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Interspecific and intraspecific variation in the susceptibility of arthropods to predation by the Argentine ant

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by

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The Thesis of Ariya Uyeno is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATION

I dedicate my thesis to my family, without whom my education would not have been possible. You've given me a lifetime of encouragement and support, for which I will be forever grateful.

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ABSTRACT OF THE THESIS

Interspecific and intraspecific variation in the susceptibility of arthropods to predation by the Argentine ant

by

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Master of Science in Biology

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Professor David Holway, Chair

The Argentine ant (*Linepithema humile*) is a facultative scavenger that opportunistically consumes both live prey and carrion. Despite its stature as a widespread and disruptive invader, how it interacts with other arthropods remains incompletely understood; published studies report a wide range of impacts on non-ant arthropods. Here, we examine interspecific and intraspecific variation in susceptibility of different arthropods to predation by the Argentine ant. We used mesh-covered enclosures containing different arthropods to measure susceptibility to predation.

Mesh screens permitted Argentine ants to move in and out of the cages, but mesh size was too small to permit escape of other arthropods. For four different arthropod taxa, the percent killed by the Argentine ant after 24 h was as follows: roaches (25%), crickets (70%), wax moth larvae (100%), and harvester ants (100%). For these same arthropods, *L. humile* consumed carrion and killed prey to a similar degree, indicating the importance of scavenging in general and, in particular, for arthropods that the Argentine ant can't readily subdue as prey. In a second experiment, we found that increasing body size protected crickets from ant predation. These findings provide experimental evidence for interspecific and intraspecific variation in susceptibility to predation by the Argentine ant and provide a potential framework for understanding the contrasting impacts resulting from this invasion. Our results further illustrate the importance of scavenging for ant invasions.

INTRODUCTION

Prey consumption is a well-studied behavior in food web ecology. Indeed, the "green" food web—involving the consumption of living biomass—is often considered the default mode of energy transfer (Evans-White and Halvorson, 2017, Moleón et al., 2014). The green food web excludes decomposition, yet eventually all living biomass is decomposed (Mougi, 2020). Decomposition directs consumable nutrients to the "brown" food web as carrion, dead animals, or detritus—decaying organic matter (Barton et al., 2012). Carrion differs from prey in several respects, including that dead organisms cannot actively fight or flee their predators (Tan and Corlett, 2012). As such, scavengers benefit from feeding on carrion without risking their survival or expending costly amounts of energy.

Although some "obligate" scavengers rely solely on carrion for nutrients, many organisms, such as eagles, coyotes, wolves, and bears, are scavenging predators and feed on both prey and carrion (Walker et al. 2021). This generalist behavior is known as facultative scavenging, the act of opportunistically feeding on carrion while also actively hunting live prey (Walker et al. 2021). Facultative scavengers maximize energy consumption while limiting potential costs and contribute to nutrient flow in both the green and brown food webs (Wilson and Wolkovich, 2011).

Recent interest in filling this gap has fueled a spike in research on invertebrate detrital interactions. Of the commonly studied invertebrate detritivores—including flies, cockroaches, and other species—ants were almost always the most dominant scavenger (Angulo et al., 2011; Tan and Cortlett, 2012; Griffiths et al., 2017; Holway and Cameron, 2021). Ants have a uniquely high biomass compared to other invertebrate scavengers (Houadria et al., 2016). Their ability to

organize in large numbers allows them to recruit to carrion quickly while also enabling them to hunt live prey larger than themselves. As a result, many ant species are generalized predators and can consume both live and dead prey (Carroll and Janzen, 1973). Their ability to communicate and organize in large numbers makes ants a compelling scavenger to study.

One particularly successful scavenging ant species is the Argentine ant (*Linepithema humile*, Formicidae, Hymenoptera). The Argentine ant is a widespread invasive species established on six continents (Wilgenburg et al., 2010). The Argentine ant is characterized by its aggressive nature and ability to form super colonies (Wilgenberg et al., 2010; Angulo et al., 2011). These unique qualities make them well-adapted to invading ecosystems, establishing new colonies, and recruiting to food sources. Their role as generalist predators and facultative scavengers means that they can quickly fill the role of other detritivores in regions they invade. It is therefore critical to study the Argentine ant's predatory behaviors to better understand the invasive species' impact on their surrounding ecosystems.

Current research has compared the scavenging behavior of the Argentine ant to that of native ants. Angulo et al. (2011) observed the scavenging behaviors of the Argentine ant in the Mediterranean. They found that, in stark contrast to the highly seasonal native ants and detritivores, the Argentine ant was more consistent in activity relative to season and temperature. Notably, the study found that Argentine ants appeared to co-opt the ecological role of the native ants at a higher and more constant rate (Angulo et al., 2011). Though studies have observed the differences between the scavenging behavior of the invasive Argentine ant and native ants, few studies have examined a direct comparison between their predation and scavenging behaviors.

Studies have shown that prey size has a direct impact on the ability of ants to scavenge carrion (Cerdá et al., 1998). Moreover, certain characteristics of individual ant species—including aggression, worker body size, and number of recruited workers—impact the size of prey they can scavenge. To better understand the predation behavior of the Argentine ant, it is important to consider the unique characteristics of this species in relation to its role in the ecosystem as a generalist predator and facultative scavenger.

In this study, susceptibility of prey species to predation or scavenging by Argentine ants was measured to investigate the role of *L. humile* as a facultative scavenger. Specifically, we observed how prey size and species affected the Argentine ant's ability to kill and consume them. We expected that the Argentine ant would prefer to consume carrion, given that scavenging presents a much lower risk to workers than predation. Moreover, we anticipated that rates of predation on live prey would decrease as prey size increases, as the cost of lost workers and energy expended may outweigh the nutrient benefit of larger prey.

METHODS

Prey enclosures

To assess interspecific and intraspecific variation in susceptibility to predation, we constructed semipermeable cages (Fig. 1) that allowed free passage of Argentine ant workers but effectively contained arthropod prey. Cages were 7.5 cm in width and 7.5 cm in height were constructed from plastic planting cups collected from Armstrong Garden Center, covered with mesh screen (Fig. 1AB). Mesh screens were hot glued to the planter cups such that the external surface of the cage was covered in screen. On one end of the cage, the screen was left unglued to the rim of the cage to allow for arthropods to be added to the cage prior trials.

All trials were conducted with free-living Argentine ant colonies at Rose Canyon Open Space Park (32°51'47.6238"N 117°12'6.6816"E). We established a linear transect of 20 stations in riparian vegetation and parallel to Rose Creek; stations were at least 5 m apart. We performed trials between March and May 2024 under mild conditions in which ants and non-ant arthropods were active both during the day and at night.

Experiment I: Interspecific variation in susceptibility to predation

We tested the ability of the Argentine ant to prey upon four different insect species: Dubia roaches (*Blaptica dubia* (Blattodea)), house cricket (*Acheta domesticus* (Orthoptera)), waxworms (*Galleria mellonella* (Lepidoptera)), and harvester ants (*Pogonomyrmex occidentalis* (Hymenoptera)) (Table 1). All four species are commercially available. Field trials were conducted with living (prey) or dead (carrion) individuals of each arthropod species. Individuals were randomly assigned to experimental group with individuals designated to the carrion group frozen until use in trials. Individual cages contained one of the four focal arthropod species that was either alive or dead. We weighed (in mg) all individuals using a Mettler AJ100 balance

before adding them to cages. To control for water loss, we placed an arthropod of each species in a cage covered with breathable mesh that was too fine to allow entry by the Argentine ant. These control cages were placed mid-transect and were in the field while experimental cages were in the field. We retrieved all cages after 24 h, determined whether or not prey was killed by the Argentine ant, and re-weighed all non-living individuals (i.e., carrion and killed prey).

We used chi-square tests to compare the four arthropod species with respect to the proportion alive or dead at the conclusion of each trial. For carrion and killed prey, we compared the amount consumed by ants (after correcting for water loss) with mixed effects models. In these analyses, day and bait station were treated as random effects, and species and treatment (prey or carrion) were treated as fixed affects. All analyses were performed in R (R core team 2023) and using packages 'vcd,' 'lme4,' and 'lmerTest.'

Experiment 2: Intraspecific variation in susceptibility to predation

This second experiment tested if predation risk depends on prey size. The methods for this second experiment were identical to those of the first experiment, expect that only live crickets (n = 157) were used. Prior to deployment in the field, we measured the thorax width of individual crickets. We retrieved all cages after 24 h, determined whether crickets had been killed by the Argentine ant, and weighed all prey that succumbed to predation. Crickets ranged in thorax width between 1-7.5 mm. We used mixed effects models to test intraspecific susceptibility to predation. In this analysis, cricket thorax width was the response variable, day and bait station were treated as random effects, and cricket state (alive or dead) was treated as a fixed variable.

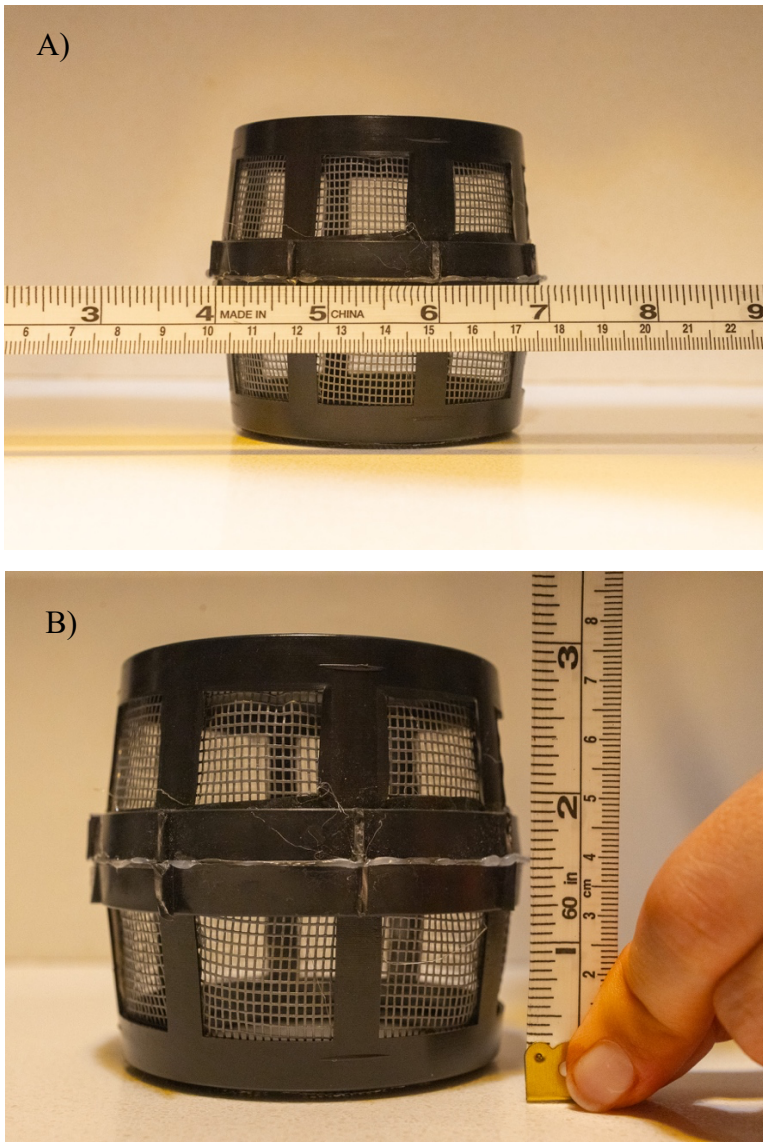

Figure 1. Images of enclosure.

Table 1. Summary of species size data.

Species	Mass range (mg)
House cricket, *Acheta domesticus*	134.2-394.5
Dubia roach, *Blaptica dubia*	42.4-495.6
Waxworm, *Galleria mellonella*	64.4-228.9
Harvester ant, *Pogonomyrmex occidentalis*	4.0-15.7

RESULTS

Experiment I: Interspecific variation in susceptibility to predation

The risk of predation significantly differed among the species of potential prey (Table 2; chi-squared test: X-squared = 38.935, df = 3, $P < 0.001$). While live waxworms and harvester ants were always recruited to and killed by Argentine ants, crickets and especially Dubia roaches were less likely to be killed (Table 2).

On an individual basis, carrion and prey that succumbed to Argentine ant predation were consumed to the same extent depending on the species considered (Fig. 2). In the mixed effects model, the effect of species was significant ($t = 10.21$, $P < 0.0001$), whereas the effect of treatment (prey / carrion) was not ($t = 0.063$, $P > 0.05$). Given that all arthropod species were consumed as carrion (i.e., were palatable), prey items that survived the trials represent energy unavailable to the Argentine ant (Table 3). Harvester ants and waxworms were mostly consumed as prey and carrion, but over a third of cricket prey biomass was unavailable to ants and most of the roach biomass was unavailable to ants (Table 3).

Experiment 2: Intraspecific variation in susceptibility to predation

The risk of predation for crickets depended significantly on their size (Fig. 3). In the mixed effects model, state (living / dead) was a significant predictor of cricket size ($t = 7.54$, $P = 0.00011$).

Table 2. Risk of predation on prey species by Argentine ants.

Preyed upon?	Cricket$_a$	Dubia roach$_b$	Waxworm$_c$	Harvester ant$_c$
Yes	14	5	20	20
No	6	15	0	0

Subscripts$_{abc}$ indicate significant difference in predation by the Argentine ant ($p < 0.01$). Species with different subscripts observed significant differences in the risk of predation, whereas species with the same subscript$_c$ experienced similar rates of predation.

Table 3. Percent of presented prey or carrion mass consumed of the four arthropod species.

	Cricket	Dubia roach	Waxworm	Harvester ant
Live prey	61.2%	14.5%	96.4%	97.5%
Carrion	98.2%	93.8%	96.9%	97.0%

The impact of prey species on Argentine ant tendency to consume live prey via predation or carrion via scavenging. Percentages are determined by the total mass consumed divided by the total original mass.

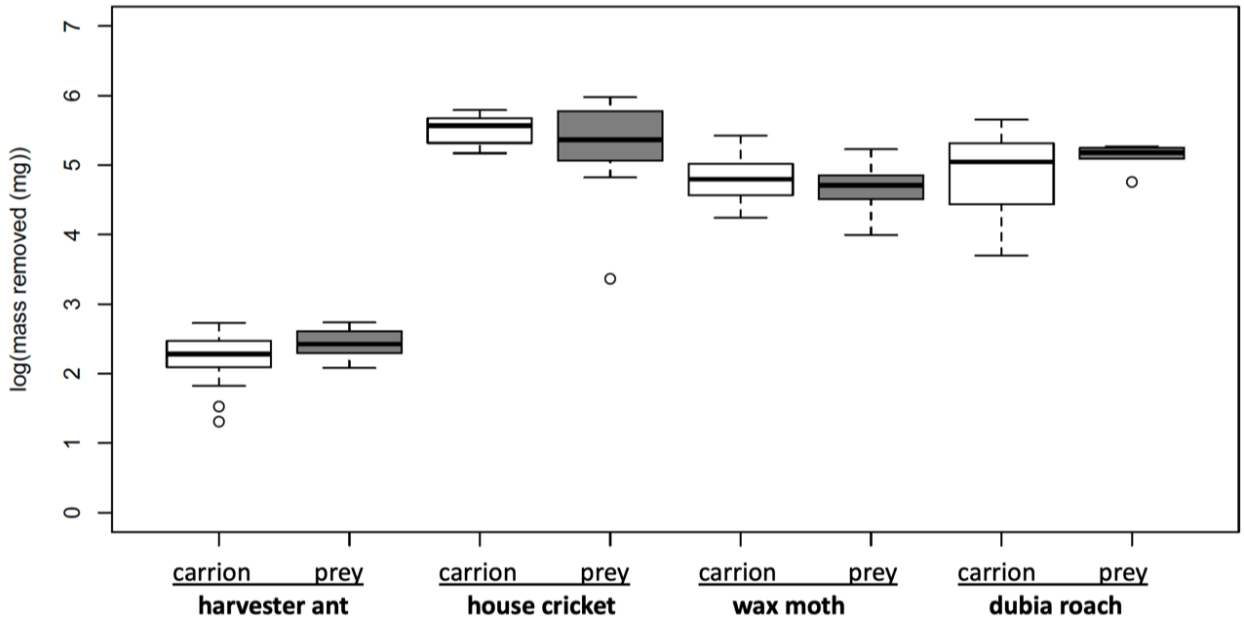

Figure 2. The log of mass removed by Argentine ants per individual bait depending on species, live prey, and carrion. Carrion are represented by open box plots, whereas prey are represented by filled boxes.

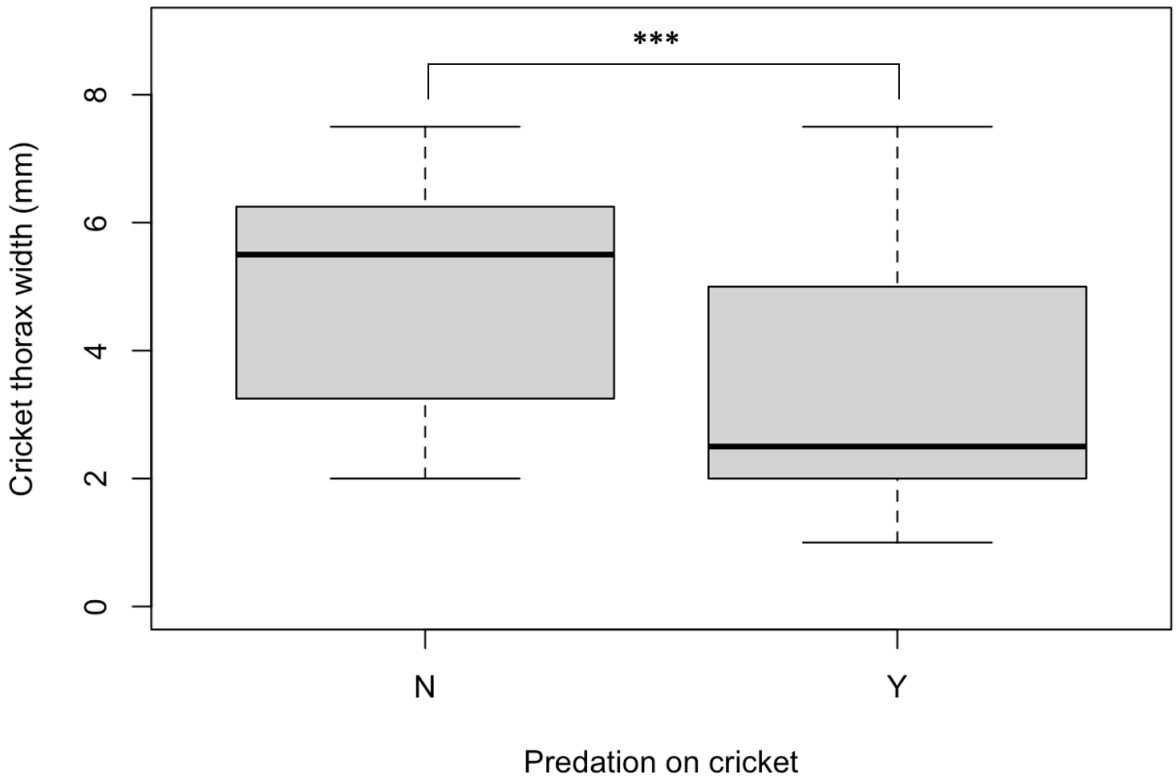

Figure 3. The relationship between prey size and the subsequent risk of predation by the Argentine ant. Prey size of the cricket was determined by cricket thorax width to remain constant in relation to body size.

DISCUSSION

The outcome of the field trials demonstrated interspecific and intraspecific variation in susceptibility to predation. Moreover, direct comparison of resources available as prey or carrion illustrated that carrion consumption was equally or more important than prey consumption, depending on the arthropod species considered. Our data thus provide evidence for the importance of scavenging for this widespread introduced species and point to potential explanations for why published studies on the impacts of this invader on non-ant arthropods have reached contrasting conclusions (Hanna et al. 2015, Holway and Cameron, 2021).

Experimental enclosures containing either prey or carrion of four different arthropod species illustrated that the Argentine ant's consumption of energy is influenced by prey species. Across all prey species, we observed that a greater overall mass of carrion was available to Argentine ants than prey. However, carrion and killed prey were consumed to the same degree. The Argentine ant scavenges arthropod carrion despite its flexibility as a generalist predator (Angulo et al., 2011; Holway and Cameron, 2021). The strength of interspecific preference differed greatly between arthropod prey species, with high levels of predation on waxworms and harvester ants, and reduced predation rates on crickets and Dubia roaches. Such variation in preference could result from the mobility or size of prey items. Waxworms were the least mobile of the species tested, which may have contributed to their high susceptibility to predation. By comparison, crickets are extremely mobile and are known to have a quick escape response to stimuli, making them more difficult to hunt while alive (Kiuchi, 2023).

Experiment 2 demonstrated size-dependent susceptibility to predation. This size selective predation behavior may result from a combination of both worker and prey size. Worker size often restricts the maximum prey size susceptible to recruitment by ants (Cerdá et al., 1998).

Smaller workers may be less fit to recruit to and subdue larger prey items; to do so could be dangerous to the colony with little reward. Moreover, small workers' prey-carrying ability can be hindered by larger prey items, making the transportation of such food sources difficult (Cerdá et al., 1998). Certain ant behaviors can mitigate this limitation, such as aggressiveness and social recruitment, both of which are exhibited by the Argentine ant. While our experiment illustrated that prey size can influence the outcome of a predator-prey interaction, crickets in either outcome category (consumed / not consumed) varied in size. This variation may have resulted from differences in Argentine ant density across bait stations as well as variation in cricket condition. Small crickets might have been more susceptible to desiccation because of their greater surface area to volume ratio, but conditions were mild throughout all the trials.

Our results challenge the traditional interpretation of Argentine ants as predators and reinforce the idea that they must be considered as scavengers, especially when examining their role in invaded ecosystems. Limitations to our experiment exist, particularly the use of enclosures to examine predation behavior. The presence of a cage may have limited the ability for prey, particularly fast-moving prey such as crickets and Dubia roaches, to escape Argentine ant predation. As such, the values we report, including risk of predation by species (Table 2) may not apply to all free-living arthropods. Although this cage effect may have resulted in an overestimated rate of predation, the enclosures remained constant in size, organization, and usage throughout the study. The cage effect is constant for each comparable set of data, and therefore did not bias our results.

This study provides evidence for interspecific and intraspecific variation in prey susceptibility to predation by the Argentine ant. This wide variation in live prey susceptibility thus provides evidence for the importance of scavenging for the Argentine ant, particularly in

situations where prey species or size may act as a limiting predation factor. The importance of scavenging for the Argentine ant may encourage a reconsideration the ecological impacts of *L. humile* as an invader. Hanna et al. (2015) found the presence of the invasive Argentine ant has little to no impact on the species abundance of native non-ant arthropods. Although the spatial distribution of the Argentine ant may not have observable impacts on the surveyed array of non-ant arthropods, the functional role of Argentine ants in the scavenging food web may be important to consider. Further research may be warranted on the direct impact of Argentine ant scavenging preference on competing non-ant scavenger arthropods. Given the apparent importance of scavenging for non-native ant species to settle in an invaded ecosystem (Holway and Cameron, 2021), future research must consider how native arthropod scavengers may be displaced.

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