

Learning in a sedentary insect predator: Antlions (Neuroptera: Myrmeleontidae) anticipate a long wait

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ABSTRACT

Pit-building antlions, the larvae of a winged adult insect, capture food by digging funnel-shaped pits in sand and then lying in wait, buried at the vertex, for prey to fall inside. The sedentary nature of this sit-and-wait predatory behaviour and, especially, antlions' innate ability to detect prey arrival, do not fit the typical profile of insects that possess learning capabilities. However, we show, for the first time, that learning can play an important role in this unique form of predation. In three separate experiments, individual antlions received, once per training day, either a vibrational cue presented immediately before the arrival of food or that same cue presented independently of food arrival. Signalling of food not only produced a learned anticipatory behavioural response (Experiment 1), but also conferred a fitness advantage: Associative learning enabled antlions to dig better pits (Experiments 2 and 3), extract food more efficiently (Experiments 2 and 3), and, in turn, moult sooner (Experiment 3) than antlions not receiving the associative learning treatment.

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The study of associative learning in insects, already well-established in the 1970s (Alloway, 1972) but limited to a handful of species, now spans social, eusocial and solitary-living insects from multiple families in all major insect orders (Papaj, 2003; North and Greenspan, 2007). Beyond those early studies of honeybees, *Apis mellifera* (e.g., Menzel, 1968; Menzel et al., 1974), fruitflies, *Drosophila melanogaster* (e.g., Murphy, 1967, 1969; Quinn et al., 1974; Spatz et al., 1974), and ants, *Formica* sp. (Schneirla, 1941, 1943), the capacity for associative learning, as well as its fitness benefits, have been demonstrated recently in two species of parasitic wasps, *Biosteres arisanus* (Dukas and Duan, 2000) and *Microplitis croceipes* (Rains et al., 2006), social wasps, *Polybia occidentalis* (London and Jeanne, 2005), moths, *Heliothis virescens* (Skiri et al., 2005), cockroaches, *Periplaneta americana* (Sakura and Mizunami, 2001; Watanabe and Mizunami, 2005), locusts, *Locusta migratoria* (Simpson and White, 1990), mosquitoes, *Ades aegypti* (Kaur et al., 2003), and grasshoppers, *Schistocerca americana* (Dukas and Bernays, 2000), to name but a few species.

However, in all the insect learning research to date, the subjects have been animals that move about their environment as

they actively forage for food, find a mate, locate a host, or avoid some noxious stimulus. Associative learning essentially improves the efficiency of that active search. Put another way, learned cues help to make searching in a changing environment more predictable. Indeed, this notion that mobility of one form or another is a closely linked characteristic of learning in the natural environment not only has pervaded the vertebrate literature (e.g., Hollis, 1982, 1997; Staddon, 1988), but also has been recognized as an important predictor of which insect species would be expected to have evolved the capacity for associative learning (e.g., Bernays, 1993).

Given this profile of active search in an unpredictable environment, extremely sedentary insects such as pit-building antlions (Neuroptera: Myrmeleontidae; see Fig. 1), the larvae of a winged adult insect, would appear to be unlikely candidates for associative learning: Not only are these sit-and-wait predators unique in the insect world for their extremely sedentary predation strategy (Mansell, 1992, 1996), but also they already are equipped with a multitude of sensory receptors designed to detect the approach of prey at optimal distances (Devetak, 1985), as well as an elaborate battery of responses designed to capture prey quickly and efficiently (Devetak et al., 2007; Griffiths, 1980, 1981, 1986; Mansell, 1999; Matsura and Takano, 1989; Mencinger-Vračko and Devetak, 2008). Nonetheless, here we show that pit-building antlions, the most sedentary of sit-and-wait insect predators, use associative learning to great advantage. This demonstration that antlions can learn,

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Fig. 1. A pit-building larval antlion (top); typical antlion pits in sand (bottom). Photography by Susan Beatrice.

and that such learning increases fitness, suggests the possibility that associative learning is even more widespread than the current census of insect research suggests. Indeed, the approach that has emerged from the field of invertebrate neurobiology suggests that many behavioural commonalities – including associative learning – while not true homologies, “may represent universality of an even higher order: that of fundamental principles of brain functionality” (Greenspan, 2007, p. 649).

Antlions are a diverse group of insects dispersed worldwide in arid, sandy regions (Grimaldi and Engel, 2005). Although not all antlion species build pits during their larval stage, those that do are sometimes called “doodlebugs” because of the winding furrows created by the larvae as they move across the sand surface, searching for a suitable pit location (Topoff, 1977). Once such a location is found, typically one that offers protection from wind and rain (Crowley and Linton, 1999; Scharf and Ovidia, 2006), the long wait for prey to wander into the pit begins. But the potentially long wait between meals in this predatory species belies the importance of prey capture during antlions’ larval stage: Larvae must acquire sufficient food reserves to sustain them during subsequent pupation, when they do not feed at all (Griffiths, 1980, 1985); in addition, larval growth has a direct and significant impact on the individual’s ability to reproduce (Gotelli, 1997; Griffiths, 1985). Thus, the larval

stage is a critical feeding stage—and efficient pit construction and prey capture are especially important.

To construct its pit, an antlion spirals backwards, using its mandibles as shovels with which to flick the excavated sand (Tauber et al., 2003; Mansell, 1999); the completed pit is conically shaped, built and sloped in such a way to form a highly efficient trap (Lucas, 1982, 1989; Fertin and Casas, 2006). To capture prey, an antlion positions itself at the vertex of the pit, with its mandibles open and its body completely under the sand, perhaps with only the antennae and part of the mandibles visible (Griffiths, 1980). When a prey item accidentally wanders over the edge of a pit, it loses footing on the steep walls and plummets toward the antlion’s open mandibles. If, however, capture is not immediate, an antlion will flick sand in the direction of its prey, which cascades down the pit walls and carries the prey to the vertex (Lucas, 1989; Mencinger-Vračko and Devetak, 2008).

Once prey is captured, an antlion uses its mandibles to inject a poison, which paralyzes or kills the prey within minutes (Yoshida et al., 1999), then to inject digestive enzymes, which liquefies the prey, and finally to pump the externally digested contents of the prey back into its gut (Griffiths, 1982; Van Zyl et al., 1998). After feeding is complete, an antlion flicks the prey exoskeleton out of the pit (Mansell, 1999) and performs routine maintenance to return the pit to its conical capture shape (Fertin and Casas, 2006).

Antlions’ larval stage is highly variable in length, in large part because it depends on the availability of food (Griffiths, 1980, 1986). During this stage, which often lasts as long as 2 years (Gotelli, 1997), antlions mature through three substages, termed instars, moulting twice (Tauber et al., 2003). Although they cease feeding and move down into the sand under their pits while moulting, antlions resume feeding in the same pit, which increases in size as the antlions grow larger. When larval antlions reach a critical mass, pupation occurs, the stage during which the larvae metamorphose into reproductively mature adults (Griffiths, 1985). To pupate, larvae spin a sticky, silken cocoon, to which sand and debris attaches (Mansell, 1999). The resulting sand-ball cocoon offers protection and shelter for roughly 30 days, after which the imago (adult) emerges. Although adults occasionally feed on small insects or nectar, the primary function of adulthood, which lasts a mere 4 weeks (Gotelli, 1993), is reproduction. Thus, despite larval antlions’ numerous adaptations to detect prey arrival well in advance and to handle it efficiently, if the capacity to learn about environmental predictors of food provided even a small net gain in an individual’s ability to obtain the necessary calories and nutrients required for reproduction, it would enjoy a selective advantage.

Here we report the results of three experiments in which we investigated the capacity of antlions to modify their predatory behaviour through associative learning. In Experiment 1, we explored whether antlions changed any component of their behaviour in the presence of a signal predicting food. In Experiments 2 and 3 we explored the effect of learning to anticipate prey arrival on several correlates of fitness, namely pit construction, prey extraction and the latency to moult.

1. Experiment 1

In Experiment 1, antlions received a vibrational signal, falling sand, at one of three different distances, namely 3, 8, or 15 cm, either immediately before food arrival or at another, randomly determined time of day. This particular cue was chosen because it did not elicit any form of observable behaviour in pilot work. Nonetheless, antlions’ bodies are covered with thousands of small bristles, or setae, containing mechanoreceptors that respond to vibrations conducted through the sand (Matsura and Takano, 1989) and are capable of detecting vibrational stimuli, such as sand disturbances, within this range of distances (Devetak, 1985; Devetak et al., 2007;

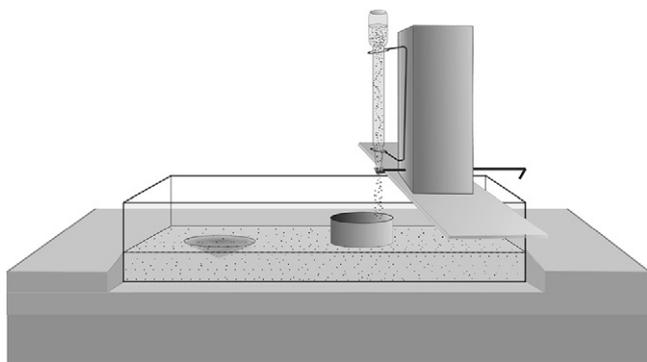


Fig. 2. Front view of the apparatus illustrating the rectangular plastic box housing the antlion in its pit. The plastic box is nestled in a foam block; the sand delivery device rests on top of the box, directly over the sand collection container. The sound-and-vibration attenuating chamber (not pictured) surrounds the apparatus on the left, right and rear sides.

Mencinger, 1998). Thus, in Experiment 1 we looked for evidence of antlions' ability to associate a behaviourally neutral vibrational cue with the arrival of a prey item.

1.1. Method

1.1.1. Subjects and apparatus

Prior to selection as subjects, larval antlions (*Myrmeleon crudelis* Walker), obtained from AntLionFarms.com (Pensacola, FL), were placed individually in plastic bowls (12.5 cm diameter \times 4.0 cm height) filled with 225 g of white marine sand (Estes, Inc., Staley, NC) and located in a room maintained at 20 °C with a 14:10 h light:dark cycle (light onset at 09:00 h). After 48 h, antlions that dug pits were removed and weighed. Pairs, matched for weight, were created; one member of each pair was randomly assigned to a learning treatment group (LRN) while its matched-weight pairmate was assigned to a control treatment group (CTL). Subjects were run in two replications, namely 12 pairs of subjects in the fall of 2005 and 11 pairs of subjects in the spring of 2006.

Following its assignment to either the LRN or CTL treatment group, each antlion was moved to a rectangular plastic box (28 cm long \times 17 cm wide \times 17 cm deep; see Fig. 2), filled with 3600 g of white marine sand, described above, such that the sand surface was 3 cm below the top of the plastic box. Each plastic box was placed in a sound-and-vibration attenuating chamber (60 cm long \times 46.5 cm wide \times 30 cm deep) made of 2.54-cm thick foam board, open to the front for viewing. The floor of the chamber was a 7.62-cm thick block of foam; the plastic box was cradled in a 2.54-cm deep pocket of that foam, centred in the middle of the foam floor. Individual chambers were located adjacent to one other on 4 shelves, with 6 chambers per shelf.

A sand delivery device, which provided the vibrational cue, consisted of a plastic dropper attached by a wire holder to a block of wood, and rested across the top of the box housing each antlion. A lever, which also extended from the block of wood, controlled the release of sand contained in the dropper. Directly underneath the dropper was a sand collection container, namely a 3.8 cm tall section of 1.5 in. PVC pipe with plastic wrap secured across the bottom; this collection container rested on the sand surface and prevented additional sand from accumulating in an antlion's box. Each antlion was given 36 h to dig a pit and was not fed until the start of the experiment.

1.1.2. Procedure

The experiment consisted of two phases, namely a 25-day Training Phase, consisting of 5 blocks of 5 days (4 consecutive training days followed by a 1-day rest interval), and a 1-day Test Phase. Each

antlion was fed one prey item per training day and on test day; no food was provided on rest days.

1.1.2.1. Training Phase. For each antlion in the LRN group, a prey item, described below, was delivered to the centre of the pit at a randomly selected time between 10:00 and 17:00 h, once per day, immediately following a signal, specifically 4.5 ml of sand dropped into the sand collection container (approximately a 5-s vibrational cue). Antlions in the CTL group were fed at the same time of day as the antlions in the LRN condition; however, each antlion in the CTL group received the vibrational signal at another randomly selected time between 10:00 and 17:00 h. Throughout training and testing, the vibrational signal was delivered to each antlion at one of three signal distances (SD), namely 3 cm from the edge of the pit (SD-Near), 8 cm from the edge of the pit (SD-Intermediate), or 15 cm from the edge of the pit (SD-Far).

Prey items were wingless fruit flies (*Drosophila* sp.) obtained from Connecticut Valley Biological Supply (Southampton, MA). Only prey items that weighed between 0.8 and 1.2 mg were used. Weighing required flies to be anesthetized briefly with CO₂: Flies were placed in a small plastic tube connected to a glass test tube filled with approximately one tablespoon of sodium bicarbonate, to which 4 ml of distilled vinegar was added. A cotton filter at the juncture of the plastic and glass tubes prevented the flies from entering the test tube. Flies were fully awake when fed to antlions. A single prey item was delivered to each antlion from a small polystyrene weigh boat; a metal spatula was used to push the prey out of the boat into the pit.

Between 21:00 and 23:00 h (i.e., shortly before light offset), both the sand delivery device and the sand collection container were removed and prepared for the following day (i.e., the sand delivery device was refilled and the collection container was emptied), and then replaced at the appropriate location.

1.1.2.2. Testing Phase. On the test day, each antlion received the vibrational signal at its assigned signal distance, followed immediately by delivery of a single prey item. All prey items weighed 0.8 mg and were delivered exactly as in training.

1.1.3. Data analysis

During training, all feeding sessions, as well as the separate vibrational cue sessions for CTL animals, were recorded onto 90-min (LP) Panasonic Mini DV tapes using Panasonic PV-GS31 Mini DV Digital Camcorders. The camcorders were positioned approximately 0.75 m from each antlion's box. LRN subjects were videotaped for 20 s prior to the presentation of the vibrational signal, continuing through subsequent prey delivery, until feeding terminated, either because the prey carcass was tossed out of the pit by the antlion, or because the prey carcass was discarded by the antlion but remained in the pit untouched for 10 min. CTL subjects were videotaped twice each day, during both signal presentation and feeding: Videotaping began 20 s prior to the vibrational cue presentation and continued for an additional 10 min; videotaping of feeding began 20 s prior to prey delivery, and continued until feeding terminated as described for LRN subjects.

Videotapes were scored individually by four researchers after both replications were completed. Each antlion received a single score each day for movement during the vibrational cue: If the antlion moved either its mandibles or head during the vibrational cue, it received a score of 1; if the antlion did not move at this time, it received a score of 0. In addition, if sand grains on the sides of the pit were disturbed by the vibrational cue, a score of 1 was recorded for that trial; if not, a score of 0 was recorded. Inter-observer agreement amongst the four scorers was 97% on both measures.

1.2. Results

Several subjects moulted during the Training Phase and thus did not complete the experiment; these individuals were excluded from analysis, resulting in 36 subjects (LRN SD-Near, $n=5$; LRN SD-Intermediate, $n=7$; LRN SD-Far, $n=6$; CTL SD-Near, $n=6$; CTL SD-Intermediate, $n=7$; CTL SD-Far, $n=5$). Despite this reduction in the number of subjects, a univariate analysis of variance (ANOVA) confirmed that the remaining subjects in each of the six treatment subgroups not only were of equal weight at the start of the experiment, all $F_s(2, 30) < 1.00$, but also did not differ in pit volume, all $F_s(2, 30) < 1.00$. Following Youthed and Moran (1969), pit volume was calculated using the equation for the volume of a cone: Volume (cm^3) = $1/3\pi \times r^2 \times h$, in which r is the pit radius and h is the pit height (depth).

1.2.1. Training Phase

A mixed measures ANOVA was used to analyze all data from the Training Phase. The ANOVA included two between subject variables, namely, treatment group (LRN and CTL) and signal distance group (SD-Near, SD-Intermediate, and SD-Far), and one within subjects variable (10 blocks of 2 training sessions).

Subjects in the LRN group moved their head and mandibles significantly more frequently in response to the vibrational cue than subjects in the CTL condition, $F(1, 30) = 11.663$, $p = 0.002$. However, that same analysis revealed a significant interaction between treatment group and signal distance group, $F(2, 30) = 8.165$, $p < 0.001$. Tukey post hoc tests revealed that LRN subjects in SD-Near moved significantly more in response to the vibrational cue than CTL subjects in SD-Near, $F(2, 30) = 19.728$, $p < 0.001$, but instances of movement between LRN and CTL animals in SD-Intermediate and SD-Far were not significantly different from one another, $F_s(2, 30) < 1.00$ (Fig. 3). Chi-square tests were used to analyze what appears to be a difference in movement during the first block of days between LRN and CTL subjects in SD-Near. Subjects' movement in response to the vibrational cue did not differ on training Day 1, either between LRN and CTL subjects across all three signal distance groups ($\chi^2 = 1.03$, $df = 1$, $N = 36$, $p = 1.00$) or between LRN and CTL subjects in SD-Near alone ($\chi^2 = 1.32$, $df = 1$, $N = 11$, $p = 0.455$).

Because of the proximity of the vibrational signal in SD-Near, sand grains on the sides of the pit were disturbed much more frequently in SD-Near subjects, an event that occurred only rarely in trials with SD-Intermediate and SD-Far subjects, $F(2, 30) = 177.24$, $p < 0.001$. However, not only did instances of pit disturbance occur equally for subjects in the LRN and CTL groups overall, $F(1, 30) = 0.851$, $p = 0.364$, but also the occurrences of pit disturbance were identical in the respective LRN and CTL signal distance subgroups, $F(2, 30) = 0.145$, $p = 0.865$. Thus, although pits were disturbed equally often for LRN and CTL animals, only LRN subjects reacted to this event by moving to position themselves above any cascading sand grains during the vibrational signal, in preparation for the arrival of prey.

1.2.2. Testing Phase

Univariate ANOVA's with two between subject variables, namely, treatment group (LRN and CTL) and signal distance group (SD-Near, SD-Intermediate, and SD-Far), were used to analyze test data. In response to the vibrational cue, animals in the LRN group moved their head and mandibles marginally significantly more than animals in the CTL group, $F(1, 27) = 3.818$, $p = 0.061$. Instances of movement did not differ between signal distance groups, $F(2, 27) = 1.383$, $p = 0.268$, nor did the ANOVA indicate an interaction between treatment group by signal distance group, $F(2, 27) = 1.383$, $p = 0.268$.

Instances in which a subject's pit was disturbed during the vibrational cue occurred equally in both the LRN and CTL groups,

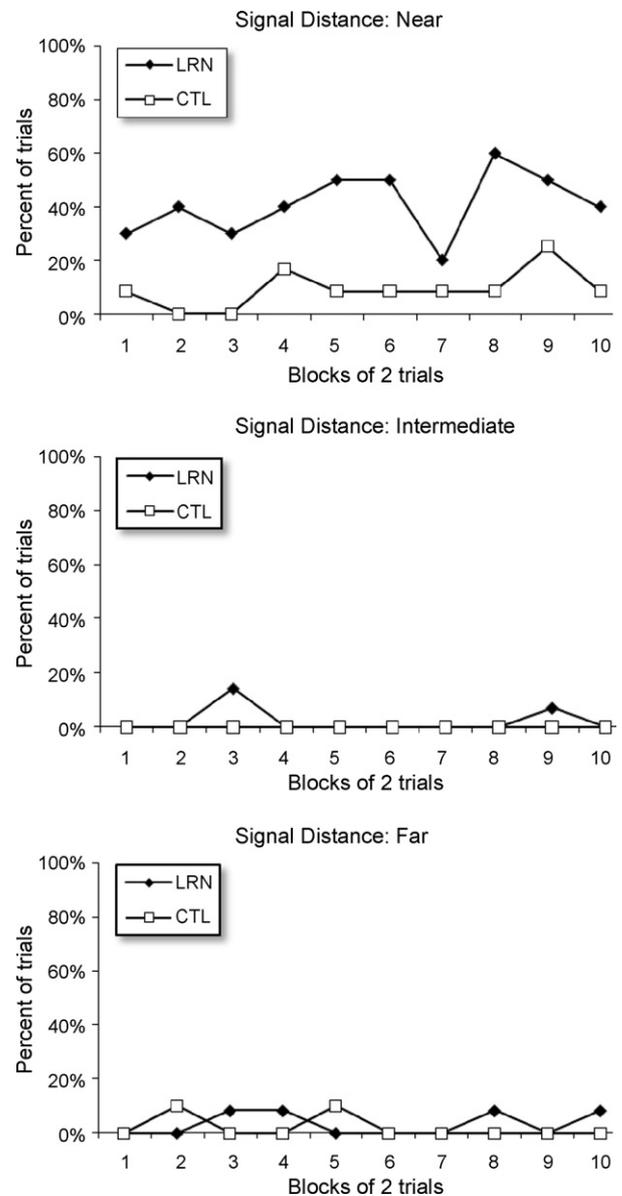


Fig. 3. Percent of trials in which subjects in the LRN and CTL treatment groups moved in response to a vibrational cue in the SD-Near condition (top panel), the SD-Intermediate condition (middle panel), and in the SD-Far condition (bottom panel) of Experiment 1.

$F(1, 28) = 2.135$, $p = 0.155$. Similar to training, the pits of subjects in SD-Near were disturbed more frequently than subjects in either SD-Intermediate or SD-Far, $F(2, 28) = 53.589$, $p < 0.001$; however, again similar to training, within these signal distance groups, instances of pit disturbance did not differ between LRN and CTL subjects, $F(2, 28) = 2.144$, $p = 0.136$.

1.3. Discussion

The results from both the Training and Test Phases support the idea that antlions learned to associate the vibrational signal with food delivery. When the signal was close to the pit (i.e., SD-Near), animals in the LRN group moved more frequently than animals in the CTL group. In fact, a difference in response rate was observed after only 2 training sessions.

The instances of pit disturbance did not differ between animals in the learning and the control groups; therefore, it is not plausible to attribute the different occurrence of head/mandible

movement between the treatment groups to any differences in sand fall. Nonetheless, it is possible that, just as animals in the LRN group learned that pit disturbances predicted food arrival, animals in the CTL group may have learned a different kind of association between pit disturbance and food, namely that pit disturbance predicted the *absence* of food arrival. Whereas the function of LRN animals' behavioural response, namely freeing the head and mandibles from sand that has fallen on top of them, is obviously to ready themselves to capture prey that enters the pit, CTL animals' response would prevent them from unnecessary energy expenditure. Whether this kind of inhibitory learning did, in fact, occur requires further study; at a minimum, however, the response of LRN animals clearly demonstrates that antlions are able to learn a stimulus-stimulus relationship, and form an appropriate anticipatory response.

2. Experiment 2

Given that antlions can learn to anticipate the arrival of food, Experiment 2 attempted to explore the possible fitness benefits of this associative learning. As in Experiment 1, one group received the associative learning treatment (LRN) while the other group received a control treatment (CTL) in which the vibrational cue did not predict food. Unlike Experiment 1, however, the vibrational cue was presented at the same distance from the edge of the pit for all subjects. In addition, because Experiment 2 was designed specifically to analyze possible fitness advantages of learning, we developed a procedure to insure that subjects in the LRN and CTL treatment groups were at exactly the same point in their larval development, namely the third instar. Finally, ants were used as prey in the Training Phase of Experiment 2. Not only are ants a frequent prey item of antlions (hence both antlion's common and scientific species name), but also ants attempt to escape from the pit if not captured efficiently and, thus, they should provide a sound basis for assessing any differences in predation success between treatment groups.

2.1. Method

2.1.1. Subjects and apparatus

Prior to selection as subjects, larval antlions (*Myrmeleon crudelis* Walker), obtained from AntLionFarms.com (Pensacola, FL), were housed individually in plastic cups (7 cm diameter × 8 cm deep), filled with 170 g of silica play sand (Pakmix, Inc., Toledo, OH) and located in a room maintained at 20 °C with a 14:10 h light:dark cycle (lights onset at 09:00 h). As soon as antlions dug pits, they were fed 2 wingless fruit flies (*Drosophila* sp.) obtained from Connecticut Valley Biological Supply (Southampton, MA) once each day at 21:00 h until they stopped feeding and disappeared under the sand in preparation for either moulting or pupating. Those that pupated were excluded from further treatment while those that moulted and successfully re-emerged as third instar larvae were removed from their cups and weighed. Pairs, matched for weight, were created; one member of each pair was randomly assigned to the associative learning treatment group (LRN) while its matched-weight pairmate was assigned to the control treatment group (CTL). Eight such third-instar pairs were created. Each pairmate was housed individually, as described in Experiment 1, within the same sound-and-vibration attenuating chamber, and with the same sand delivery device, located at 4.5 cm from the edge of the pit, and sand collection container (see Fig. 2).

2.1.2. Procedure

The experiment was run in blocks of 3 consecutive training sessions of 48 h each, followed by a 24 h rest period. Thus, each block of 3 sessions represented a single week of the experiment. For each antlion in the LRN group, a single ant, described below, was

delivered to the centre of the pit, once per training session (i.e., once every 48 h, 3 times per week), at a randomly selected time during one of the two light cycles, and immediately following the vibrational signal, specifically 4.5 ml of sand dropped into the sand collection container (approximately a 5-s vibrational cue). Antlions in the CTL group were fed at the same time as the antlions in the LRN group; however, each antlion in the CTL group received the vibrational signal at another randomly selected time during one of the two light cycles per each training session.

During training, subjects were fed Argentine ants (*Linepithema humile*) obtained from AntsAlive.com (Hurricane, UT). Only prey that weighed between 2.0 and 4.5 mg were used; ants were anesthetized briefly for easy of weighing by 5 min of refrigeration but were fully awake when fed to antlions. A single prey item was delivered to each antlion from a small polystyrene weigh boat; a metal spatula was used to push the prey out of the boat into the pit.

Prey weight was recorded before the prey item was fed to an antlion, and again after it had been discarded. All feeding sessions, as well as the separate vibrational cue sessions for CTL animals, were recorded onto 90-min (LP) Panasonic Mini DV tapes using Panasonic PV-GS31 Mini DV Digital Camcorders.

At 12:00 h following each light onset, the depth and diameter of each pit was recorded by two researchers to the nearest 0.1 cm. In addition, at the end of each training session, both the sand delivery device and the sand collection container were removed and prepared for the following training session (i.e., the sand delivery device was refilled and the collection container was emptied), and then replaced at the appropriate location. For each subject, individual training sessions continued until it stopped feeding and disappeared under the sand to construct a cocoon, in preparation for its metamorphosis into an adult.

2.1.3. Data analysis

Videotapes were scored individually by four researchers at the completion of the experiment. Dependent measures obtained from these videotapes included (1) capture time, i.e., the amount of time that elapsed between food presentation and prey capture; and, (2) feed time, i.e., the amount of time taken to consume prey. Inter-observer agreement was analyzed using a two-way mixed model intraclass correlation using an absolute agreement model; this analysis revealed little variability in scores across different researchers, $ICC = 0.93$, $p < 0.05$.

As in Experiment 1, pit volume was calculated using the equation for the volume of a cone: $\text{Volume (cm}^3\text{)} = 1/3\pi \times r^2 \times h$, in which r is the pit radius and h is the pit height (depth). The prey carcass was weighed immediately upon terminus of feeding by the antlion. Following Griffiths (1982), several feeding measures were calculated. The amount of mass extracted from the prey was calculated by subtracting the final weight (W_f) of the carcass from the initial prey weight (W_i). *Extraction time*, the time it took the antlion to consume the prey, was measured in seconds from the time the antlion captured the prey until the prey carcass was thrown from the pit or discarded by the antlion inside the pit for 10 min. *Extraction rate*, or mass consumed per unit time, was calculated by dividing the amount of prey mass ingested by the amount of time it took to ingest: $(W_i - W_f)/\text{Extraction Time}$. *Extraction efficiency*, the percent of prey mass ingested, was calculated by dividing the mass extracted by the initial weight: $(W_i - W_f)/W_i$. *Extraction efficiency rate*, or efficiency of extraction per unit of time, was calculated by dividing extraction efficiency by the extraction time: $((W_i - W_f)/W_i)/\text{Extraction Time}$.

2.2. Results

Data for all dependent measures were analyzed from only the first 42 training sessions (14 blocks of 3 sessions each), during which

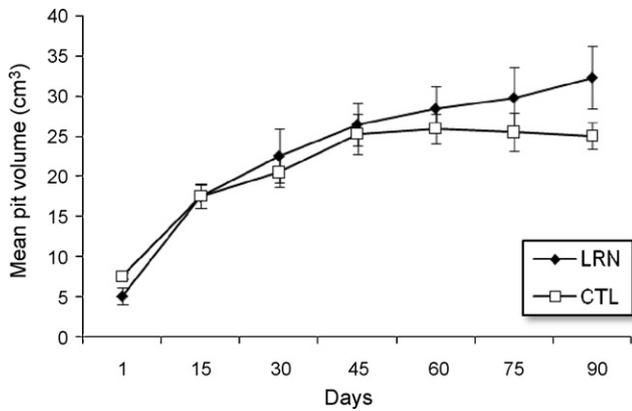


Fig. 4. Mean \pm standard error pit volume (cm^3) of subjects in the LRN and CTL treatment groups on Days 1, 15, 30, 45, 60, 75, and 90 of Experiment 2.

all subjects were feeding. During the subsequent training block, the first subject, a LRN subject, cocooned.

Independent sample *t*-tests confirmed that at the start of the experiment, not only were subjects in the two treatment groups of equal weight, $t(14) = 0.040$, $p = 0.969$, but also subjects did not differ in pit volume, in pit depth, or in pit diameter, all t s < 1.00 .

2.2.1. Pit measures

Analyses of pit measurements (specifically, depth, diameter, and volume) included scores from the first day of training and then every 15 days until the first subject cocooned (14 weeks later). Mixed measures ANOVAs, including one between subject variable, namely, treatment group (LRN and CTL), and one within subject variable, namely pit measurements taken every 15 days, was performed. Three subjects initially constructed pits in the corner of their experimental box, such that their pits were touching two walls, and accurate measurements could not be obtained. Therefore, pit measures were analyzed for 6 animals in the LRN treatment group and 7 animals in the CTL treatment group.

As Fig. 4 illustrates, the volume of pits constructed by animals in both treatment groups increased significantly over days, $F(1, 6) = 60.811$, $p < 0.001$. However, as that figure also reveals, antlions in the LRN treatment already were constructing significantly larger pits than animals in the CTL treatment by the time the first subject cocooned, groups by days $F(1, 6) = 2.29$, $p = 0.046$. Moreover, whereas Fig. 4 suggests that CTL antlions reached an asymptote in pit size, LRN animals show no such evidence of nearing asymptotic pit volume. Matching this pattern of results, the analysis of pit depth also revealed an increase in both groups over days $F(1, 6) = 56.195$, $p < 0.001$, as well as a significant groups by days interaction, $F(1, 6) = 3.383$, $p = 0.006$, with animals in the LRN treatment constructing pits of greater depth than animals in the CTL treatment. Unlike these analyses of pit volume and depth, however, the diameter of pits constructed by animals in the LRN and CTL treatments did not differ, group, $F(1, 11) = 0.386$, $p = 0.547$; groups by days, $F(1, 6) = 0.348$, $p = 0.365$. However, pit diameter increased significantly for all animals over the 14 weeks of training, days, $F(1, 6) = 69.232$, $p < 0.001$.

2.2.2. Feeding measures

Because of the relatively small number of subjects and the high variability within groups, non-parametric Mann–Whitney *U*-tests were performed to assess whether the LRN and CTL treatment groups differed in their feeding ability across the 42 training sessions. Extraction efficiency, which is the simple percent of prey mass extracted, did not differ between the LRN and CTL treatment group, $U(N_1 = 14, N_2 = 14) = 62$, $p > 0.05$. However, extraction

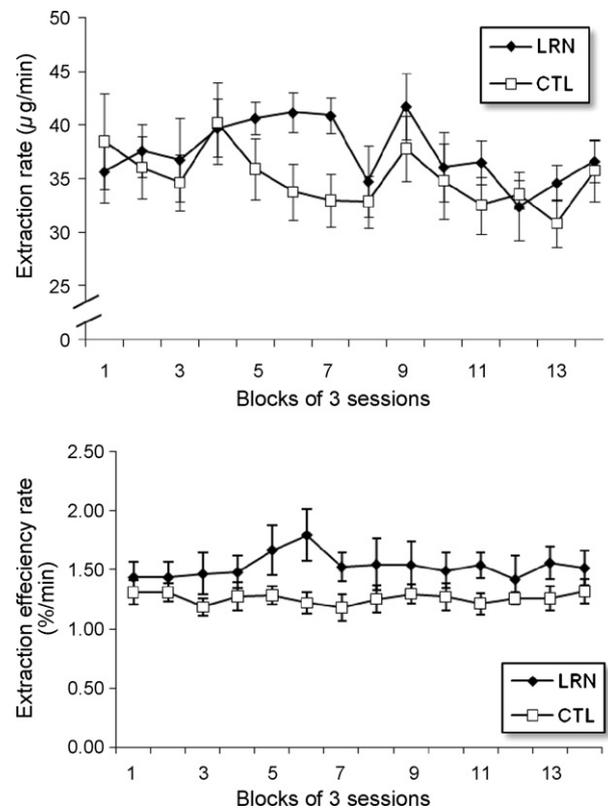


Fig. 5. Mean \pm standard error extraction rate (top panel) and mean \pm standard error extraction efficiency rate (bottom panel) of prey consumed by subjects in the LRN and CTL treatment groups in Experiment 2.

rate, namely the rate at which animals extracted prey contents, was significantly faster in LRN antlions than it was in CTL animals, $U(N_1 = 14, N_2 = 14) = 55$, $p < 0.05$ (Fig. 5, top panel). Also, LRN antlions exhibited a greater extraction efficiency rate, that is they extracted a greater percent of prey mass per unit time, compared to CTL animals, $U(N_1 = 14, N_2 = 14) = 0$, $p < 0.01$ (Fig. 5, bottom panel).

2.3. Discussion

Because antlions do not build pits that exceed the size of prey that they can capture, pit size is highly correlated with antlion size, such that larger antlions construct pits with larger volumes (Day and Zalucki, 2000; Griffiths, 1986; Mansell, 1996). Data from our own lab confirm these findings: When data from both Experiments 1 and 2 were combined to analyze the intercorrelations between antlions' initial weight and initial pit measurements, that analysis revealed that initial weight was highly correlated with all initial pit measurements (see Table 1). Thus, in our lab, too, pit size is an accurate indicator of antlion size, which in turn provides indirect evidence that associative learning has a positive influence on growth rate. That is, in the current study, all subjects initially constructed pits that were the same size. However, by the end of training, animals that had the benefit of learned cues predicting prey arrival constructed larger pits than animals that did not have that benefit of learning.

Consistent with these data, it is hardly surprising that antlions receiving the LRN treatment not only were able to extract prey contents faster, but also were able to extract a greater percent of prey mass per unit time, than subjects receiving the CTL treatment. That is, learned signals of prey arrival improved antlions' digestive efficiency which, in turn, enabled individuals to grow faster than antlions that did not enjoy the benefit of learned signals.

Table 1
Intercorrelations between antlions' initial weight and initial pit measurements ($n = 52$ antlions).

Variable	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) Initial weight	–	0.576*	0.657*	0.672*	0.706*	0.657*	0.740*
(2) Pit volume–T1		–	0.877*	0.909*	0.867*	0.857*	0.828*
(3) Pit volume–B1			–	0.822*	0.941*	0.887*	0.933*
(4) Pit depth–T1				–	0.917*	0.789*	0.809*
(5) Pit depth–B1					–	0.875*	0.921*
(6) Pit diameter–T1						–	0.960*
(7) Pit diameter–B1							–

Note: T1 = Trial 1; B1 = Block 1, consisting of 3 training trials

* $p < 0.01$

3. Experiment 3

A third experiment, conducted with second instar antlion larvae, explored whether the fitness benefits derived from learning and observed in Experiment 2, namely larger pit size and more efficient prey extraction, not only might be obtained by second instar larvae, but also might translate into a faster time to moult. As in Experiments 1 and 2, one group received the associative learning treatment (LRN) while the other group received a control treatment (CTL) in which the vibrational cue did not predict food. More importantly, however, Experiment 3 included a test day in which all animals in both groups were treated identically, for a direct, better controlled comparison of the treatment groups. Finally, animals in both groups were monitored for an additional 7 days for evidence of moulting.

3.1. Method

3.1.1. Subjects and apparatus

Subjects were obtained, housed and selected as subjects for LRN and CTL groups as described in Experiment 1, except that the room was maintained on a slightly shorter, 13.5:10.5 h light:dark cycle (light onset at 08:30 h). Following its random assignment to either the LRN or CTL treatment group, each of 24 antlions was moved, in its same plastic bowl, to a 2.5-cm foam pad within a sound-and-vibration-insulating chamber, similar to that described above. For this experiment, the sand delivery device was modified to sit on the foam pad, outside the antlion's bowl. Individual chambers were located adjacent to one other on 4 shelves, with 6 chambers per shelf as described in Experiment 1.

3.1.2. Procedure

The experiment consisted of three phases, namely a 16-day Training Phase, a 1-day Test on Day 17, and a 7-day monitoring period, lasting until Day 24. During training, the LRN and CTL treatments were conducted as described in Experiment 1. However, because ants were not available as prey items, wingless fruit flies (*Drosophila* sp.), like those used in Experiment 1, were used. On Day 17, each antlion in both LRN and CTL groups received the same procedure, namely the vibrational signal followed immediately by delivery of a single prey item. The day after the test, each antlion's bowl was covered with perforated plastic wrap and it was left to moult without any further feeding. Antlions were monitored daily for evidence of moulting, namely the discarded exoskeleton that the antlion flings out of its pit.

3.1.3. Data analysis

All feeding sessions, and vibrational signal sessions for CTL, were recorded as described for Experiment 1, except that subjects were videotaped for only 10 min post-food delivery. Thereafter, experimenters monitored pits for discarded carcasses.

The formulae for measuring pit volume, extraction efficiency, extraction rate, and extraction efficiency rate were identical to

those used in Experiments 1 and 2. Data were analyzed in blocks of three training sessions using non-parametric Mann–Whitney *U*-tests.

3.2. Results and discussion

During the experiment a total of ten antlions had to be eliminated because they did not build a pit or eat. These included 5 LRN and 5 CTL subjects. Fourteen subjects remained; these included 7 LRN subjects and 7 CTL subjects.

3.2.1. Training

Although Mann–Whitney *U*-test comparisons revealed that the pit volume of antlions in the LRN and CTL groups did not differ on Block 1 of training, Mann–Whitney $U(N1 = 7, N2 = 7) = 21$, $p > 0.05$, by the end of the Training Phase on Day 16, LRN subjects were constructing significantly larger pits, Mann–Whitney $U(N1 = 7, N2 = 7) = 1$, $p < 0.05$ (see Fig. 6).

Concerning antlions' feeding behaviour, although compared to CTL animals, antlions in the LRN group appeared to develop slightly higher extraction measures (extraction efficiency and extraction efficiency rate) over days of training, Mann–Whitney *U*-tests revealed that only extraction rate was significantly higher for LRN antlions by the end of training, Mann–Whitney $U(N1 = 7, N2 = 7) = 4$, $p < 0.05$.

3.2.2. Test

When measured under identical conditions on the day of the test, Day 17, antlions receiving the LRN treatment exhibited greater extraction rate, Mann–Whitney $U(N1 = 7, N2 = 7) = 4$, $p < 0.05$, and extraction efficiency, Mann–Whitney $U(N1 = 7, N2 = 7) = 7.5$, $p < 0.05$, than animals receiving the CTL treatment. In addition, LRN antlions had significantly bigger pits, not only in terms of pit volume, Mann–Whitney $U(N1 = 7, N2 = 7) = 1.5$, $p < 0.05$, but also pit diameter, Mann–Whitney $U(N1 = 7, N2 = 7) = 0$, $p < 0.05$.

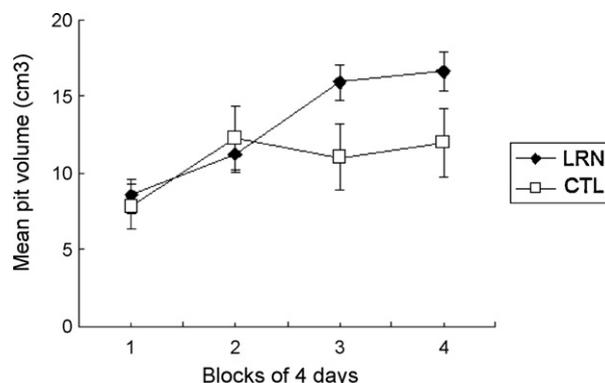


Fig. 6. Mean \pm standard error pit volume (cm^3) for subjects in the LRN and CTL treatment groups during the Training Phase of Experiment 3.

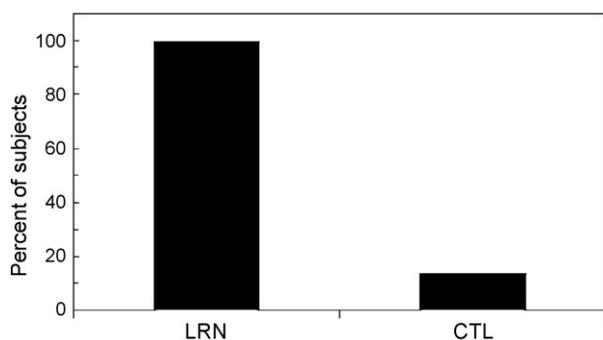


Fig. 7. Percent of subjects in the LRN and CTL treatment groups moulting by Day 24 of Experiment 3.

Finally, a Chi-square analysis revealed that antlions receiving the LRN treatment moulted significantly sooner than antlions in the CTL treatment, $\chi^2 (1, N = 14) = 10.5, p < 0.01$; that is, by Day 24, all seven of the antlions receiving the LRN treatment had moulted, whereas only one of the 7 antlions in the CTL treatment had moulted (see Fig. 7).

4. General discussion

Taken together, the three experiments reported here demonstrate that antlions, extremely sedentary animals that do not fit the typical profile of insects that possess learning capabilities, nonetheless are capable of learning to associate environmental cues with the arrival of prey. More importantly, this ability to learn provides antlions with several potential fitness advantages. Specifically, when an environmental signal predicts the appearance of a prey item, antlions exhibit a learned response, here a movement that frees the head of sand, in anticipation of prey arrival (Experiment 1). This particular learned response – and likely others, either ones that we were unable to measure, such as digestive changes, or those that might appear under slightly different circumstances – enables antlions to extract the contents of their prey more efficiently (Experiments 2 and 3). In turn, this increased feeding efficiency results in faster larval growth, as reflected in increased pit volume (Experiments 2 and 3), and faster moulting (Experiment 3), which reduces the amount of time that antlions remain in the larval stage, a very vulnerable period in which antlions themselves are subject to predation (Crowley and Linton, 1999).

Antlions that had the benefit of learned cues differed slightly across Experiments 2 and 3 in exactly how their feeding efficiency improved—for example, in terms of extraction rate, extraction efficiency, and extraction efficiency rate. These differences might be the result of the different prey items used (ants vs. fruit flies) or the difference in larval stage of the subjects (3rd vs. 2nd instar). In any case, LRN antlions in both experiments dug larger volume pits than CTL animals, a highly reliable index of their larger size (Day and Zalucki, 2000; Griffiths, 1986; Mansell, 1996; see also Table 1). But how might we make sense of the fact that the differences in extraction measures for LRN antlions across Experiments 2 and 3 nonetheless produced the same outcome in terms of increased larval growth? Obviously, higher extraction efficiency (Experiment 3), which is the percent of prey mass extracted, is directly related to caloric intake and, thus, growth. However, increases in rate, too – either extraction rate or extraction efficiency rate – could aid growth, albeit indirectly: Higher extraction rates (i.e., consuming prey faster) may indicate more efficient extraoral digestion in antlions, perhaps through increased secretion of digestive enzymes as has been suggested by Griffiths (1982; see also Lucas, 1985). That is, a physiological conditional response to the vibrational cue may have been anticipatory enzyme production. This response would be

similar to the prototypical classically conditioned salivary response (Pavlov, 1927), recently demonstrated via salivary neurons of cockroaches (Watanabe and Mizunami, 2005), and which in humans and other mammals results in increased caloric extraction (Hollis, 1982).

Yet another advantage of learning to anticipate prey arrival concerns the effects of larval weight and growth rate on mortality and reproduction. Faster larval growth may increase fitness indirectly by reducing generation time and, thus, exposure to larval mortality (Crowley and Linton, 1999). Although antlions can attempt to escape predators by moving down into the sand, they cannot quickly do so and, thus, they remain very vulnerable throughout the larval stage. Furthermore, larvae that pupate at smaller weights become adults with greatly reduced fitness, not only because their reproductive organs sometimes are developmentally incomplete, but also because the mean size and fat content of their eggs are smaller (Griffiths, 1985). Finally, because antlions are forced to endure prolonged periods of starvation, for example, during cold winter months in northern climates or during the rainy season in southern climates, greater larval weight helps to insure that they survive (Griffiths, 1985). Thus, compared to CTL animals, the faster growth rate of LRN antlions – reflected in their significantly larger pits – provides additional benefits.

Although we have emphasized the benefits of increased larval weight, using pit volume per se as a mere index, larger pits in themselves provide several advantages. In short, larger pits capture larger prey, which is more energetically profitable. In fact, capture of a single large prey can result in substantial larval growth, which can be sufficient to bring a 3rd instar larva to a size at which it can pupate (Griffiths, 1986). Larger pits also have higher encounter rates with prey. In a study conducted by Griffiths (1980), an increase of 2 mm in pit diameter translated into a 10% increase in capture success. Extrapolating from this result to our own data, wherein the mean difference in pit diameter between LRN and CTL animals on the last day of training in Experiment 2 was 5.2 mm, LRN antlions would be expected to obtain a 25% increase in capture success.

Finally, in nature, using learned signals to extract prey contents more quickly would provide antlions with yet another advantage, namely the ability to be ready for additional prey items that fall into the pit. Although antlions sometimes are able to catch a second prey item while extracting the contents of a dead prey item (Griffiths, 1980), to do so they first must toss the first prey item aside, which sometimes results in a failure to relocate it. A bigger risk, however, is that the second prey item escapes from the pit, which is more likely in the pit's current condition, having already been disturbed by the first capture. Because capturing prey involves a relatively large expenditure of energy for so sedentary a predator as an antlion, the escape of a second item would represent considerable energy loss (Griffiths, 1980).

In sum, we have shown that learning to utilize cues associated with food availability could have enormous fitness advantages for antlions, a sessile predator. In some ways, our results are similar to those of Dukas and Bernays (2000), who examined the fitness-related benefits of learning in foraging grasshoppers (*Schistocerca americana*) mentioned earlier in this paper. In their study, grasshoppers learned to associate cues, namely coloured background cards and artificial flavourings, with balanced and unbalanced diets, and subsequently used those cues to locate the balanced diet more reliably than animals in the control treatment. This ability to use learned cues resulted in a 20% higher growth rate and a 7% shorter instar duration than control animals. Although similar to our own findings, antlions are sedentary insects, a characteristic that is unlike grasshoppers and, for that matter, all other insects studied thus far for their learning abilities. Already armed with an arsenal of supposedly innate prey-detecting and prey-capturing responses, antlions would not appear to be a typical candidate

for learning. However, with antlions added to the list of insects capable of learning, many other unlikely candidates soon may be discovered.

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