

Consequences of the instar stage for behavior in a pit-building antlion



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ARTICLE INFO

Article history:

Received 8 April 2013

Received in revised form 7 November 2013

Accepted 21 November 2013

Available online 4 December 2013

Keywords:

Behavioral profiles

Behavioral syndromes

Consistency

Morphology

Personality

Trap-building predators

ABSTRACT

Pit-building antlion larvae are opportunistic predators that dig conical pits in loose soils, and prey on small arthropods that fall into their traps. We investigated different behavioral traits of second and third instar larvae selected for similar body masses, while also exploring the behavioral consistency and personalities of the third instar stage. Second instar larvae constructed smaller pits than third instar larvae. The former also responded more slowly to prey and exploited prey less efficiently. Notably, all these instar-based differences disappeared after molting into the third instar stage. In addition, third instar larvae exhibited consistent behavior in their pit size, response times to prey and to less extent in relocation distances. We detected two axes of behavior. The first axis included a correlation between pit size, response time and prey exploitation efficiency, thus reflecting investment in foraging activity. The second axis seemed to represent a trade-off between response time and relocation distance, implying that individuals that responded more slowly to prey, relocated over larger distances. These results point to coordinated behavior reflecting different levels of investment in foraging, while also emphasizing the importance of instar stage, in addition to body mass, when studying the behavior of such organisms characterized by a complex life cycle.

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1. Introduction

Individuals within a population often differ consistently in their behavior, and such differences can persist over time and across varying ecological contexts (e.g., Sih et al., 2004; Dingemans et al., 2010). Behavioral variation can be maintained by different processes including frequency-dependent selection and changing environmental conditions (Réale et al., 2007; Pruitt and Riechert, 2012). Notably, most studies of animal personalities and syndromes, rather than emphasizing initial/known individual differences in body size, development stage or age, demonstrate how similar individuals that experience the same environmental conditions behave consistently and differently (e.g., Kortet and Hedrick, 2007; Modlmeier and Foitzik, 2011). However, especially in field-collected animals, it is often difficult to determine whether apparently similar individuals are indeed similar. For instance, differences related to developmental history usually remain uncovered, although they may dramatically affect various behaviors, such as activity level and boldness (Tremmel and Müller, 2013).

In arthropods, individuals of two successive instar stages may possess very similar body masses and dimensions. When the

behavior of such individuals is studied while erroneously ignoring the instar stage, consistent individual differences based on personality might be detected. However, such consistency and behavioral differences are stage-dependent, and they may disappear when all individuals progress into the same instar stage. Although behavioral differences based on the instar stage and independent of body size may exist, they are rarely reported. Three examples include sharp between-instar differences in the predation avoidance behavior of grasshoppers in the presence of spider predators (Danner and Joern, 2003), foraging behavior differences between instar stages of a plant bug reflected in its feeding time and number of plants it attacks (Zink and Rosenheim, 2005), and differences in the latency to attack prey and resume movement after disturbance in spiders (Sweeney et al., 2013). In such cases, ignoring the instar stage and studying insect personality and consistency might lead to erroneous results.

In addition, morphology and body shape have consequences for behavior, even when the general body size is similar. For instance, sit-and-wait and widely foraging predators differ in their body shape (stocky vs. streamlined, respectively; Huey and Pianka, 1981; Meiri, 2010). Yet, the vast majority of personality studies ignore body shape, even when accounting for general body size or mass (e.g., Kortet and Hedrick, 2007; Modlmeier and Foitzik, 2011; Tremmel and Müller, 2013). Incorporating morphology could lead to a better understanding of behavioral differences among individuals. For instance, morphology can indicate on body condition

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(e.g., [Stevenson and Woods, 2006](#)), which is likely to have a strong influence on behavior (e.g., [Tucker et al., 2007](#)).

In this research, we investigated, for the first time, personalities and behavioral consistency of pit-building antlion larvae. These opportunistic predators hunt their prey by constructing conical pits in loose soils, while waiting for small arthropods to overstep their pit margins. Because they are not yet in a reproductive stage, they are an excellent model system to study foraging-related behavior. Our goals are: (1) To investigate behavioral differences between second and third instar larvae having similar body masses in spite of their different developmental stage. (2) Examine behavioral consistency of third instar larvae; consistency is the first step in determining animal personality ([Bell et al., 2009](#)). (3) Determine the personality (defined here as the nature of the correlation among behavioral traits) of third instar larvae. (4) Investigate possible effects of morphology on behavior.

In insects there is a tight positive correlation between body size and reproductive success ([Honek, 1993](#)). State-dependent models, considering both survival and reproduction, predict that when foraging aims at future reproduction and not mere survival, there is a benefit to increase effort and take further risks ([Bednekoff, 1996](#)). This especially holds true for sit-and-wait predators in advanced larval/juvenile stages, facing a minor starvation risk combined with uncertainty in respect to accumulating sufficient resources for reproduction. Antlion species develop through three instar stages. The third instar is the longest, comprising more than half of the larval period, and providing the main opportunity for the antlion to accumulate resources for reproduction. Thus, third instar larvae should exhibit high foraging activity and more risk-prone behavior compared to second instar larvae, which are perhaps more occupied with survival. Regarding antlion personality, we predicted that there should be a positive correlation between behaviors related to foraging, including pit size and response time to prey, and a negative correlation between these two traits and relocation tendency. We also posited that head width (hereafter, HW) and mandible length (hereafter, ML) should be positively correlated with pit size, as pits are constructed by throwing sand using the mandibles.

2. Methods

2.1. Study species

We collected *Myrmeleon hyalinus* (Neuroptera: Myrmeleontidae) larvae under different tamarisk trees and brought them to the laboratory. Larvae were identified according to the key presented in [Simon \(1988\)](#). The collection site, Nahal Secher (31°06'N, 34°49'E), is a sandy area 15 km south of Beer-Sheva, Israel. *M. hyalinus* is the most abundant pit-building antlion in Israel. The larvae inhabit a wide range of sandy soils, but they prefer constructing their pits in shaded areas located under trees or bushes ([Simon, 1988](#)). *M. hyalinus* attains body masses of up to 0.06 g before pupating ([Scharf et al., 2008a](#)). Individuals spend most of their lives as larvae (lasting ~1 year), enter pupation (lasting ~1 month), and then emerge as short-lived and weak-flying adults (lasting ~1 week) ([Scharf et al., 2009a](#)).

2.2. Experimental design

The study included two experiments, of which the first included two phases: (1a) investigating the behavioral consequences of the instar stage by comparing the behavior of second and third instar larvae. (1b) Testing whether the differences between the instars diminish when the larvae are all at the third instar, and exploring the behavioral consistency and syndromes in third instar larvae.

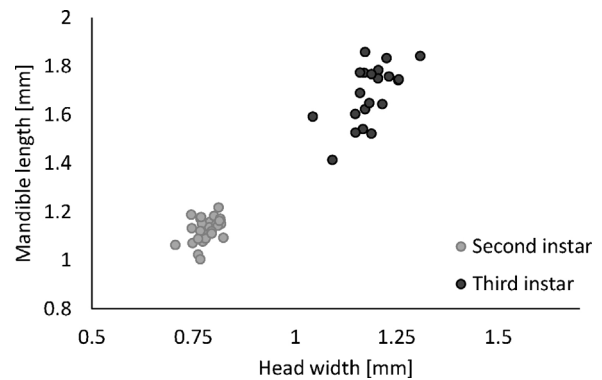


Fig. 1. Mandible length vs. head width of second and third instar larvae. The separation between the two instar stages is clear, based on this figure. Note that body mass of all antlion larvae was very similar.

(2) Studying within-instar stage behavioral differences of second instar larvae differing in body mass.

Prior to all experiments, antlions were fed weekly with one flour beetle larva (mean larva mass of 2.5 mg) for three successive weeks, weighed using an analytical scale (CP224S, Sartorius AG, Göttingen, Germany; accuracy of 0.1 mg), and then starved for seven days in small plastic cups (diameter of 4.5 cm, filled with about 3 cm of sand). This procedure allowed standardizing the physiological state of the larvae prior to the experiment ([Scharf et al., 2009b](#)). All experimental tests were conducted under identical day/night photoperiod (12:12 h), temperature of 21.9 °C and relative humidity of 49.3% (averages of three daily measurements in the test room). Larvae were photographed using digital camera (Micropublisher 5.0, QImaging, Surrey, BC, Canada) connected to a Nikon stereoscope (SMZ 800, Nikon, Kawasaki, Japan). Using the program ImageJ (a public domain Java image processing program, National Institute of Mental Health, Bethesda, Maryland, USA) we measured ML, HW and abdomen width (hereafter, AW).

2.2.1. Experiment 1

We selected 50 individuals with similar initial body mass (mean mass \pm 1 SD: 6.0 ± 0.5 mg, $N = 50$; range: 5.1–6.9 mg). Body mass of 5.0–7.0 mg is the range in which *M. hyalinus* second instar larvae molt and progress to the third and final instar stage ([Scharf I, unpublished data](#)). The morphological trait discriminating best between the larval stages is HW ([Simon, 1988](#); [Scharf et al., 2008a](#)). By plotting the HW against ML, we could distinguish between second ($N = 29$) and third instar ($N = 21$) larvae ([Fig. 1](#)). The study included two phases: At the beginning, individuals were similar in mass (second instar: mean mass \pm 1 SD: 5.8 ± 0.5 mg, $N = 29$; third instar: mean mass \pm 1 SD: 6.2 ± 0.5 mg, $N = 21$). The overlap in body mass between the instars is limited, and although we selected small third instar larvae and large second instar larvae, there was a significant difference in average body mass between the two groups (t -test: $t_{41} = -2.79$, $P = 0.0075$); however, the effect size was very small (~6.5%). In comparison, differences within the whole mass range of second instar larvae could reach ~300% (this study). At the end of the first phase, the larvae were fed once a week until all second instar larvae progressed to the third stage and reached similar body masses (individuals collected as second instar: mean mass \pm 1 SD: 8.5 ± 1.2 mg, $N = 24$; individuals collected as third instar: mean mass \pm 1 SD: 9.2 ± 1.3 mg, $N = 16$). Six individuals died and four were either heavier or lighter more than 50% than the group's average; therefore, they were excluded from the second phase of the experiment in order to maintain similar average and low variance of body mass between the two groups.

2.2.2. Experiment 1, phase 1

In the first phase of the experiment, we placed the larvae in round plastic cups (diameter = 8.5 cm, depth = 6 cm) filled with sand and recorded the following behavioral traits: (a) pit diameter (b) response time to prey (i.e., a single flour beetle larva), (c) relocation activity, and (d) prey exploitation efficiency. Pit diameter was recorded using a digital caliper (± 0.1 mm). Response time to prey was measured as in Scharf et al. (2010), by placing a flour beetle larva at the edge of the antlion pit and measuring the time it took for the antlion to respond by throwing sand. If the antlion did not respond within 60 s, we assumed it was uninterested in the prey, and we entered this maximal value as its response time. Scharf et al. (2010) have shown that antlions usually either respond fast to prey (i.e., less than 20 s) or do not respond at all. Thus, we chose a value that is three times higher than the common response time. In order to measure the antlions' relocation activity, we placed each individual into separate 25 cm \times 17 cm aluminum tray, filled with 3 cm of sand in a dark room. Scharf et al. (2008b) have shown that antlion larvae prefer to hunt during the day and to relocate their pits at night. After 48 h, before disturbance, we recorded the relocation activity reflected in relocation distance (measured with a caliper). Subsequently, we destroyed traces and documented their relocation activity after 24 h, i.e., after disturbance. This manipulation mimics the disturbances often occurring in nature by wind/rainfall, relocating conspecifics, or larger animals moving nearby (Barkae et al., 2010).

Prey exploitation efficiency was measured by dividing the difference in antlion body mass before and after feeding, by the prey body mass [(antlions mass after feeding – antlion mass before feeding)/prey mass] (Scharf et al., 2009c). Antlions were weighed few hours after feeding, and prey exploitation efficiency values ranged between 0 (no consumption) and 0.9 (near-complete consumption of the flour beetle). Prey exploitation could be perceived in two ways. It can be either seen as similar to giving-up mass or density (hereafter, GUD), i.e., the resources remaining in a patch after foraging ceased (Brown, 1988). Thus, we may consider each flour beetle as a patch, and the decision to stop consuming one prey item and continue to the next one as parallel to moving into a new patch (Lucas, 1985; Samu, 1993). Alternatively, it may point to the efficiency at which the antlion converts the prey mass into its own body mass, having less to do with the GUD. Yet, both can be perceived as metrics of foraging efficiency. Pit diameter and response time were documented twice with an interval of six/seven days. Relocation activity was documented four times, before and after disturbance with intervals of one to six days. Prey exploitation efficiency was measured only once.

In summary, the experimental procedure of the first phase was as follows: We first weighed all larvae, and then separately placed them in round plastic cups. On the next day we measured pit diameters and provided a flour beetle larva to each antlion (after weighting the prey). We measured the response time to prey and weighed the antlion larvae again on the next day, to calculate the prey exploitation efficiency. We repeated this procedure twice, except for measuring prey exploitation efficiency. Finally, we documented relocation activity in larger trays (25 cm \times 17 cm aluminum tray). Larvae were maintained between experiments in round cups under the same room conditions. In order to make sure that second instar larvae would not progress into the third stage, during the first phase we consistently checked around each individual's pit for molts. The first phase of the experiment lasted four weeks.

2.2.3. Experiment 1, phase 2

During the second phase of the experiment (lasting for two and a half weeks, starting 10 weeks after the first phase), we aimed at testing whether the differences between the instar stages are consistent over time, even after the second instar larvae molted into

the third instar stage, hence comparing early third instar larvae (the ones recently molted) and late third instar larvae (the ones that were already collected as third instar). We repeated all behavioral tests in a similar manner, with the exception of measuring prey exploitation efficiency twice with an interval of five days. In addition, we photographed all larvae once more and measured HW, ML and AW.

2.2.4. Experiment 2

This experiment took place under identical day/night photoperiod (12:12 h), temperature of 25.2 °C and relative humidity of 74.4% (averages of three daily measurements in the test room). It also included two phases: First, sixty second instar larvae were divided into two groups based on body mass differences (early second instar: mean mass \pm 1 SD: 3.1 \pm 0.65 mg, $N=30$; late second instar: mean mass \pm 1 SD: 5.9 \pm 1 mg, $N=30$). The second phase, included the early second instar larvae that turned into late second instar larvae after they were fed (mean mass \pm 1 SD: 5.2 \pm 0.86 mg, $N=26$). One of those individuals died and three others progressed to the third instar stage. Therefore, they were excluded from the second phase of the experiment. We recorded the same behavioral traits similar to the first experiment: pit diameter, response time to prey, prey exploitation efficiency and relocation activity. Each behavioral trait was observed once in each phase of the experiment.

2.3. Statistical analysis

2.3.1. Experiment 1: behavioral consequences of the instar stage

We first averaged the repetitions of different behaviors to obtain a single value representing each behavioral trait during the experimental phases. Pit diameter and prey exploitation efficiency were normally distributed. Response time to prey and relocation activity were square-root transformed to correct for their deviation from normality (many low values and skewed to the right). We also repeated the analysis performed on the raw data (no transformations) and got qualitatively similar results. In order to test for behavioral differences between instar stages, we used four separate repeated-measures ANOVAs, with pit diameter, response time to prey, relocation distances and prey exploitation efficiency as the response variables. The phase of the experiment was used as the within-subject factor. These analyses allowed us to test whether instar-related differences diminished when larvae progressed to the third instar stage. Thus, a significant phase \times instar interaction indicated a moderation of the instar-based differences. Finally, we performed a discriminant analysis using the Z-score transformed behavioral traits as response variables and instar stage as the explanatory variable. The results provided us with the axis best separating between the second and third instar stages within the multi-dimensional state space defined by the behavioral traits documented.

2.3.2. Experiment 1: behavioral consistency and personality

In this analysis, we used only the data of the second experimental phase when all larvae were third instar. We first applied Pearson correlation tests in order to detect whether *M. hyalinus* activity is consistent over time, between two successive measurements of behavior (for pit size and exploitation efficiency). Response times and relocation distances were not normally distributed. Hence, we used the Spearman rank correlation. In order to detect inter-correlations in behavioral traits, we applied principal component analysis (PCA) to the four behavioral traits obtained during the second experimental phase (all larvae in their third instar stage). As mentioned above, to avoid pseudo-replications we used trait averages. As accepted, prior to the PCA we applied a Z-score transformation. We referred only to PCs exhibiting eigenvalues larger than one and in each PC only to loadings larger than 0.3 (Tabachnick

and Fidell, 2007). We then correlated the first and second PCs with head width, mandible width and abdomen width. Doing so allowed us to test for possible links between the morphology and behavior of third instar larvae.

2.3.3. Experiment 2: within-instar behavioral differences

In the first phase of the experiment, we used five separate one-way ANOVAs in order to test for behavioral differences within-second instar stage (i.e., early vs. late second instar larvae). In the second phase, we conducted the statistical analysis on the same individuals, comparing early second instar stage (from the first phase) with early that turned into late second instar larvae (from the second phase). Therefore, we used five separate repeated-measures ANOVA for each behavioral trait, with time as the within subject factor. Response time to prey, prey exploitation efficiency and relocation activity were not normally distributed, and we applied the square-root transformation.

All statistical analyses were performed using SYSTAT v. 12 (SYSTAT Software); principal component analysis was performed in MATLAB v.7.8 (Mathworks).

3. Results

3.1. Experiment 1: behavioral consequences of the instar stage

During the first phase of the experiment, third instar antlion larvae constructed larger pits compared to second instar larvae, but this difference diminished during the second experimental phase, when all larvae already turned into their third instar stage (a significant phase \times instar interaction: $F_{1,35} = 5.26$, $P = 0.028$; Fig. 2a and b). Third instar larvae responded faster to prey during the first experimental phase, but this difference disappeared after the second instar larvae turned into third instars (phase \times instar interaction: $F_{1,38} = 11.60$, $P = 0.0016$; Fig. 2c and d). Relocation distances did not differ between second and third instar larvae ($F_{1,38} = 1.46$, $P = 0.23$), but were longer during the first experimental phase ($F_{1,38} = 17.06$, $P = 0.0002$). The two-way interaction term was not significant ($F_{1,38} = 2.06$, $P = 0.16$). Finally, during the first experimental phase third instar larvae demonstrated higher prey exploitation efficiency than second instars, but this difference diminished during the second experimental phase (phase \times instar interaction: $F_{1,38} = 8.09$, $P = 0.0071$; Fig. 2e and f). According to the discriminant analysis, pit diameter was the best behavioral trait separating between the instars (F -to-remove = 12.27), followed by consumption efficiency (F -to-remove = 3.52) and relocation distance (F -to-remove = 2.53). The worst separating trait was response time (F -to-remove = 0.85). The first canonical axis, which provides the maximal separation among instars, is given by: $CV1 = 0.847 \times \text{Pit diameter} - 0.258 \times \text{Response time} - 0.383 \times \text{Relocation distance} + 0.536 \times \text{Exploitation efficiency}$ (Wilk's $\lambda = 0.558$, $P < 0.0001$, $df = 4$, 43).

3.2. Experiment 1: behavioral consistency and personality

During the second experimental phase when all individuals were third instars, pit diameter and response time to prey were correlated between the two successive trials ($r = 0.600$, $P = 0.0001$ and $r_s = 0.516$, $P = 0.0008$, respectively). Relocation distances before and after disturbance in each trial were positively correlated (first trial: $r_s = 0.678$, $P < 0.0001$; second trial: $r_s = 0.391$, $P = 0.014$). Relocation distances after disturbance were significantly correlated between the first and second trials, but there was no significant correlation between relocation distances before disturbance (after: $r_s = 0.476$, $P = 0.0022$; before: $r_s = 0.206$, $P = 0.21$). Prey exploitation efficiency measurements were not significantly correlated ($r = 0.203$, $P = 0.21$).

Table 1

Results of the PCA performed on the behavioral traits obtained during the second phase of the experiment. Only PCs with eigenvalue larger than one are presented.

	PC1	PC2
Eigenvalue	1.448	1.247
Variance explained	36.2%	31.2%
Pit diameter	-0.6807	0.0844
Response time	0.3189	0.6320
Relocation distance	0.0064	0.7366
Prey exploitation efficiency	-0.6595	0.2255

The first PC of the PCA explained 36.2% of the variance and was composed of pit diameter, response time and prey exploitation efficiency (Table 1). Antlions with high loading on this axis built small pits, showed low prey exploitation efficiencies and responded slowly to prey. This PC thus represented the investment in foraging. The second PC explained 31.2% of the variance, pointing at a correlation between long response times and relocation distances (Table 1). In other words, antlions with high loading on this axis responded slowly to prey and relocated over longer distances. PC1 and PC2 were not significantly correlated with any of the morphological traits measured (PC1: head width: $r = -0.124$, $P = 0.46$; mandible length: $r = -0.171$, $P = 0.30$; abdomen width: $r = 0.022$, $P = 0.89$; PC2: head width: $r = -0.068$, $P = 0.68$; mandible length: $r = -0.107$, $P = 0.52$; abdomen width: $r = -0.044$, $P = 0.79$).

3.3. Experiment 2: within-instar behavioral differences

In the first phase of the second experiment, early second instar larvae responded faster to the prey ($F_{1,58} = 10.20$, $P = 0.0023$) and relocated their pits over longer distances following a disturbance ($F_{1,56} = 7.05$, $P = 0.010$) than the late second instars. However, we could not detect any differences between those groups in their pit diameter ($F_{1,46} = 0.46$, $P = 0.50$), prey exploitation efficiency ($F_{1,56} = 1.91$, $P = 0.17$) or relocation activity before disturbance ($F_{1,56} = 1.80$, $P = 0.19$). In the second phase of the experiment, we could not detect any differences within the same individuals, early and late second instar larvae, in their pit diameter ($F_{1,23} = 2.81$, $P = 0.11$), response time to prey ($F_{1,25} = 0.66$, $P = 0.42$), prey exploitation efficiency ($F_{1,25} = 0.76$, $P = 0.39$) and relocation activity before ($F_{1,25} = 2.28$, $P = 0.14$) or after the disturbance ($F_{1,25} = 1.44$, $P = 0.24$).

4. Discussion

Although having similar body masses, second and third instar larvae demonstrated distinct personalities. Early third instar larvae constructed larger pits and responded faster to prey than late second instar larvae. Third instar larvae also exploited higher proportions of the prey provided. These differences disappeared after the second instar larvae molted and progressed into the third larval stage. We highlight to this end two important points: (1) We often assume that animals, especially those collected from the field, are very similar, based on general body size. But they are not. (2) Between-instar behavioral differences should be compared using a set of multiple traits, similar to the tools used for personality research. Regarding the third instar larvae, pit size was correlated with prey exploitation efficiency, emphasizing the importance of pit construction for hunting in pit-building antlions. Long response times to prey were positively correlated with relocation distances, implying that antlions that are unresponsive to prey can later relocate over longer distances.

The most interesting result of this study is the between-instar behavioral differences, which are unrelated to body mass. In other words, stronger behavioral differences were detected between second and third instar larvae very similar in body mass than between small/early and large/late second instar larvae. The body

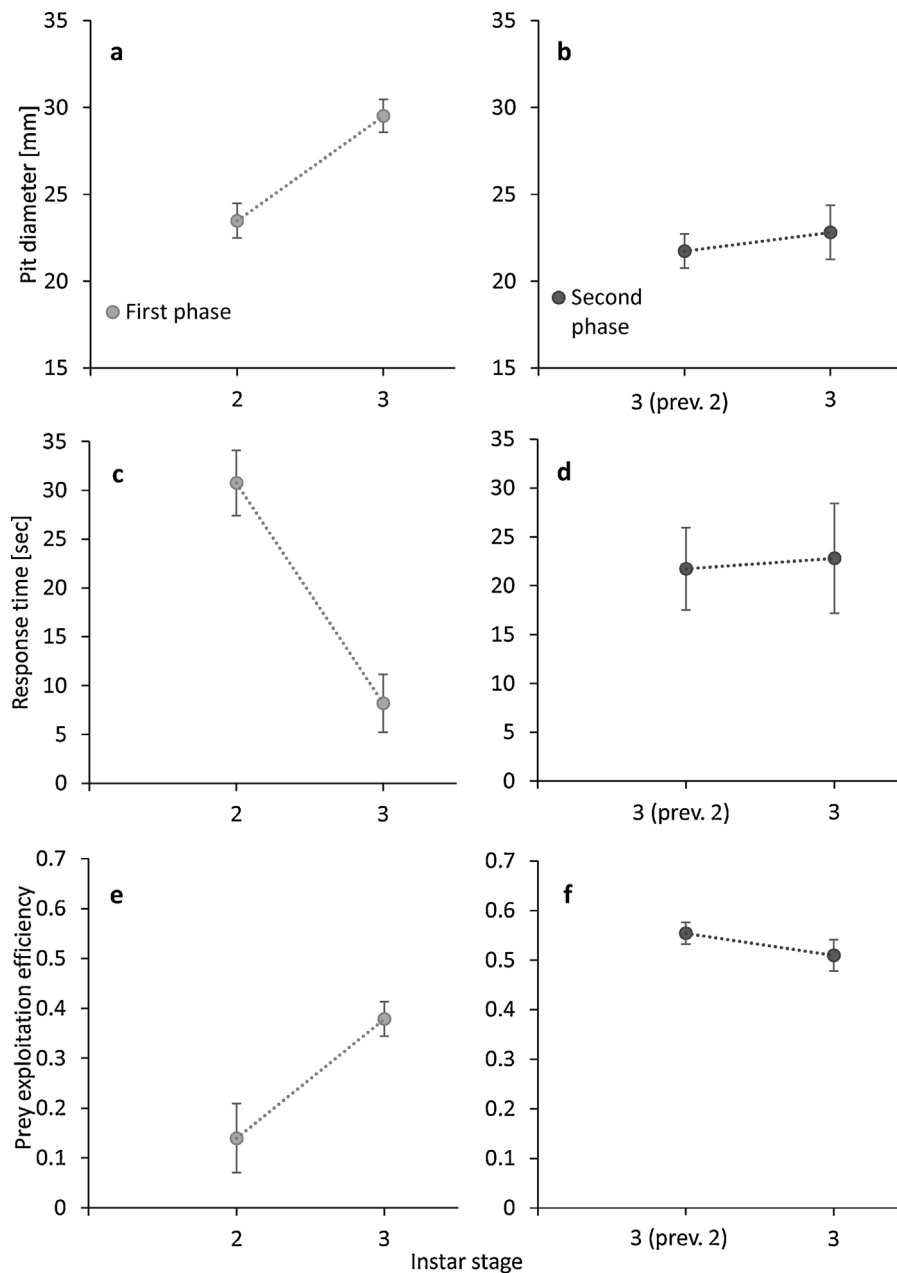


Fig. 2. (a and b) Pit diameter, (c and d) response time to prey, and (e and f) prey exploitation efficiency of the second and third instar larvae during the first (a, c, e) and second (b, d, f) experimental phases. During the second phase, the former second instar larvae already turned into third instars. Note that the initial differences detected during the first phase disappear when all antlion larvae turned into third instars. Means \pm 1 S.E. are presented.

mass difference in this case was over 60%. It thus demonstrates the large contribution of the developmental stage to behavior in insect larvae. It also supports state-dependent models of organisms with complex life cycle, exhibiting risk prone behavior prior to entering the reproductive stage and which is not aimed at avoiding starvation (Bednekoff, 1996). The second and third instar stages are best behaviorally distinguished based on their investment in foraging. In other words, pit size, followed by prey exploitation efficiency, is the best single behavioral trait distinguishing between the instars, in contrast to relocation distance, which is not related to investment in foraging.

It has been known that heavier or larger larvae construct larger pits (e.g., Griffiths, 1980; Allen and Croft, 1985; Scharf et al., 2009b). However, this link is better explained by head and mandible size, the main difference between late second and early third instar stages, and not total body mass. Thus, whenever studying the

behavior of insect larvae in general and antlions in particular, it is important not only to document body mass – an important trait in its own right – but to take the instar stage into account in the experimental design and analysis. Between-instar behavioral differences are known in other insects, but are rarely reported.

We studied in parallel the consequences of body mass and instar stage for antlion behavior, and reached a conclusion that the instar stage predicts better antlion behavior than the variation in body mass within each instar. Earlier studies of antlion or wormlion behavior controlled more often for body mass than the instar stage (e.g., Hauber, 1999; Day and Zalucki, 2000; Farji-Brener, 2003; Devetak, 2008; Scharf et al., 2010; Barkae et al., 2012; but see, e.g., Lucas, 1985; Tsao and Okuyama, 2012). This study advises against it, although it is difficult to determine if incorporating the instar stage would have a dramatic effect in each experiment and system. The results of the second experiment comparing the behavior of early

and late second instar larvae showed that behavior changes, but quite moderately and not for all traits documented. These moderate differences within the second instar compared with the stronger differences between instars do not support a possible effect of an oncoming molting on the behavior of the second instar larvae. However, we cannot completely rule out that a small component of the reduced activity shown by the large/late second instar larvae is induced by an oncoming molting, similar to other insects (e.g., Zilberstein and Ayali, 2002; Wang et al., 2010).

Third instar larvae demonstrated consistent behavior in respect to the size of the pit constructed and the response time to prey. In addition, there was mostly a significant correlation of relocation distances, but this analysis might be biased by many antlions that did not move at all (>60% in the second phase of the experiment). Pit size and response time are both measures of foraging activity and the consistent behavior may stem from inter-individual differences in metabolic rate (Biro and Stamps, 2010). This could be compared to the common positive correlation in many animals between activity and aggression levels (Réale et al., 2007). Antlions of different instar stages differ in other aspects as well, such as the angle of the pit walls (Griffiths, 1986), the strength of correlation between body mass and pit size (Tsao and Okuyama, 2012), and the frequency of sand throwing while attacking prey (Nonato and Lima, 2011).

The PCA on the behavioral traits of the third instar larvae demonstrated a clear link among foraging-related behaviors. First, larvae that built large pits, responded fast to prey and consumed more of each prey item provided. In other words, these antlions invested more in foraging activity and were either better in translating the prey into body mass gained or simply more interested in feeding by having lower GUDs. Antlions at the extreme side of this axis could perhaps be simply better, more efficient predators than antlions at the other extreme, showing long response times and small pits. Variance of behavioral traits is often kept in the population by context-specific performance trade-offs, frequency-dependent selection, shifting selection pressures or a different balance point between current and future reproduction (e.g., Wolf et al., 2007; Pruitt and Riechert, 2012). It is possible that slow responding antlion larvae constructing smaller pits have lower metabolic rate and cope better with starvation periods (as suggested for antlions: Scharf et al., 2009c; and in general for other insects: e.g., Djawdan et al., 1997). Interestingly, aggression toward prey is not always positively correlated with consumption efficiency. Spiders that responded fast and aggressively to prey, left higher levels of GUDs, resulting in “superfluous killing” of prey (Maupin and Riechert, 2001; Pruitt et al., 2008). The difference between the antlion system here and other spider systems could be related to food availability (lower for antlions, higher for spiders) and consequent expectation for the next encounter with prey.

When all antlions were at the third instar stage, we could not detect any effect of morphology on behavior. This is surprising as pit diameter is correlated with antlion body size/mass (e.g., Griffiths, 1980; Scharf et al., 2009b). However, it is probably based on between-instar differences, and we thus suggest that within-instar morphological differences have only weak effects on behavior. Still, the effect of morphology on behavior should be studied in a broader context and under several conditions (e.g., satiation and hunger) to reach a more comprehensive understanding. In addition, pit construction and size is very plastic, depending on various factors, such as hunger level (e.g., Tsao and Okuyama, 2012), density (e.g., Day and Zalucki, 2000), substrate (e.g., Devetak et al., 2005; Barkae et al., 2012), temperature (Rotkopf et al., 2012) and prior experience (e.g., Guillette et al., 2009). We suggest that such and similar biotic and abiotic factors override the contribution of morphology, within the instar stage, to pit size.

Based on the detected inter-instar differences in behavior, it is possible that additional instar-based differences exist. For instance, investigating physiological differences among instars, such as metabolic rate, would contribute to the understanding of the observed behavioral differences. Furthermore, even when all larvae were at the third instar stage, consistent behavioral differences were detected. Studying the physiological basis of such behavior should be important, as well as consistency in behavior across different contexts. In the meantime, we demonstrated here strong behavioral differences between instar stages in a pit-building antlion, and call for the incorporation of morphology in the research of animal personalities.

Acknowledgements

The research leading to this manuscript was partially funded by the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement no. [333442] to IS.

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