td>
<td>0.5289</td>
</tr>
<tr>
<td>Time × Plant size</td>
<td>5, 168</td>
<td>0.16</td>
<td>0.9753</td>
</tr>
<tr>
<td>Time × Abundance of honeydew-excreting insects</td>
<td>5, 168</td>
<td>0.49</td>
<td>0.7855</td>
</tr>
<tr>
<td>Time × Number of nectary bodies</td>
<td>5, 168</td>
<td>0.61</td>
<td>0.6885</td>
</tr>
<tr>
<td>Time × Nectar treatment × Abundance of HEI</td>
<td>5, 168</td>
<td>1.00</td>
<td>0.4214</td>
</tr>
<tr>
<td>Time × Nectar treatment × Number of nectary bodies</td>
<td>5, 168</td>
<td>0.53</td>
<td>0.7503</td>
</tr>
</tbody>
</table>
Fig. 4. Behavioral responses of *Anoplolepis gracilipes* and other dominant ant species to prey larvae introduced near *Morinda citrifolia* nectary bodies. Discovery was defined as antennation with no further aggressive behaviors. Attacks occurred when workers bit, stung, or sprayed formic acid at larvae, but did not remove them from plants. Removals occurred when workers either carried prey off plants or physically ejected the larva from the plant. Error bars represent ±1 SE of the mean. Asterisks represent results from logistic regressions, with * indicating $P < 0.05$ and ** indicating $P < 0.01$.

Fig. 5. Time to discovery, attack and removal of prey by *Anoplolepis gracilipes* versus other dominant ant species. Shorter bars represent more rapid responses. Error bars represent ±1 SE of the mean. Trials lasted a maximum of 150 seconds. Asterisks represent results from survival analyses, with *** indicating $P < 0.001$, and **** indicating $P < 0.0001$. 

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time (Fig. 6c, d), and removed attacked prey in 76% less time (Fig. 6e, f) than on plants with the lowest nectar level (0% treatment). Overall, prey removal by Anoplolepis gracilipes increased from 19% to 81% from the lowest to the highest nectar level. In contrast, the aggressive behaviors of other dominant ants did not show significant responses to nectar manipulations (Fig. 6).

The strongest responses of A. gracilipes to increasing nectar levels occurred in terms of the proportion of larvae that were discovered ($P = 0.0068$, $r = 0.9987$; Fig. 6a, Table 5) and the time it took workers to discover prey items ($P = 0.0037$, $r = -0.8634$; Fig. 6b, Table 6). Once larvae were discovered, there was a marginally significant positive effect of the nectar treatment on the proportion of larvae attacked by A. gracilipes ($P = 0.0594$, $r = 0.8634$; Fig. 6c) and a marginally significant negative effect of the nectar treatment on time to attack ($P = 0.0606$; $r = -0.8615$; Fig. 6d). Once attacked by A. gracilipes workers, 94% ($\pm 2.4\%$) of larvae were removed from M. citrifolia plants regardless of nectar level (Fig. 6e). The time it took A. gracilipes workers to remove larvae dropped significantly as nectar levels increased ($P = 0.0481$, $r = -0.8814$; Fig. 6f).

### Evidence for community-level indirect effects mediated by ants

We predicted that there would be cascading, community-wide consequences resulting from differences in the dynamics of M. citrifolia-ant mutualisms when A. gracilipes dominated plots versus when it did not (Fig. 1A). This prediction was supported in terms of the $\beta$-diversity of plant-associated arthropods. There was a significant three-way interaction between A. gracilipes invasion status, ant access to the plants, and nectar availability of M. citrifolia (PERMDISP for three-way interaction: $P = 0.009$), indicating that there was an indirect interaction between these EFN-bearing plants and local arthropod communities that was mediated by ants. Furthermore, the effect of ants on these indirect interactions was different when A. gracilipes dominated local ant assemblages (Fig. 7). Specifically, at reduced nectar levels, arthropod $\beta$-diversity did not change when ants were allowed access to plants.

Table 5. Analyses of proportions of prey that were discovered, attacked or removed across sites invaded and uninvaded by Anoplolepis gracilipes. Logistic regression with a binomial distribution and a chi square test were used to compare the proportions of prey discovered, the proportion of discovered prey that were attacked and the proportion of attacked prey that were removed. Because sites were either invaded or uninvaded by A. gracilipes, site was used as a nested factor.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Prey discovered</th>
<th>Discovered prey attacked</th>
<th>Attacked prey removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. gracilipes invasion status</td>
<td>$\chi^2$  0.216, $P = 0.6418$</td>
<td>$\chi^2$  0.8705, $P = 0.3508$</td>
<td>$\chi^2$  0.0023, $P = 0.9616$</td>
</tr>
<tr>
<td>Nectar treatment</td>
<td>$\chi^2$  6.140, $P = 0.0132$</td>
<td>$\chi^2$  0.2762, $P = 0.5992$</td>
<td>$\chi^2$  0.3366, $P = 0.5618$</td>
</tr>
<tr>
<td>Invasion status × Nectar treatment</td>
<td>$\chi^2$  14.86, $P = 0.0001$</td>
<td>$\chi^2$  4.8001, $P = 0.0285$</td>
<td>$\chi^2$  0.1961, $P = 0.6579$</td>
</tr>
<tr>
<td>Forager recruitment</td>
<td>$\chi^2$  9.000, $P = 0.0027$</td>
<td>$\chi^2$  1.2926, $P = 0.2556$</td>
<td>$\chi^2$  0.0033, $P = 0.9545$</td>
</tr>
<tr>
<td>Site (Invasion status)</td>
<td>$\chi^2$  12.81, $P = 0.2347$</td>
<td>$\chi^2$  8.534, $P = 0.5769$</td>
<td>$\chi^2$  3.03, $P = 0.9807$</td>
</tr>
</tbody>
</table>

Table 6. Analyses of times to discovery, attack and removal of prey items across sites invaded and uninvaded by Anoplolepis gracilipes. To examine the time it took ants to perform aggressive behaviors, we conducted survival analyses with a Weibull distribution and Wald $\chi^2$ test statistics.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Time to discover prey</th>
<th>Time to attack of discovered prey</th>
<th>Time to removal of attacked prey</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. gracilipes invasion status</td>
<td>$\chi^2$  2.997, $P = 0.0834$</td>
<td>$\chi^2$  0.2994, $P = 0.5843$</td>
<td>$\chi^2$  0.7080, $P = 0.4001$</td>
</tr>
<tr>
<td>Nectar treatment</td>
<td>$\chi^2$  7.387, $P = 0.0066$</td>
<td>$\chi^2$  7.421, $P = 0.0064$</td>
<td>$\chi^2$  0.1309, $P = 0.7175$</td>
</tr>
<tr>
<td>Invasion status × Nectar treatment</td>
<td>$\chi^2$  39.90, $P &lt; 0.0001$</td>
<td>$\chi^2$  24.279, $P &lt; 0.0001$</td>
<td>$\chi^2$  7.147, $P = 0.0075$</td>
</tr>
<tr>
<td>Forager recruitment</td>
<td>$\chi^2$  16.84, $P &lt; 0.0001$</td>
<td>$\chi^2$  5.265, $P = 0.0218$</td>
<td>$\chi^2$  2.104, $P = 0.1469$</td>
</tr>
<tr>
<td>Site (Invasion status)</td>
<td>$\chi^2$  30.47, $P &lt; 0.0007$</td>
<td>$\chi^2$  27.443, $P = 0.0022$</td>
<td>$\chi^2$  16.919, $P = 0.0762$</td>
</tr>
</tbody>
</table>
Fig. 6. Behavioral responses of dominant ant species to experimentally manipulated nectar levels. Filled circles represent responses of *Anoplolepis gracilipes* and empty circles represent other dominant ant species. Error bars represent $\pm 1$ SE of the mean. All relationships between nectar level and behavioral responses of non-*A. gracilipes* ants were ns. Significant relationships ($P < 0.05$) are depicted with a solid line and marginally significant trends ($0.05 < P < 0.10$) are depicted with a dashed line.
regardless of whether the ants were *A. gracilipes* or other ant species. However, at ambient nectar, the presence of *A. gracilipes* decreased β-diversity, while the presence of other ant species increased β-diversity. These β-diversity differences between ant access treatments were stronger at sites dominated by *A. gracilipes* than they were at sites dominated by other ant species (Fig. 7).

**DISCUSSION**

When exotic species are introduced to novel habitats, they often form beneficial associations with indigenous species. However, we know little about the ways that these positive interactions affect invaders themselves or their interactions with other community members. Trait-mediated indirect interactions via mutualistic associations may contribute strongly to the negative impacts associated with species invasions. In this study, we predicted that a highly invasive ant species would exhibit a strong response to increasing nectar levels in terms of recruitment and aggressive behaviors. We also predicted that these responses would be much weaker in co-occurring less-invasive ant species.

Fig. 7. Evidence for indirect effects of plants on the arthropod community mediated by ants: among-plot dissimilarity (β-diversity) of plant-associated arthropods on *Morinda citrifolia* at sites dominated by *Anoplolepis gracilipes* (black bars) and those dominated by other ant species (gray bars). Filled bars represent plots with ants permitted and empty bars represent plots in which ants were excluded from *M. citrifolia* plants using a sticky barrier. There was a significant *A. gracilipes* invasion status × ant access × nectar level interaction (PERMDISP: *P* = 0.009); asterisks are from significant post-hoc pairwise comparisons of ant (+) and ant (−) treatments within each *A. gracilipes* status × nectar level *P* < 0.05, **P** < 0.01.
We expected that differential responses of highly invasive and less invasive ants would then lead to different consequences for the plant-associated arthropod community (Fig. 1A). In fact, we found that both A. gracilipes and other non-native dominant ant species responded positively to increasing nectar levels in terms of forager recruitment. However, A. gracilipes uniquely increased the degree of aggressiveness as nectar levels increased (Fig. 1B), resulting in more rapid attacks on and greater removal of plant-associated arthropods. Furthermore, the β-diversity of the arthropod community responded to the M. citrifolia–ant mutualism differently when A. gracilipes dominated local ant assemblages vs. when other ant species were dominant. Thus, trait-mediated indirect effects between native nectar-producing plants and plant-associated arthropods were present and differed depending on the identity of the ant partner, and were likely at least partially responsible for differences in the structures of communities dominated by A. gracilipes vs. dominated by less-invasive ants.

Of necessity, these experiments were conducted at sites already invaded by exotic ant species, and these species were not uniformly distributed across islands, e.g., A. gracilipes was more abundant and widespread on Savaii than on Tutuila. Since it would be unethical to transport this highly invasive species to uninvaded sites in Tutuila, we could not create a more balanced design by experimentally manipulating the identity of the dominant ant species at these sites. Consequently, there could be confounding effects of island on these results, although tests within A. gracilipes or other dominant ant species found no evidence for behavioral differences between islands.

**Effects of nectar subsidies on forager recruitment**

Carbohydrate-rich resources, such as plant nectar or hemipteran honeydew, may promote ant invasions by providing a high-energy food that fuels greater activity and growth and furthers the establishment of dominant supercolonies (the ‘carbohydrate subsidy hypothesis’, Lach 2003, Savage et al. 2009). If these carbohydrate-rich resources are, in fact, important factors in the progression of ant invasions, then highly invasive species should respond more strongly to variation in nectar resources than co-occurring less invasive species. In a previous study in northeastern Savaii (Savage et al. 2011), we found that recruitment responses of A. gracilipes were much stronger than a less-invasive ant, *Pheidole megacephala*. In this study, we manipulated plant nectar and tested the recruitment responses of a larger number of ant species (including four previously untested dominant species) across a much wider array of sites in both Savaii and Tutuila. The carbohydrate subsidy hypothesis was supported at sites invaded by *A. gracilipes*: *A. gracilipes* workers recruited strongly to increasing nectar levels, and other ant species were rarely observed on the plants. This difference in the response of *A. gracilipes* to variation in carbohydrates is important, because the availability of these resources often varies substantially across multiple spatiotemporal scales. For example, Eubanks (2001) found patchy distributions of the red imported fire ant (*Solenopsis invicta*) in agricultural systems of the South-eastern United States. He suggested that the presence of attended, honeydew-excreting aphids explained much of this variation—a supposition that was later supported by manipulative experiments (Kaplan and Eubanks 2005). Similarly, our broad-scale surveys across the Samoan Archipelago showed that the patchy distribution of *A. gracilipes* was strongly, positively correlated with the dominance of EFN-bearing plants (Savage et al. 2009).

However, our prediction that other ant species would display weaker recruitment responses to nectar availability was not supported in sites where *A. gracilipes* was absent. In fact, the pooled response of other dominant ants to increasing nectar availability was also strong and positive. There is likely variation among these less invasive ant species in their recruitment response to increasing nectar availability. However, the purpose of this study was to assess differences between *A. gracilipes* and co-occurring ant species because (1) our previous surveys (Savage et al. 2009) indicated that *A. gracilipes* was the only species with a strong positive association with EFN-bearing plants and (2) the carbohydrate subsidy hypothesis explicitly predicts that highly invasive ant species respond more strongly to carbohydrate resources (*A. gracilipes* is the most invasive species in Samoa). Therefore, we did not have sufficient replication at the level of individ-
ual, non-A. gracilipes species to determine how these species differed from each other. Despite these caveats, the strong, positive pooled responses of these other species suggest that while recruitment responses likely contribute to dynamics between highly invasive A. gracilipes, native plants, and co-occurring arthropod communities, recruitment, in and of itself, cannot explain why A. gracilipes is associated with greater invasive impacts than are the other exotic dominant ant partners of M. citrifolia (Fig. 7; Savage et al. 2009, Savage et al., in preparation).

If long-term responses of ants to nectar differ from short-term responses, the connections we identify between EFN, ant behavior, and community-level consequences may be different. We note that manipulations of ant access and nectar availability over six months (Savage et al., in preparation) resulted in similar patterns of ant recruitment to those reported in this study, that is, recruitment to supplemented nectar treatments stayed high while recruitment to reduced nectar treatments stayed low (Savage, unpublished data). However, it is likely that sustained increases in nectar availability (in contrast to the pulses used in this experiment) would lead to increased colony growth (also see Oliver et al. 2008). Larger colony size could have important effects on co-occurring community members (i.e., density-mediated indirect effects), especially in the context of invasions. For example, Holway and Case (2001) found that increased Linepithema humile (Argentine ant) colony size enhanced both exploitative and interference competitive abilities of this highly invasive ant species.

Additionally, our experimental nectar supplements (the 150 and 200% levels) only manipulated one component of M. citrifolia nectar (sucrose) and did not manipulate other nectar components such as amino acids, perhaps leading to unrealistic results. Some previous studies have indicated that carbohydrate-rich diets lead to increased activity in the short-term, but are nutritionally incomplete and limit the ability of ant colonies to grow (Buschinger and Pfeifer 1988, Porter 1989). However, recent studies have challenged this assertion (e.g., Kay et al. 2010, Wilder et al. 2010). For example, Byk and Del-Claro (2011) showed that the abundance and mass of queens, workers, and eggs all increased when Cephalotes pusillus ants were permitted access to EFN. Some of these benefits could be due to the presence of trace amounts of amino acids in nectar and honeydew (Bluthgen et al. 2004), although recent evidence suggests that the effects of amino acids are not as strong as the effects of carbohydrates. For example, Wilder et al. (2011) recently demonstrated that access to carbohydrates (without amino acids) reduced worker mortality in L. humile, while access to amino acids (without carbohydrates) increased worker mortality (Wilder et al. 2011). Most importantly, if amino acids were driving ant responses to EFN, then we would expect to see differences in the shape of ant responses moving from reduced to ambient nectar (0 and 50% to 100%), versus from ambient to supplemented nectar (100% to 150 and 200%). Instead, the slopes of ant responses to nectar manipulations were consistent across nectar levels (Figs. 3 and 6), suggesting that the supplemental nectar treatments manipulated the component of M. citrifolia nectar most relevant to the ant species in this study.

Effects of nectar subsidies on ant aggression

In addition to increased forager recruitment, another trait-mediated pathway whereby carbohydrate-rich resources may influence ants and their impacts on other species is through increased levels of aggression (Fig. 1). Based upon the carbohydrate subsidy hypothesis, we predicted that highly invasive ants would respond to increasing carbohydrate levels by increasing the likelihood or speed of attacks on co-occurring arthropods. Again, this prediction is contingent upon the assumption that less invasive ants do not respond in the same way to carbohydrate availability. In this study, we explicitly tested this prediction for the first time, by manipulating levels of plant nectar and observing the aggression displayed by highly invasive and co-occurring less-invasive ants.

We found that the highly invasive ant A. gracilipes displayed unique increases in aggression in response to increasing nectar availability, a response not found for other dominant ant species in Samoa. As nectar levels increased, there was a general increase in the likelihood of prey discovery and attack and a general reduction in the amount of time it took A. gracilipes workers to discover, attack and remove prey. The strongest responses of A. gracilipes were in terms...
of prey discovery, which may be due to higher overall activity levels when nectar availability is high. However, the independent effects of nectar on *A. gracilipes* attack and removal suggests that increased carbohydrate availability can influence other aspects of aggressive behaviors, at least for this species. Interestingly, other exotic, dominant ant species did not display these nectar-dependent aggressive responses, even in the absence of *A. gracilipes*. Thus, our findings support the prediction that highly invasive ant species respond more strongly to carbohydrate availability in terms of increased aggression than less invasive ants. Furthermore, this trait difference apparently has consequences for co-occurring arthropods (Figs. 1B and 7) and thus could potentially explain the observed community-level effects of *M. citrifolia-A. gracilipes* mutualisms.

At first glance, any changes in aggressive responses of *A. gracilipes* to increasing nectar resources could be simply explained by the forager recruitment responses that we demonstrated in the first experiment. With more ants recruiting to nectar, the likelihood of prey discovery should be higher, due to increased encounter rates. In fact, we found that increased forager recruitment did explain some of the variation in ant aggression across nectar levels. However, we also found that significant effects of nectar on aggression remained even after accounting for these numerical effects. Furthermore, other ants did not express these aggressive responses despite displaying similar recruitment responses to *A. gracilipes*. These patterns indicate that nectar uniquely influences per-capita worker aggression in *A. gracilipes*.

Together with our findings, results from other recent studies suggest that carbohydrates can strongly influence ant behaviors with consequences for co-occurring arthropods, and that these effects can be conditional on ant identity. For example, Grover et al. (2007) found that both activity levels and intraspecific aggression of *L. humile* were higher for lab colonies that were fed a diet rich in carbohydrates than under a protein-rich diet. Similarly, Pringle et al. (2010) showed that native plant-inhabiting ants in a neotropical lowland rainforest were more aggressive towards plant-feeding herbivores when fed a diet rich in carbohydrates. However, Kay et al. (2010) examined the aggression exerted by *L. humile* towards heterospecific ants and found no influence of diet, although carbohydrate-rich diets resulted in greater colony growth (a density-mediated effect). In the deserts of the Southwest United States, Ness et al. (2009) demonstrated that four species of native ants were more aggressive towards novel prey when fed supplemental carbohydrates than when fed protein or given no supplements; however, no invasive ant species were tested in this study. Finally, Lach and Hoffmann (2011) demonstrated that invasive *A. gracilipes* workers were more likely to attack prey than workers of one native, dominant ant species (*Oecophylla smaragdina*), but only on plants bearing EFN; attack rates of the species did not differ on plants that did not secrete extrafloral nectar.

In most of the cases in which *A. gracilipes* has been reported to have detrimental impacts on co-occurring community members, this species has also associated with plants or insects that secrete carbohydrate-rich food in the form of nectar or honeydew (Addison and Samways 2000, Holway et al. 2002, Hill et al. 2003, O’Dowd et al. 2003, Lester and Tavite 2004, Savage et al. 2009). The amplification of aggressive behaviors that we detected in this study may provide a mechanism that underlies this pattern. Similar tests of aggression in response to carbohydrate availability across the invaded range of *A. gracilipes* will help to elucidate the generality of these findings. Furthermore, it will be important to conduct studies that manipulate nectar over longer time spans in order to ascertain the relative importance of density and trait-mediated effects of nectar on *A. gracilipes* invasions.

**Conclusions**

Mutualisms are common interspecific interactions that can influence the structure and dynamics of communities and the functioning of ecosystems (Bronstein 1994, Stachowicz 2001, Rudgers and Clay 2008). However, we know surprisingly little about the mechanisms that underlie many of these community-wide effects. Importantly, indirect, trait-mediated pathways are likely to be important mechanistic components of the effects of mutualisms on communities, just as they are for antagonistic interactions. In this study, we demonstrated that (1) resources
exchanged in mutualisms between ants and EFN-bearing plants can change behaviors that ants exert towards other community members and (2) these changes are more extreme for invasive *A. gracilipes* than co-occurring less invasive ants. In studies of antagonistic interactions, variation in aggression has been shown to influence both species co-existence (Frye 1983, Logan 1984, Morrison 1996) and the displacement of native species by exotics (Carpintero and Reyes-Lopez 2008). Thus, if they represent a widespread pattern, our findings suggest that trait-mediated indirect interactions associated with novel mutualisms between invaders and native species could contribute to the success and detrimental impacts of species invasions.

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