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April 2014

Exploring the Link Between Rescue Behavior and Experience  
in the Pavement Ant, *Tetramorium* sp. E

by  
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A Thesis  
Presented to the Faculty of Mount Holyoke College  
in partial fulfillment of the requirements  
for the degree of Bachelor of Arts  
with Honors

Program in Neuroscience and Behavior  
Mount Holyoke College  
South Hadley, Massachusetts

## ACKNOWLEDGEMENTS

Thank you to Karen Hollis, my wonderful thesis advisor, whose support, insight, and encouragement made this project worthwhile. I would also like to thank Janelle Gagnon for assisting me with my statistical analyses. Thanks as well to my thesis committee members Gary Gillis and Stan Rachootin, not only for providing welcome feedback on my writing, but for teaching courses that kindled my interest in scientific research.

Thank you to the following members of the Hollis lab team – Gen Borrino, Alyssa Dodd, Thu Pham, Lindsey Bannish, Emily Jue Wang, and Beatriz Diaz Alvarez – for spending many long hours ant-watching, offering valuable feedback and suggestions for my project, and keeping the research process fun. Thank you, Sandra Lawrence and the rest of the Psych 411/412 class, for keeping me grounded and sane when deadlines loomed.

Finally, to my family, I thank you for your endless love and encouragement, in this as in all things.

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## ABSTRACT

Rescue behavior, a specific form of altruism, has been recorded in diverse animal species. Pavement ants (*Tetramorium* sp. E) are capable of freeing nestmates trapped by antlions (*Myrmeleontidae* spp.), a common predator of ants and other insects. Although rescue behavior in *T. sp E* and other ant species is well-documented, the potential effects of previous rescue experience on patterns of future rescue behavior are unknown. Ants and many other insects show evidence of task specialization based on repeated prior exposure to certain stimuli, and in rats, individuals that have previously rescued a conspecific become more likely to perform rescue behavior later, given the opportunity. These findings suggest that ants may modify their expression of rescue behavior according to whether they have engaged in rescue behavior in the past. In this project, the effects of previous experience on subsequent patterns of rescue behavior were investigated by allowing ants to encounter a nestmate trapped by an antlion in two back-to-back experimental trials. The behavioral responses of ants that had engaged in rescue behavior during their previous confrontation with the antlion were compared to the responses of naïve ants that did not engage in rescue behavior during the initial encounter. Experienced and inexperienced ants presented with a second opportunity to rescue a nestmate did not differ in their likelihood to perform rescue behavior, did not perform more or fewer types of rescue behavior, did not demonstrate different latencies to engage in rescue behavior, did not spend different amounts of time performing rescue

behavior, and did not exhibit shorter or longer periods of sustained rescue behavior. Ants that had performed rescue behavior in the initial trial displayed shorter bouts of sustained rescue behavior and performed less limb-pulling behavior in the second trial than in the initial trial. Taken together, these findings suggest that, rather than becoming more effective rescuers as a result of prior experience, *T. sp. E* individuals that have previously performed rescue behavior show increased reluctance to rescue in the future as a byproduct of predator avoidance behavior.



## INTRODUCTION

The phenomenon of altruism has long fascinated biologists. An altruistic behavior is broadly defined as any activity an organism performs that benefits another individual, the recipient, while incurring some cost to the individual performing the behavior, the altruist (Hamilton, 1964). Somewhat counterintuitively, these seemingly unprofitable actions may benefit the altruist by increasing its inclusive (gene-level) fitness, helping to explain the surprising evolutionary persistence of 'selfless' behaviors (Dawkins, 1976). Altruistic behaviors have been reported across a wide range of species. Common vampire bats (*Desmodus rotundus*), for example, have been observed regurgitating blood meals from their own stomachs and offering the meals to hungry bats nearby, including unrelated individuals (Wilkinson, 1984). Fed bats often reciprocate in the future, thereby helping their former donors avoid starvation (Wilkinson, 1984). Adult female individuals of many species, such as red squirrels (*Tamiasciurus hudsonicus*), will adopt orphaned juveniles, raising them among their own litters (Gorrell, McAdam, Coltman, Humphries and Boutin, 2010). Crucially, juveniles are likely to be adopted only if they are closely related to their adopter. Altruistic behavior, therefore, is consistent with the predictions of inclusive fitness theory, since a female may be able to offset the potential fitness costs of caring for another individual's offspring, provided that the juvenile shares most of her own genes (Gorrell et al., 2010).

Altruism is not limited to mammals. Eusocial insects (order Hymenoptera) are among the best-studied practitioners of altruism (Wilson, 2008). In ants, the existence of “helpfulness behaviors” has been known for over 70 years; for example, early myrmecologists observed the ant species *Formica fusca* and *Lasius flavus* carrying and grooming injured nestmates, pulling trapped ants out from between walls, and digging nestmates out of obstructive clumps of dirt (Lafleur, 1940). Recently, a specific type of altruism known as precision rescue behavior was described in ants (Nowbahari, Scohier, Durand and Hollis, 2009). Nowbahari et al. (2009) observed that a Mediterranean ant species, *Cataglyphis cursor*, attempted to free nestmates trapped by simple snares the researchers constructed from paper and string. The ants frequently performed several different behaviors during their rescue attempts, such as picking up sand with their mandibles and transporting it away from the snare, biting the snare, pulling on the limbs of the nestmate, or pulling on the snare (Nowbahari et al., 2009). In a follow-up experiment examining five ant species occupying diverse ecological niches, Hollis and Nowbahari (2013) found that precision rescue behavior is neither unique to *C. cursor* nor universal among ant species. The study species that nested in loose, sandy soils prone to creating cave-ins tended to rescue nestmates more readily than species that lived in hard soils, where the risk of entrapment by environmental debris was lower (Hollis and Nowbahari, 2013). These findings suggest that ecology can strongly influence a species' propensity to perform rescue behavior. Physiology can also affect

patterns of rescue behavior in ants. In a study designed to investigate whether *C. cursor* individuals belonging to different castes give and receive different levels of aid in a rescue situation, Nowbahari, Hollis and Durand (2012) demonstrated that developmentally mature ants (foragers) engage in more rescues of nestmates, and are rescued more often in turn, than members of the immature castes (nurses and inactives).

Despite these recent advances in our understanding of rescue behavior dynamics in ants, much more research remains to be conducted in this area. Anecdotal reports of animals rescuing conspecifics or even heterospecifics abound, but only three scientific studies (Siebenaler and Caldwell, 1956; Vogel and Fuentes-Jiménez, 2006; and Bartal, Decety and Mason, 2011) have documented rescue behavior in non-ant species (dolphins, capuchin monkeys, and rats, respectively). Rescue behavior may in fact be much more widespread, but the current paucity of scientific research makes it difficult to draw conclusions about the potential factors – for example, social, ecological, or physiological factors – that influence the expression of rescue behavior. Furthermore, because few studies of rescue behavior were designed to reuse the same individuals across multiple trials, little is known about how animals process and integrate memories of previous rescue attempts, or whether individuals rely on these memories to make decisions about rescue behavior in the future. However, one study found that rats that were exposed to an artificial trap in a laboratory setting were more likely to rescue conspecifics from the traps, and succeeded in

freeing trapped rats more quickly, than rats that had never encountered that particular type of trap (Bartal et al., 2011). These results suggest that, at least in rats, individuals may become more “efficient” rescuers due to previous exposure to a rescue stimulus, such as a trap or a predator. Further research is needed to determine whether these observed shifts in rescue behavior as a result of experience are extensible to species other than rats. Ants, which readily display rescue behavior and are capable of learning to perform complicated, multi-step tasks (as described below), are therefore ideal candidates for testing hypotheses about the role of prior experience in shaping future patterns of rescue behavior.

### Learning in Ants

Learning is widespread and common among insects, including ants (Dukas, 2008). For example, the the desert-dwelling ants *Melophorus bagoti* use visual and spatial cues to create sophisticated mental maps of the landscape, which help them navigate to and from the nest (Schwarz and Cheng, 2011). Experimental evidence suggests that *M. bagoti* are capable of forming sequential memories of the landmarks they encounter while foraging, and that these ants, instead of blindly relying on memories to inform navigational decisions, discriminate between memorized cues based on their perceived degree of reliability (Schwarz and Cheng, 2011). *C. cursor*, the same ant species used in several studies of precision rescue behavior, is also capable of memorizing and discriminating between different visual

patterns (Chameron, Schatz, Pastergue-Ruiz, Beugnon and Collett, 1998). Furthermore, there is evidence that memories of individual experiences have the potential to shape future patterns of non-rescue behavior in ants (Ravary, Lecoutey, Kaminski, Chaline and Jaisson, 2007; Langridge, Sendova-Franks and Franks, 2008). Ants of the species *Cerapachys boroi* that never encountered food while foraging were less likely to perform foraging-related tasks in the future, preferring instead to stay in or around the nest, while ants that were given extra food to encounter while foraging became more likely, over time, to forage and venture outside the nest (Ravary et al., 2007). Similarly, in colonies of *Temnothorax albipennis*, individuals who previously completed a specific task, namely transporting items from one nest to another, were more likely to transport items in the future, and also tended to transport a larger quantity of items and begin transporting items earlier than inexperienced nestmates (Langridge et al., 2008). Another study conducted using *T. albipennis* found that previous foraging success was associated with increased willingness to forage in the future (Robinson, Feinerman and Franks, 2012). These studies provide ample evidence that experience with different tasks, such as foraging, navigation and item transportation, lead to modifications in ant behavior. In this respect, they are similar to Bartal et al.'s (2011) finding that familiarity with a rescue stimulus leads to improved rescue performance in rats. Taken as a whole, these studies suggest that ants may change their behavior by drawing on memories formed during previous rescue situations.

## Antlions and Antipredator Behavior in *Tetramorium* sp. E

Antlions (*Myrmeleontidae* spp.) are common predators of many ant species, including *Tetramorium* sp. E, the pavement ant. As larvae, antlions dig conical pits in loose sandy soils to entrap prey. When a prey item, such as an ant, travels over the edge of the pit, the sloped walls cause the ant to fall into the bottom of the pit, where the antlion closes its mandibles around the prey and injects a paralytic venom (Taylor, Visvader, Nowbahari and Hollis, 2013). Ants typically remain alive for several minutes after they are seized by an antlion, giving nearby nestmates ample time to attempt to rescue the trapped ant (Taylor et al., 2013).

Antlions are capable of eliciting a wide range of antipredator behaviors in ants. In a recent laboratory study, *T. sp. E* ants exposed to nestmates trapped by antlions engaged in one or more rescue behaviors, such as attacking the antlion or pulling on the limbs of the trapped ant, in approximately one-third (36%) of all trials (Taylor et al., 2013). The presence of antlions can lead to large-scale, colony-wide modifications in ant behavior; one study found that ants avoided foraging in areas with high densities of antlions, even when extra food was placed in the so-called 'antlion zone' by the researcher (Gotelli, 1996). In another study, individual ants (*T. sp. E*) that fell into antlion pits and successfully escaped were subsequently less likely to approach both the pits they had already encountered and pits belonging to novel antlions (McNew, 2013), indicating

that prior experience can modify the expression of antipredator behavior in pavement ants.

## The Present Study

The present study is designed to assess how prior rescue experience influences patterns of rescue behavior in the pavement ant, *T. sp. E.* Antlions are used as the rescue stimulus, providing an obstacle from which ants might attempt to rescue their nestmates. Since *T. sp. E.* are capable of modifying certain types of behavior, such as avoidance, in response to encountering antlions (McNew 2013), I hypothesized that ants that attempted to free a trapped nestmate from an antlion would exhibit different patterns of rescue behavior during future rescue attempts than ants that encountered an antlion without performing rescue behavior, or ants that did not encounter an antlion at all. Because ants rarely succeed in completely freeing their nestmates from antlions (Taylor et al., 2013), prior rescue success, per se, might not strongly influence an individual ant's willingness to perform rescue behavior in the future. Instead, ants might use the information they have gleaned from previous encounters with antlions to adjust their rescue 'strategy' by performing different rescue behaviors during their next confrontation with an antlion. Alternatively, ants may become more reluctant to approach the antlion a second time, consistent with McNew's (2013) observations of predator avoidance behavior in *T. sp. E.* If ants tend to avoid antlions after an initial encounter, the number of rescue behavior attempts

would decline as a result, since ants must approach the antlion in order to get close enough to the captured ant to perform rescue behavior.

To investigate potential modifications in rescue behavior patterns between initial and subsequent rescue attempts, ants in this study were provided with the opportunity to encounter a nestmate trapped by an antlion and to perform rescue behavior. These ants were then grouped into three categories according to their behavior during the initial trial, and were given a second opportunity to encounter a nestmate trapped by a different antlion. All instances of rescue behavior were timed and recorded in each of the two trials, and this temporal and behavioral information was used to evaluate four general measures of rescue performance: the latency to begin performing rescue behavior, the number of different rescue behaviors exhibited during an encounter with an antlion, the length of the longest continuous display of rescue behavior, and the overall duration of rescue behavior during a trial. This last measurement, the duration of rescue behavior, was further divided into three sub-measurements, one for each of the three different types of rescue behavior an ant could display during a rescue attempt: limb-pulling, sand displacement, and attack behavior (Table 1). These seven measures of rescue performance were used to identify differences in rescue behavior patterns between ants with prior rescue experience and ants without prior rescue experience.



## METHOD

### Subjects

Eight colonies of *Tetramorium* species E were collected during the fall of 2013 from the grounds of Mount Holyoke College. The colonies were moved into the laboratory, where each colony was individually housed in a 20-gal aquarium tank. Species identity was confirmed via microscopy. Tanks were filled with the soil from which the colony was excavated and coated with fluon (Insect-A-Slip) to keep ants from escaping. Throughout the study, each colony was provided with cotton balls soaked in honey and water *ad libitum* and given 2-3 mealworms twice per week. Tanks were misted twice daily to prevent the ants from becoming dehydrated. Each colony was maintained on a 15h:9h light-dark cycle. Four of the eight ant colonies were used in rescue behavior trials.

Two ants were used in rescue behavior trials: the “victim”, an ant that was offered to an antlion, and the “rescuer”, an ant that was placed in the vicinity of the antlion’s pit and allowed to rescue the captured victim. Victims and rescuers were always taken from the same colony to minimize the potential for heterocolonial conflict. Victims and rescuers were selected randomly from the ants that were visible on the colony’s surface at the beginning of each trial. The colony from which the ants used in trials were taken was randomly selected at the start of each day and rotated to ensure equal representation of each of the four colonies in the experiment.

Larval antlions were ordered from [antlionfarms.com](http://antlionfarms.com) and housed individually in white plastic bowls filled with sand. Antlions were fed with mealworms when not being used in trials. An antlion that was used in a trial was permitted to eat the captured ant in lieu of a mealworm.

## Procedure

To investigate changes in rescue behavior patterns over time, rescuer ants were reused across two sequential trials, or phases: Phase 1, the initial trial, intended to provide ants with the opportunity to acquire rescue experience; and Phase 2, the follow-up trial, during which the ants were given a second opportunity to engage in rescue behavior.

To conduct a Phase 1 trial, two ants were removed from the same colony using insect forceps and transferred to small white plastic bowls filled with sand. One ant was chosen at random to be the victim; the other was designated as the rescuer. At the beginning of the trial, the victim ant was rolled for approximately 10 seconds between the experimenter's fingers in order to stun it, allowing for easier capture by the antlion. After rolling, the victim ant was dropped into a pit containing an antlion. The antlion was given two minutes to seize the victim ant in its mandibles; if the antlion did not capture its victim before the time limit, the trial was stopped and discarded from analysis. Once the victim was captured successfully, the rescuer ant was immediately transferred via insect forceps into the bowl containing the antlion. Rescuers were placed approximately halfway

between the wall of the bowl and the perimeter of the antlion's pit. The behavior of the rescuer was recorded for 10 minutes, starting from the point at which the antlion grabbed the victim, using a Canon Vixia HF R32 HD camcorder. At the conclusion of the 10-minute recording period, the rescuer ant was removed from the bowl containing the victim and transferred to a new plastic bowl filled with sand. The rescuer ant was held in this bowl for 4 minutes to give it time to recover from, and process, its previous experience. The victim ant was left in the bowl with the antlion.

To conduct a Phase 2 trial, a new victim ant was removed from the same colony as the rescuer and initial victim immediately prior to the end of the rescuer ant's 4-minute recovery period. At the 4-minute mark, the victim was rolled for approximately 10 seconds between the experimenter's fingers, then dropped into a pit containing a new antlion. As in Phase 1, the antlion was given two minutes to seize the victim ant in its mandibles. Upon a successful capture, the rescuer ant was immediately transferred to the bowl containing the new antlion and victim. As before, the rescuer was placed halfway between the wall of the bowl and the edge of the pit. The behavior of the rescuer was recorded for 10 minutes, starting from the point at which the antlion grabbed the victim, using the same type of camcorder used in Phase 1. At the conclusion of the 10-minute recording period, the victim was left in the pit of the antlion, and the rescuer was removed and discarded.

## Analysis of Video Data

Trial recordings were saved to a memory card in the camcorders, transferred to a computer, and viewed in Windows Media Player or VLC. A time-interval sampling design was used to record rescue behaviors shown during video playback. In this design, each 10-minute trial was divided into 30 intervals, each 20 seconds long. During playback, one experimenter called out each instance of rescue behavior as it occurred in the video, while another experimenter, using a stopwatch, marked each behavior down in its appropriate time interval. Under the time-interval sampling design, if the same type of rescue behavior was observed multiple times within the same 20-second interval, it was recorded only once per interval. If a rescuer ant displayed more than one type of rescue behavior within the same interval, both behaviors were marked down in the same interval. The three types of rescue behavior investigated in this study, limb-pulling, sand displacement, and attack, were coded using the standard operational definitions described in Table 1.

In addition, the behavior of each rescuer during its Phase 1 trial was observed and used to determine its group designation, or Natural Group. Rescuer ants that did not fall into the pit in Phase 1 (thereby avoiding encountering an antlion) were designated as Non-Rescuers. Rescuer ants that fell into the pit and had the opportunity to interact with the antlion and victim, but did not perform any of the three target rescue behaviors, were

designated as Observers. Lastly, rescuers that fell into the pit and performed rescue behavior were designated as Rescuers.

### Inter-Rater Reliability

Inter-rater reliability was determined by calculating Cronbach's coefficient alpha using the R statistical package 'psy' (v. 1.1). Two of the five raters exhibited substantial agreement across all three rescue behaviors (alpha = 0.87); these individuals were therefore used to evaluate rescue behavior in each trial, while the other three experiments were used to record the raters' observations.

### Statistics

A repeated-measures ANOVA, with one within-subjects factor (Phase: 1 or 2) and one between-subjects factor (Natural Group: Non-Rescuer, Observer, or Rescuer), was used to analyze the seven dependent variables: (a) the total duration of rescue behavior during a trial, defined as the number of intervals during which at least one of the three types of rescue behavior was recorded; (b) the number of different types of rescue behavior exhibited during a trial, ranging from a minimum of 0 (no rescue behavior) to a maximum of 3 (all three types of rescue behavior); (c) the latency to perform rescue behavior, defined as the number of intervals, converted into seconds, that elapsed before the rescuer began exhibiting rescue behavior; (d) the longest continuous bout of rescue behavior, defined as the largest number of

intervals over which the rescuer performed rescue behavior consecutively; and the durations of (e) limb-pulling, (f) sand displacement, and (g) attack behavior, defined as the number of intervals in each trial in which the respective behaviors were performed. Post hoc comparisons using Newman-Keuls tests were performed to identify the significant relationships between individual conditions. Primary statistical analyses were performed in SPSS v. 21.0.

Table 1. Operational Definitions of Target Behaviors

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<u>Behavior</u>	<u>Operational definition</u>
Limb-pulling	Ant grabs limb of the ant victim with mandibles and drags it backwards with frequent antennation. The antennae are facing forward, in the characteristic non-aggressive posture.
Sand displacement	Ant picks up, using its mandibles, one or more particles of sand/substrate covering the filter paper or ensnared ant victim, and moves it at least one body length from its original position; or, the ant positions itself within 2 cm of, and facing, the ensnared victim, and flicks sand backward, away from the victim, using its anterior legs. The antennae are facing forward, in the characteristic non-aggressive posture.
Attack	Ant engages in either of two aggressive behaviors: Biting/dismembering, closing its mandibles around part of the antlion's body, sometimes while also moving away from the antlion; or, stinging, directing its stinger toward the antlion, often while the gaster (abdomen) is flexed, curved underneath the body. All attack components are performed with the antennae flexed backward, in the characteristic aggressive posture.

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Adapted from Taylor, Visvader, Nowbahari, & Hollis, 2013.

## RESULTS

A total of 45 paired (Phase 1 and Phase 2) trials were recorded, 5 of which were ultimately excluded from the analysis due to problems during the trial, such as the victim escaping from the antlion or the antlion refusing to grab the victim within the two-minute time limit. The 40 remaining trials were used to examine potential differences in rescue behavior patterns between Phase 1 and Phase 2 among the three different Natural Groups (Non-Rescuer, Observer, and Rescuer).

### Statistical Analyses

Repeated-measures ANOVAs revealed interaction effects between the Phase and Natural Group factors in five of the seven measurements of rescue performance: total duration of rescue behavior,  $F(2,37) = 7.072$ ,  $p = 0.003$  (Figure 1); number of different types of rescue behavior  $F(2,37) = 6.165$ ,  $p = 0.005$  (Figure 2); latency to perform rescue behavior,  $F(2,37) = 10.454$ ,  $p = 0.000$  (Figure 3); longest bout of rescue behavior,  $F(2,37) = 11.721$ ,  $p = 0.000$  (Figure 4); and duration of limb-pulling behavior,  $F(2,37) = 10.155$ ,  $p = 0.000$  (Figure 5). No interaction effects were observed for duration of sand displacement behavior,  $F(2,37) = 0.053$ ,  $p = 0.948$  (Figure 6), or duration of attack behavior,  $F(2,37) = 2.624$ ,  $p = 0.086$  (Figure 7).

None of the measurements revealed a statistically significant Phase effect (total duration of rescue behavior,  $F(1,37) = 1.859$ ,  $p = 0.181$ ; number



of different types of rescue behavior,  $F(1,37) = 0.486$ ,  $p = 0.49$ ; latency to perform rescue behavior,  $F(1,37) = .296$ ,  $p = 0.59$ ; longest bout of rescue behavior,  $F(1,37) = 2.285$ ,  $p = 0.139$ ; duration of limb-pulling behavior,  $F(1,37) = 1.85$ ,  $p = 0.182$ ; duration of sand displacement behavior,  $F(1,37) = 0.051$ ,  $p = 0.823$ ; duration of attack behavior,  $F(1,37) = 2.497$ ,  $p = 0.123$ ).

All rescue performance measurements had statistically significant Natural Group effects (total duration of rescue behavior,  $F(2,37) = 8.082$ ,  $p = 0.001$ ; number of different types of rescue behavior,  $F(2,37) = 32.143$ ,  $p = 0.000$ ); latency to perform rescue behavior,  $F(2,37) = 25.838$ ,  $p = 0.000$ ; longest bout of rescue behavior,  $F(2,37) = 14.744$ ,  $p = 0.000$ ; duration of limb-pulling behavior,  $F(2,37) = 6.638$ ,  $p = 0.003$ ; duration of sand displacement behavior,  $F(2,37) = 5.949$ ,  $p = 0.006$ ; duration of attack behavior,  $F(2,37) = 11.177$ ,  $p = 0.000$ ). Because ants were, by definition, retroactively assigned to specific Natural Groups according to their own behavior in Phase 1, these statistically significant effects are not experimentally meaningful, and are therefore excluded from the discussion of the results.

### Individual Comparisons

Post hoc comparisons using Newman-Keuls tests revealed that ants in the Rescuer group spent on average 5.727 intervals (out of a possible 30) performing one or more rescue behaviors during Phase 1, which was not significantly different from the total rescue duration of the Rescuer group

during Phase 2 ( $q(2,2) = 5.371, p > 0.05$ ) (Fig. 1). The Non-Rescuer group displayed rescue behavior during 0.429 intervals on average during Phase 2, which was not a significant increase from Phase 1, during which, by definition, the Non-Rescuer group performed no rescue behavior ( $q(3,2) = 0.939, p > 0.05$ ). A similar pattern was observed in the Observer group across Phase 1 and Phase 2, where the Phase 2 Observers spent on average 0.5 intervals performing rescue behavior ( $q(3,2) = 1.094, p > 0.05$ ). The Phase 2 Rescuer group spent 3.273 intervals performing rescue behavior, compared to the 0.5 and 0.429 intervals the Observer and Non-Rescuer groups, respectively, spent performing rescue behavior in Phase 2, but these differences were not statistically significant (Phase 2 Non-Rescuers x Phase 2 Rescuers,  $q(3,2) = 6.224, p > 0.05$ ; Phase 2 Non-Rescuers x Phase 2 Observers,  $q(2,2) = 0.155, p > 0.05$ ; Phase 2 Observers x Phase 2 Rescuers,  $q(2,2) = 6.068, p > 0.05$ ).

Ants in the Rescuer group in Phase 2 did not perform significantly more or fewer types of rescue behavior than Rescuer group ants did during Phase 1 (Phase 2 mean = 0.818 behaviors; Phase 1 mean = 1.636 behaviors;  $q(2,2) = 4.652, p > 0.05$ ) (Fig. 2). Similarly, the Non-Rescuer and Observer groups did not employ more or fewer rescue behaviors in Phase 2 than Phase 1 (Non-Rescuers: Phase 2 mean = 0.143 behaviors;  $q(3,2) = 0.813, p > 0.05$ ; Observers: Phase 2 mean = 0.375 behaviors;  $q(3,2) = 2.132, p > 0.05$ ). As with the total rescue duration variable, there were no differences in the number of different types of rescue behavior displayed between any of the three Natural

Groups in Phase 2 (Non-Rescuers x Rescuers,  $q(3,2) = 3.838$ ,  $p > 0.05$ ; Non-Rescuers x Observers,  $q(2,2) = 1.319$ ,  $p > 0.05$ ; Observers x Rescuers,  $q(2,2) = 2.519$ ,  $p > 0.05$ ).

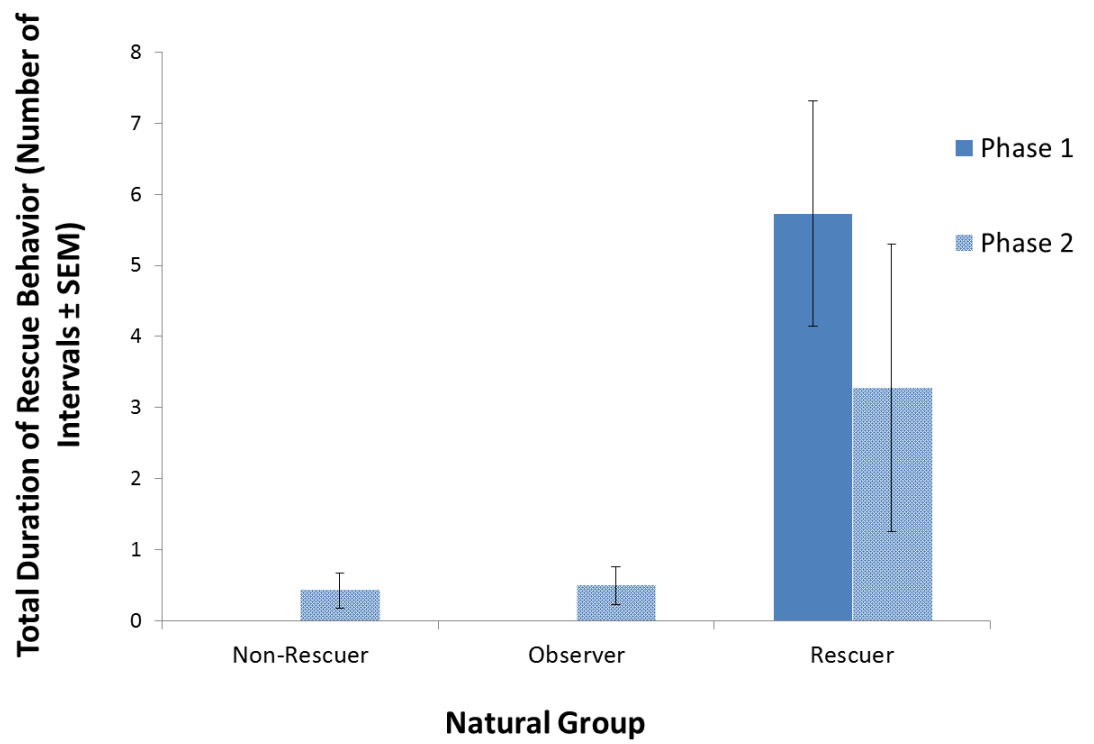
Ants in the Phase 2 Rescuer group took 436 seconds on average to begin performing rescue behavior, compared to the 351 seconds observed in the Phase 1 Rescuer group (Fig. 3); however, this difference was not statistically significant ( $q(3,2) = 5.77$ ,  $p > 0.05$ ). Likewise, the differences in latency between the Non-Rescuer group in Phase 2 and Phase 1 (mean 557 seconds vs. 600 seconds, respectively) and between the Observer group in Phase 2 and Phase 1 (463 vs. 600 seconds) were not significant (Non-Rescuers:  $q(3,2) = 1.054$ ,  $p > 0.05$ ; Observers:  $q(3,2) = 3.38$ ,  $p > 0.05$ ). The latency to perform rescue behavior did not vary between the three Natural Groups in Phase 2 (Non-Rescuers x Rescuers,  $q(3,2) = 2.971$ ,  $p > 0.05$ ; Non-Rescuers x Observers,  $q(2,2) = 2.328$ ,  $p > 0.05$ ; Observers x Rescuers,  $q(2,2) = 0.643$ ,  $p > 0.05$ ).

The length of the longest bout of rescue behavior among the Rescuer group decreased from 2.82 intervals in Phase 1 to 1.09 intervals in Phase 2 ( $q(2,2) = 6.804$ ,  $p < 0.05$ ) (Fig. 4). The differences in the length of the longest bout of rescue behavior between the Non-Rescuer group in Phase 2 (0.29 intervals) and Phase 1 (0 intervals), and between the Observer group in Phase 2 (0.5 intervals) and Phase 1 (0 intervals) were not statistically significant (Non-Rescuers:  $q(3,2) = 1.141$ ,  $p > 0.05$ ; Observers:  $q(3,2) = 1.967$ ,

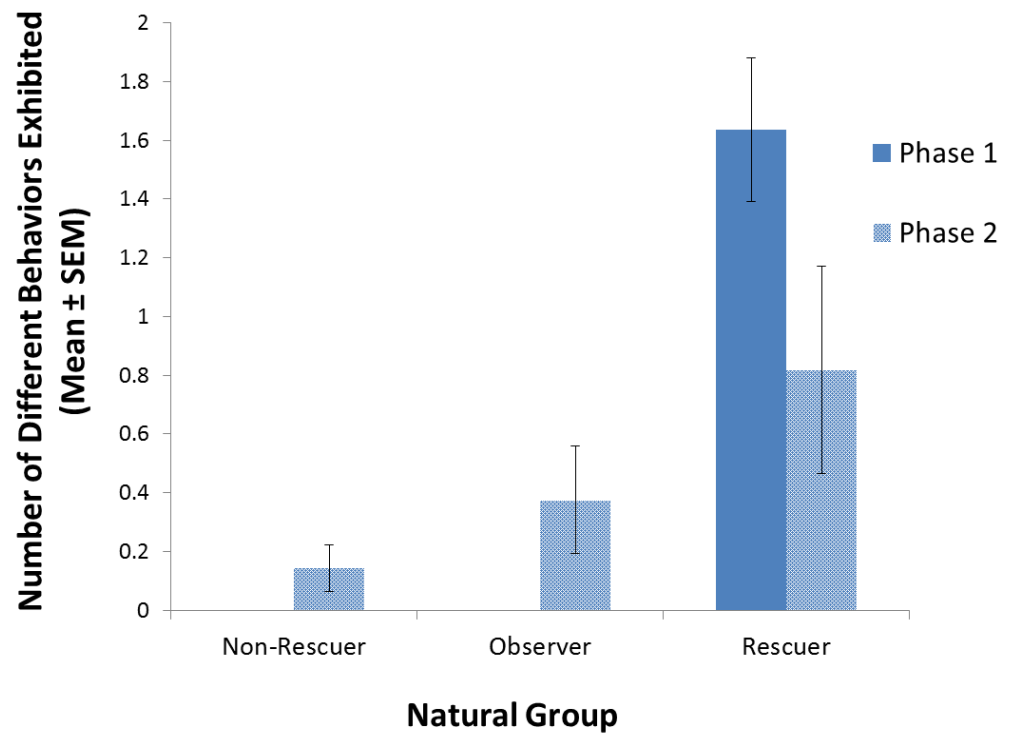
$p > 0.05$ ). No significant differences in the lengths of the longest bouts of rescue behavior were observed between the three Natural Groups in Phase 2 (Non-Rescuers x Rescuers,  $q(3,2) = 3.146$ ,  $p > 0.05$ ; Non-Rescuers x Observers,  $q(2,2) = 0.826$ ,  $p > 0.05$ ; Observers x Rescuers,  $q(2,2) = 2.321$ ,  $p > 0.05$ ).

The Rescuer group displayed less limb-pulling behavior in Phase 2 (mean 2.091 intervals) than in Phase 1 (mean 4.091 intervals) ( $q(2,2) = 6.217$ ,  $p < 0.05$ ) (Fig. 5). No statistically significant variation in the duration of limb-pulling behavior across Phase 1 and Phase 2 was observed in either the Non-Rescuer group or Observer group (Non-Rescuer Phase 2 mean = 0.429,  $q(3,2) = 1.334$ ,  $p > 0.05$ ; Observer Phase 2 mean = 0.5,  $q(3,2) = 1.554$ ,  $p > 0.05$ ). Likewise, the duration of limb-pulling behavior did not differ between the three Natural Groups in Phase 2 (Non-Rescuers x Rescuers,  $q(3,2) = 5.167$ ,  $p > 0.05$ ; Non-Rescuers x Observers,  $q(2,2) = 0.221$ ,  $p > 0.05$ ; Observers x Rescuers,  $q(2,2) = 4.945$ ,  $p > 0.05$ ).

*Figure 1.* Total duration of rescue behavior (mean  $\pm$  SEM) for each Natural Group across Phase 1 and Phase 2, measured as the total number of 20-second intervals in a trial during which an ant performed any one of the three types of rescue behavior (limb-pulling, sand displacement, and attack).

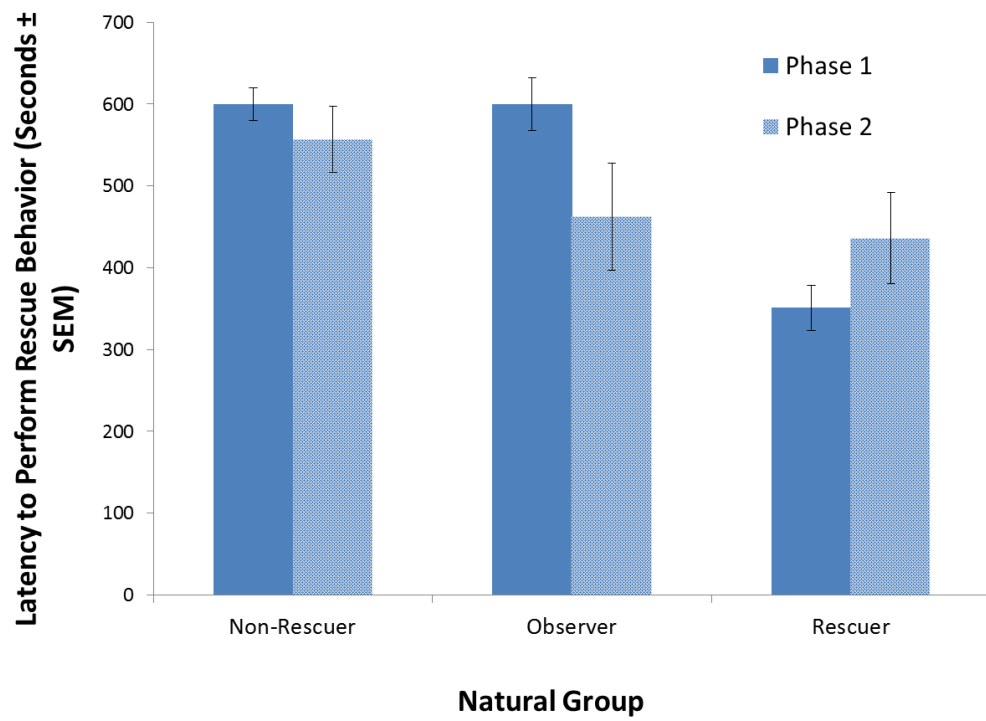


*Figure 2.* The number of different types of rescue behavior observed during trials (mean  $\pm$  SEM) for each Natural Group in Phase 1 and Phase 2. Measurements range in value from 0 (no rescue behavior exhibited during a trial) to a possible maximum of 3 (limb-pulling, sand displacement, and attack exhibited during the same trial).

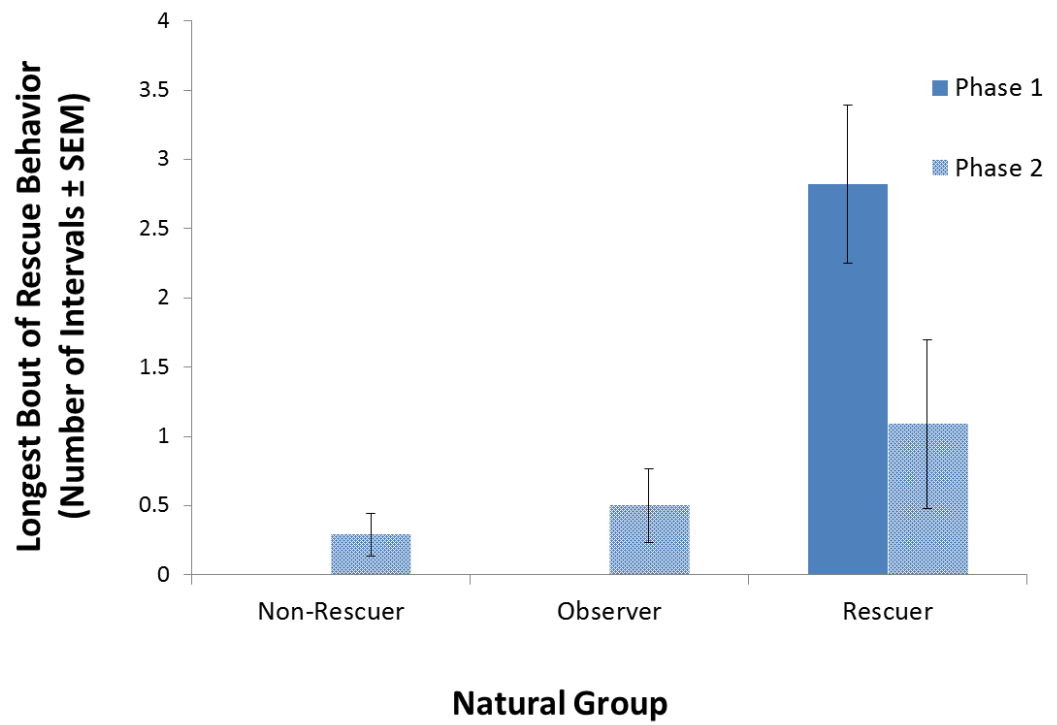




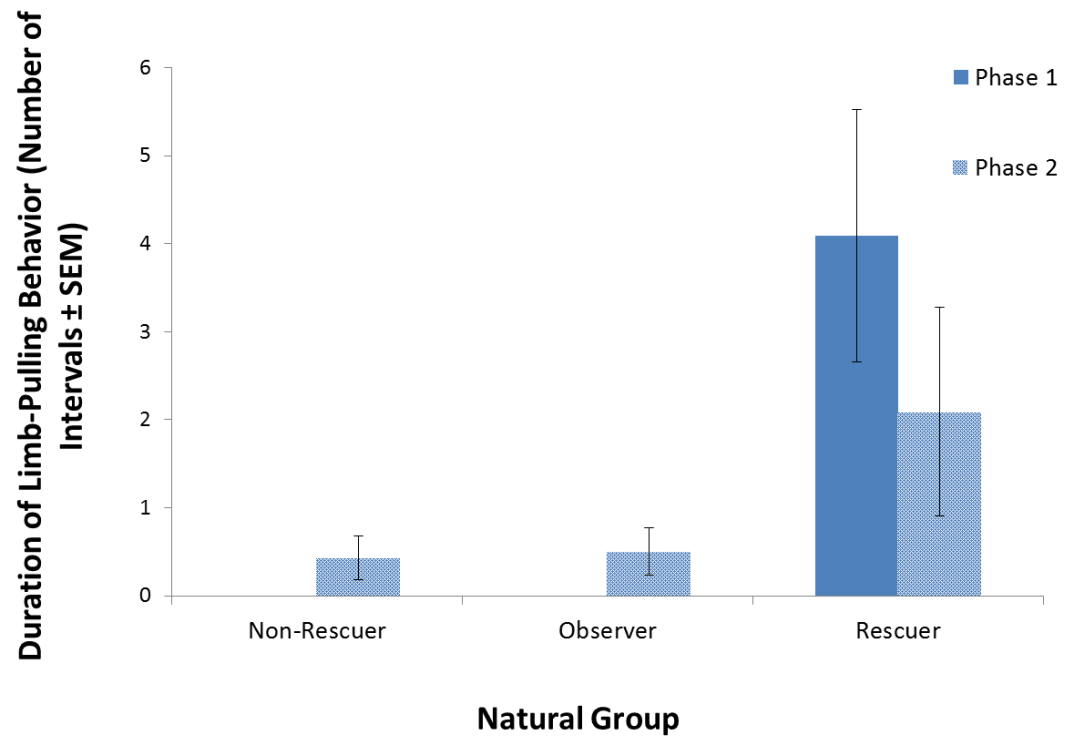
*Figure 3.* Latency to perform rescue behavior (mean seconds until first rescue behavior exhibited  $\pm$  SEM) for each Natural group between Phase 1 and Phase 2.



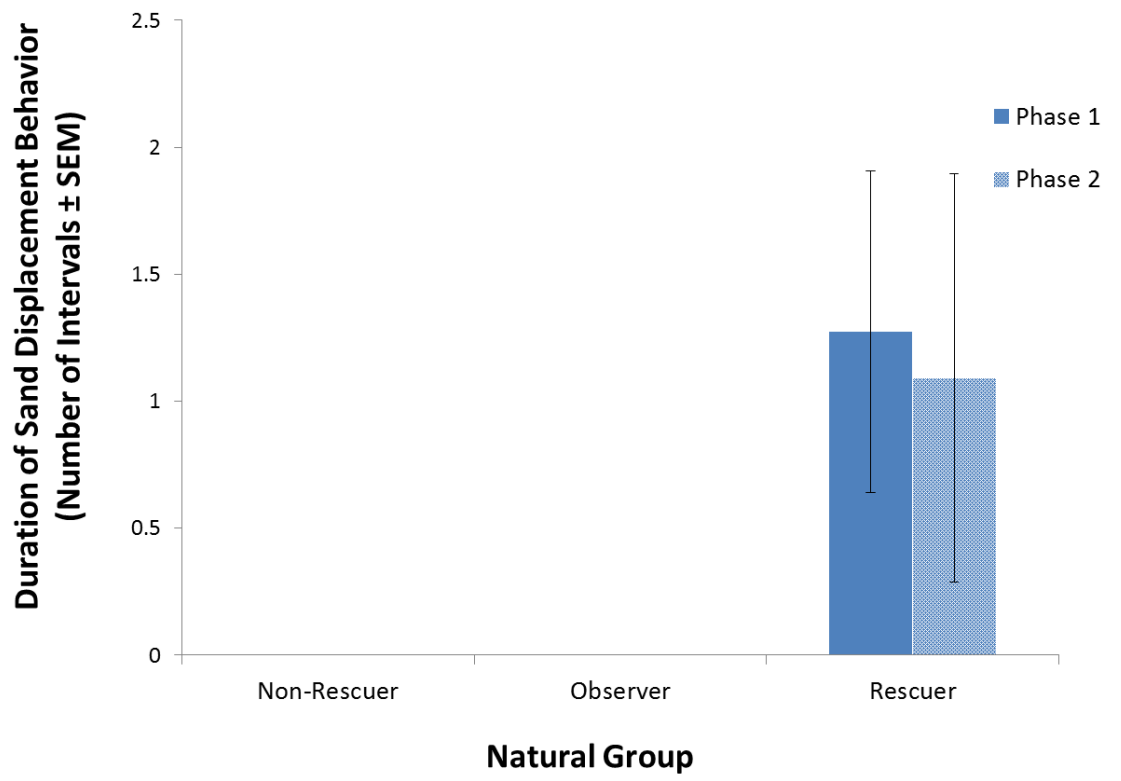
*Figure 4.* Length of the longest continuous bout of rescue behavior (mean number of intervals  $\pm$  SEM) for each Natural Group in Phase 1 and Phase 2.



*Figure 5.* Duration of limb-pulling behavior (mean  $\pm$  SEM) for each Natural Group in Phase 1 and Phase 2, measured as the total number of intervals during a trial in which an ant demonstrated limb-pulling behavior.

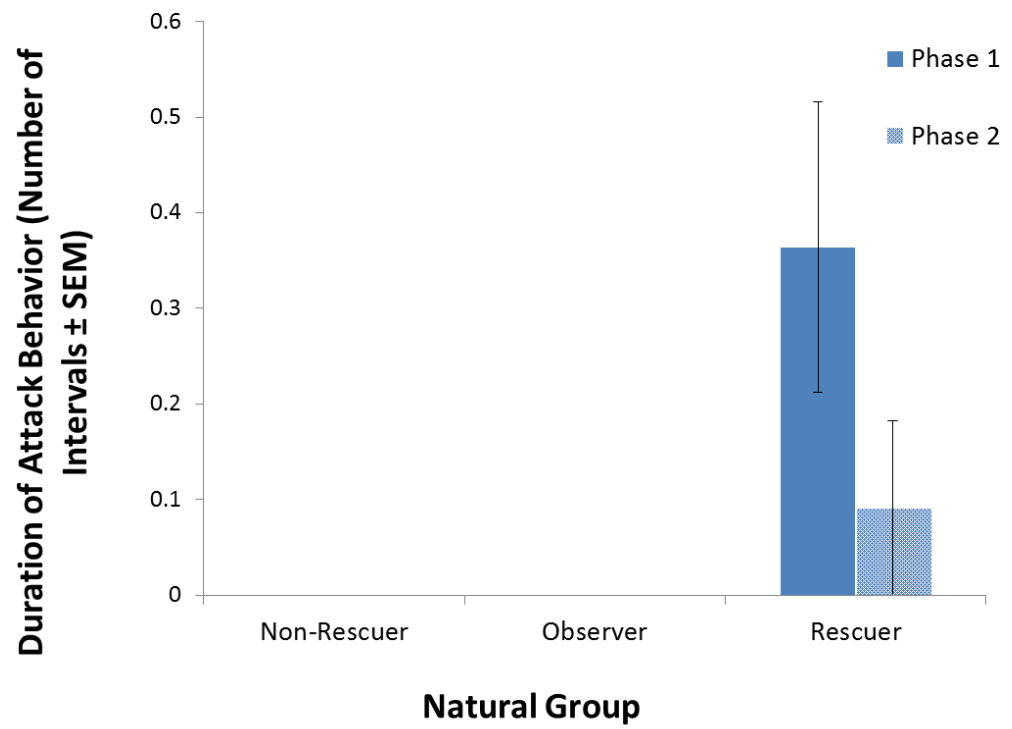


*Figure 6.* Duration of sand displacement behavior (mean  $\pm$  SEM) for each Natural Group in Phase 1 and Phase 2, measured as the total number of intervals during a trial in which an ant demonstrated sand displacement behavior.





*Figure 7.* Duration of attack behavior (mean  $\pm$  SEM) for each Natural Group in Phase 1 and Phase 2, measured as the total number of intervals during a trial in which an ant demonstrated attack behavior.



## DISCUSSION

There was little observed variation in rescue behavior during Phase 2 among the different Natural Groups. Ants without prior rescue experience (i.e., the Non-Rescuer and Observer groups) occasionally performed rescue behavior during Phase 2, but their behavioral profiles were similar to those of the Rescuer group. In particular, the finding that ants with prior (Phase 1) rescue experience were no more likely to perform rescue behavior in Phase 2 than ants without prior rescue experience (as measured by the total duration of rescue behavior) suggests that, in *T. sp. E*, rescue experience has no direct influence on an ant's willingness to perform rescue behavior during future encounters with antlions.

The two observed differences in behavioral patterns arose from comparisons of the performance of the Rescuer group across Phase 1 and Phase 2. Ants that performed rescue behaviors during Phase 1 tended to display shorter longest bouts of rescue behavior and demonstrated less limb-pulling behavior in Phase 2. These findings may reflect an overall decrease in the ants' willingness to engage in sustained rescue behavior when presented with additional opportunities to assist a trapped nestmate. It is worth noting that, although the ants used as rescuers in Phase 1 were given four minutes to recover before being used again in Phase 2 trials, this timeframe was a somewhat arbitrary decision. In a natural setting, a rescuer ant that has escaped from an antlion's pit may take as long as it likes to recover before returning to the same pit or entering a different one, as antlions are largely

immobile and cannot capture prey outside the pit. Therefore, experiments investigating the link between prior rescue experience and future rescue behavior in the field may yield different results.

The finding that none of the seven rescue performance measurements varied significantly between Phase 1 and Phase 2 in the Non-Rescuer and Observer groups, but that some variation in performance was observed between Phase 1 and Phase 2 in the Rescuer group, suggests a possible role for predator avoidance in shaping patterns of rescue behavior. During the Phase 1 trials, the Non-Rescuer and Observer groups had the opportunity to encounter the perimeter of the antlion's pit (in the case of the Non-Rescuer group) or the antlion itself (in the Observer group), but did not perform behaviors like limb-pulling or attacking that required sustained physical contact with either the antlion or the victim. Performing rescue behavior, as opposed to simply observing the antlion and victim or not encountering them at all, is associated with some degree of predation risk (indeed, several trials were discarded from this experiment because the rescuer managed to provoke the antlion into releasing its victim and capturing the rescuer instead). Previous research on antipredator behavior in *T. sp. E* has shown that ants that have previously been seized by antlions modify their behavior to avoid encountering antlions in the future; for example, by displaying longer latencies to fall into pits than naïve ants (McNew, 2013). Although the ants in the Rescuer group were not permitted to be captured by antlions during Phase 1, it is possible that the direct physical contact involved in two

of the three forms of rescue behavior (limb-pulling and attacking) was sufficient to produce the behavioral modifications in Phase 2 observed in this study. Alternatively, the energetic cost associated with performing sustained bouts of rescue behavior may have resulted in the observed declines in rescue performance among the Rescuer group in Phase 2 compared to Phase 1.

An important consideration for the present study was that rescuer ants very seldom succeed in rescuing their nestmates from antlions. Studies of task specialization and learning in ants (Langridge et al., 2008; Ravary et al., 2007) have shown that ants are not only more likely to perform behaviors that have led to past successes, but also become less likely to perform behaviors associated with task failure, based on prior experience. In Bartal et al.'s (2011) study of prior experience and rescue behavior in rats, only individuals that succeeded in rescuing a conspecific from an artificial trap became more likely to free conspecifics from traps during future opportunities; empty traps, or traps containing toy rats, were no less likely to be opened by experienced rats than naïve rats. These findings may help explain the observed decreases in limb-pulling behavior and in the length of the longest bout of rescue behavior among the Phase 2 Rescuer ants, as these were the only group of ants in the present study which had not just prior rescue experience, but universally unsuccessful prior rescue experience. Therefore, the Rescuer ants in Phase 2 may have become more reluctant to perform rescue behavior after an unsuccessful initial attempt. In contrast, the

Phase 2 Non-Rescuer and Observer groups, which did not experience unsuccessful rescue attempts during Phase 1, displayed unchanged patterns of rescue performance during Phase 2, consistent with the above findings.

Inclusive fitness theory predicts that an individual may not perform altruistic behavior if it perceives that the associated cost or risk outweighs the selective benefits. In the context of this experiment, antlions represent a direct and major threat to any ant that attempts to rescue a nestmate. It is possible that “lower-stakes” experiments using non-lethal traps, such as the clumps of dirt detailed in LaFleur’s (1940) rescue experiments using *F. fusca* and *L. flavis*, or the artificial snares used to trap *C. cursor* (Nowhabari et al., 2009), might reveal changes in rescue behavior patterns as a result of previous encounters with the rescue stimulus. In addition to posing less risk to the rescuers, another potential benefit to using artificial traps is that they can be constructed to allow the rescuer to successfully rescue a trapped ant by performing precise, targeted rescue behaviors. For example, an ant may be able to bite through a thin piece of string connected to an apparatus holding a victim in place, similar to Nowbahari et al.’s (2009) snare design. Further research on rescue behavior using artificial rescue stimuli may help disentangle the potential effects of predator avoidance, exhaustion, and prior experience in modifying the expression of rescue behavior in ants.

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