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Predator-prey system of antlions and ants: hunting strategies and rescue behaviours

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Chapter I Summary

Present dissertation focuses on the interaction of antlions and ants. These two organisms co-occur in sandy locations and are likely engaged in a coevolutionary arms race. Thesis consists of Introduction, General discussion and four main chapters in the form of four published papers investigating the apparent adaptations and counter adaptations which increase the efficiency of antlions' capture of ants and ants' evade of capture by antlions. In the first two chapters devoted to the hunting strategies of antlions, the connection between learning abilities, behavioural asymmetry, and efficiency of prey capture was investigated. In the next two chapters devoted to the rescue behaviours of ants, the conditions under which these behaviours are less likely expressed and the source of the signal eliciting them were studied. Present research demonstrates that antlions learn to associate vibrations with prey occurrence and that they master this task at different speed depending on the level of behavioural asymmetry. Further, it shows that the level of behavioural asymmetry is connected to the efficiency of prey capture. In case of ants, it was demonstrated that individuals of low value are rescued from antlion grasp less willingly than normal nestmates of high value. Additionally, the source of the "call for help" signal, responsible for rescue elicitation, was found not to originate from the mandibular glands of ants facing the threat of antlion predation. Overall, these results broaden our knowledge about the interaction of antlions and ants as well as, more generally, the interaction of predators and prey. Importantly, the thesis underlines the importance of learning abilities in the predator and secondary means of capture avoidance in the prey, both of which are highly plastic features, mostly overlooked in studies about predator-prey interactions.

Chapter II Introduction: antlions and ants

The diverse field of behavioural ecology seeks to understand animal behaviour by focusing on studying how ecological factors shape species' behavioural characteristics (Davies et al. 2012). Here, an attempt is made to investigate the predator-prey interaction of larval antlions and sand-dwelling ants, which may be engaged in a coevolutionary arms race. The focus of this dissertation are the apparent adaptations and counter adaptations which increase the efficiency of antlions' capture of ants and ants' evade of capture by antlions.

Antlions

Larval antlions are sit and wait predators that build traps as means of getting hold of prey, mostly ants (Hollis et al. 2015, Hollis 2016). These predators are sedentary and they rarely relocate after their pitfall trap in a suitable sandy location is established (Scharf & Ovadia 2006). Their hunting techniques draw interest for a long time now, focusing mostly on description of their behaviour as sit and wait foragers (Turner 1915, Druce 1923, Haub 1942, Mansell 1988, Matsura 1987, Eltz 1997, Napolitano 1998): motionless wait and constant readiness for instantaneous capture of ants that stumble inside traps. Initial assessments of antlion larvae being highly stereotypic in their behaviour had to be revised when it was discovered that they display behaviours which are clearly plastic and elicited only under appropriate conditions. Antlions can detect approaching prey from a distance of a few centimetres by detecting the vibrations caused by the potential prey, as well as direct prey inside their trap by bombarding it with sand particles and thus confusing it (Alcock 1972, Devetak 1985, Eisner et al. 1993, Mencinger 1998, Fertin & Casas 2006, 2007, Devetak et al. 2007, Mencinger-Vračko & Devetak 2008). Recently, antlions' predatory behaviour was discovered to include also the ability to anticipate prey based on vibrations. Guillette and co-authors (2009) presented antlions with a vibrational signal paired with the arrival of prey in their pit and found that larvae exposed to such presentations of cue and food started to display preliminary hunting behaviour in

response to the cue. This enabled larvae to extract prey contents faster and to a greater percentage, which in turn increased the size of their traps due to increase in larvae body mass. Increased body size (and thus trap size) provides benefits in terms of general increase in prey encounter rate and the ability to capture larger prey (Youthed & Moran 1969, Wilson 1974, Griffiths 1980, Heinrich & Heinrich 1984, Hauber 1999, Day & Zalucki 2000, Scharf et al. 2010, Humeau et al. 2015) as well as, importantly, decrease in time spent as larva (Hollis et al. 2011).

Building on these studies on learning in antlions (Guillette et al. 2009, Hollis et al. 2011), Kuszewska and co-authors (2016) have found that antlions learn the meaning of differing cues associated with prey of different sizes and, after mastering this discrimination task, start ignoring smaller in favour of larger prey, presumably reducing energetic costs of hunting. In addition, it was also showed that antlions learn to associate a vibrational cue with the loss of prey, responding to the learned cue by prey burial under the sand in order to prevent its disappearance (Kuszewska et al. 2016). In this particular study, it was observed that there were huge between-individual differences in learning speed among larvae. It was obvious that to master the task at hand, different larvae need different number of training trials. Thus, in the first study presented here, the hypothesis that differences in learning speed are associated with the level of behavioural asymmetry of larvae was tested (Miler et al. 2017; Chapter III of this dissertation). The idea was that brain lateralization occurs in these insects and, similarly as in other higher-order animals, reflects on their behavioural asymmetry and cognitive performance (Levy 1977, Vallortigara & Rogers 2005, Dadda et al. 2015). It was predicted that 1) behavioural asymmetry occurs in these insects, and 2) behaviourally asymmetric individuals, presumably with high brain lateralization, learn quicker. Further, in the second study presented here (Miler et al. 2018; Chapter IV of this dissertation), conducted due to finding that only low numbers of larvae are highly behaviourally asymmetric, the potential reasons for this low

occurrence of behavioural asymmetry were investigated. It was hypothesized that there is a life history trade-off between brain lateralization and another fitness-relevant trait, i.e. hunting efficiency (see Dadda et al. 2009 and Chivers et al. 2017 for examples on fish). The prediction was that some larvae develop high brain lateralization, evidenced by behavioural asymmetry, at the cost of lower vibration sensitivity and thus lower hunting efficiency. Overall, in these two papers, antlions' ability to use learning in adaptive ways, presenting a superb ability to put predatory pressure on their main prey, the ants, was investigated.

Ants

Some ant species face the threat of predatory antlions. These sand-dwelling ants are known to display the ability to rescue their captured nestmates (Czechowski et al. 2002; Hollis & Nowbahari 2013a). According to Hollis & Nowbahari (2013b), rescue behaviour occurs between the rescuer, which takes non-rewarded risks by fitted to the circumstances rescue engagement, and the victim, which is in a situation of severe fitness loss. Several studies have been so far conducted on ant rescue behaviour, usually with the use of the so-called entrapment bioassay, which is a laboratory simulation of antlion larva capture and/or entrapment under fallen debris (the victim is tied to a piece of paper, and such an ant is then placed in an arena, partially covered with sand particles). Nowbahari and co-authors (2009) showed that *Cataglyphis cursor* ants rescue their nestmates, but ignore or react aggressively towards ants from other colonies or species. Later on, Nowbahari and co-authors (2012) demonstrated that among different castes of *C. cursor* ants (inactives, nurses and foragers), foragers are best able to both give and to receive help. However, as showed recently, individuals younger that inactives, the so-called callows, i.e., the new born ants, receive high levels of rescue (Nowbahari et al. 2016). Overall, these to-date results indicate that some ants are capable of highly

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sophisticated, ecologically tuned rescue behaviour, which presumably is a tactic of evading antlion predation.

Building on these to-date studies, it was tested whether ants of low value for the colony are rescued less willingly than other, higher-value individuals (Miler 2016; Chapter V of this dissertation). As mentioned, foragers are rescued most willingly among the tested ant castes (Nowbahari et al. 2012), but foragers can live for weeks and can differ strongly in age, physiological state and thus value (Tofilski 2002). From the colony perspective, rescuing old and sick foragers makes no sense. Therefore, by manipulating life expectancy of foragers of *Formica cinerea*, the hypothesis that antlion victims with lower life expectancy will receive lower levels of rescue behaviours from their nestmates was tested. The ability to "call for help", i.e. to emit a signal eliciting rescue (Czechowski et al. 2002), may plausibly decrease in ants as their life expectancy decreases, and this may explain why would old and sick ants receive lower levels of rescue. The issue of how ants signal their need for help is, unfortunately, very poorly studied. A preliminary study was thus conducted, in which the source of the rescue-eliciting signal in *F. cinerea* was looked for (Miler & Kuszewska 2017; Chapter VI of this dissertation). With these two studies, several new research avenues were opened (See General discussion). The issue of how and in what conditions ants rescue each other needs more research and these studies were aimed at expanding our knowledge in this area.

Chapter III Larval antlions with more pronounced behavioural asymmetry show enhanced cognitive skills

Research

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Larval antlions with more pronounced behavioural asymmetry show enhanced cognitive skills

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Brain lateralization is hypothesized to improve the efficiency of information processing. Here, we found that some *Myrmeleon bore* antlion larvae showed individual asymmetry in righting from a supine to normal position over one side of their body, which can be considered a reflection of greater brain lateralization. We demonstrated that these behaviourally asymmetrical individuals showed improved learning abilities, providing novel evidence that brain lateralization leads to beneficial effects on cognitive functions.

1. Introduction

Brain lateralization is a well-known phenomenon, with certain brain functions involving one of the hemispheres more than the other [1]. Although it has long been studied exclusively in humans and other vertebrates [2], lateralization research involving insects is increasingly popular, with bees recently proposed as models for such studies [3]. Sophisticated types of behavioural asymmetry, i.e. left/right sidedness in behaviour, which presumably reflect brain lateralization, are found in bees [4–6]. Nevertheless, other insects can also be used to study individual asymmetries (i.e. individual side biases) and the underlying brain lateralization, along with the benefits of lateralization, which are not fully understood. Functional improvements due to decreased interference in the brain and the promotion of parallel processing have been proposed as the main explanations for selection on the existence of brain lateralization [7,8], but the evidence is still scarce. Recent experimental studies show that chicks with asymmetric thalamofugal visual projections to their forebrains are better at performing two tasks simultaneously [9], whereas locusts with probable lateralization of some brain regions involved in limb movement show increased motor control due to sidedness in forelimb use [10]. In addition, insects can be used for more detailed studies of population asymmetries (i.e. unequal proportions of left- and right-side biased individuals) [11,12]. Population-wide asymmetries are hypothesized to stem from benefits provided by shared sidedness that outweigh the associated costs [13,14]. Studying both social and non-social insects can thus elucidate the reasons underlying population asymmetries.

Here, we used antlion larvae to study behavioural asymmetry and the effect of the brain lateralization responsible for that asymmetry on larval cognitive functioning. Antlion larvae build pitfall traps for hunting ants in sandy areas [15]. They were long believed to show little or no behavioural plasticity, an opinion now challenged by accumulating evidence of their complex learning abilities [16–18]. They are completely non-social; their interactions with conspecifics are limited to cannibalism (e.g. [19]). Both of these points make antlion larvae interesting organisms for studying individual- and population-level behavioural asymmetries and brain lateralization. We tested them for asymmetry in righting from a supine to normal position (see electronic supplementary
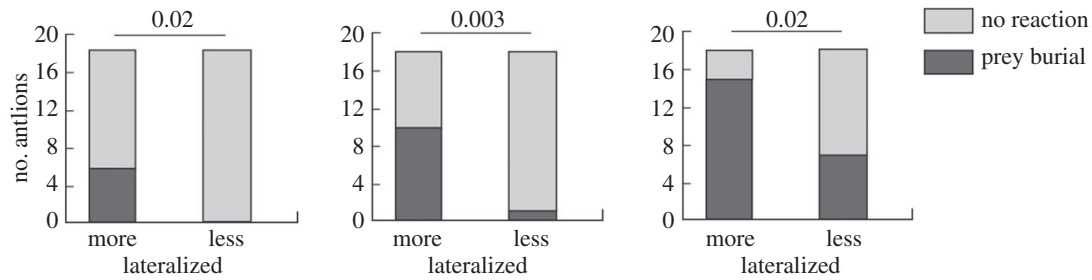
Figure 1. Behaviour of more and less lateralized larvae of the antlion *Myrmeleon bore* trained to associate a vibrational cue with prey disappearance. Numbers of larvae burying prey on two consecutive tests (dark bars) and not burying prey on two consecutive tests (light bars) are shown. The results of the first/second, second/third and third/fourth tests are shown from left to right. *P*-values from FETs are indicated above the bars.

material, Video), a behaviour investigated in previous lateralization studies (e.g. [20]). Considering the neural simplicity of antlion larvae, the strength of their behavioural asymmetry should indicate the level of their general brain lateralization, thus providing means of detecting individuals with more lateralized brains. We hypothesized that more biased individuals will perform better in a cognitive task than less biased individuals.

2. Methods

We collected 200 *Myrmeleon bore* larvae from the Błędowska Desert (Poland) and assessed preferences in their direction of righting behaviour in 20 trials (approximately 10 min between trials). In each trial, a single larva was placed inside an Eppendorf tube, which was then gently shaken. This resulted in the larva falling on its back, and we noted the direction (left or right) in which it righted itself. Then, we categorized larvae into two groups: (i) more lateralized (left turns occurring in 0–5 or 15–20 trials) and (ii) less lateralized (left turns occurring in 6–14 trials). Afterwards, each larva was weighed. Twenty weight-matched groups of four larvae each were created, with each group comprising two more and two less lateralized individuals. These larvae were individually housed in paper boxes (13 × 13 × 4.5 cm) filled with sand, fed a single ant prey and left to acclimatize and build traps for 48 h. Within each group, one more lateralized and one less lateralized individual was assigned to the training treatment, and the other two larvae were assigned to the non-training treatment.

The experiment consisted of four blocks, each involving 2 days of training followed by a day of testing. Ants were placed in antlion pits four times per training day, at 2 h intervals between 10.00 and 18.00. During two of the four daily feedings, the prey was carefully taken from the larvae after capture, using forceps. Larvae in the training group were presented with a vibrational cue immediately following prey capture and before prey disappearance; larvae in the non-training group were presented with the vibrational cue 5–10 min before or after prey capture, giving them no opportunity to learn the association between the cue and prey removal. Vibrational cues were delivered by 4.5 ml of sand falling from a funnel with attached plastic pipette tip directed towards the edge of the antlion pit. A small container (a metal pipe 4 cm in diameter) below the pipette prevented additional sand from accumulating in the box and enabled the conduction of vibrations. On each of the four test days, larvae received prey followed immediately by the vibrational cue. Larvae attempt to prevent prey loss by prey burial; thus, we noted whether each larva buried its prey in each test during the 3 min following the presentation of the cue. Larvae that initiated prey burial (i.e. started moving down into the sand) but stopped before finishing (i.e. the moment

when the larva and its victim were no longer visible on the surface of the sand) were treated as showing no response.

Data from the first, second and third tests were used, after corrections using data from the second, third and fourth tests (respectively). Prey burial can occur spontaneously; corrections were made to exclude false-positive reactions, i.e. larvae that displayed prey burial in the former but not in the latter test were treated as if they showed no reactions in the former test. Thus, we only considered that larvae had buried prey if the prey were buried in two consecutive tests. Fisher's exact tests (FETs) were used to check for between-group differences in the number of larvae that buried prey. In addition, we performed memory tests. On days 3, 6 and 9 after the fourth test, with no reinforcement between tests, additional tests of prey burial were performed with trained larvae. We compared the results from the third test with the results from each memory test using FETs with alpha values adjusted down using experiment-wide Bonferroni correction for multiple comparisons to assess between-group differences in the memory of the learned association. In the experiment, two groups of four larvae were excluded due to a lack of functional traps. One additional group of four larvae was excluded from the third memory test for the same reason. Statistical analyses were conducted using two-tailed tests in STATISTICA v. 11 (StatSoft, Poland).

3. Results

We found that only 24% of the larvae showed either a left or a right asymmetry in righting (48 of 200 individuals, 38 right-sided and 10 left-sided). We did not analyse population-level asymmetry owing to the small overall number of asymmetric individuals found.

We present results only for the trained groups (more versus less lateralized larvae) because they are the focus of this study; the non-training treatment was designed solely to ensure that only trained individuals learned (see electronic supplementary material, Appendix, for results from the non-training treatment). The occurrence of prey burial was significantly higher among more lateralized larvae than among less lateralized ones in all three tests (figure 1). In the memory tests, we found that both more and less lateralized larvae performed worse at 6 and 9 days after the end of the experiment, and the two groups showed similar rates of memory loss (figure 2).

4. Discussion

Our results show that some antlion larvae display individual behavioural asymmetry, which implies that they possess pronounced brain lateralization. Furthermore, we demonstrate that more lateralized individuals have greater learning
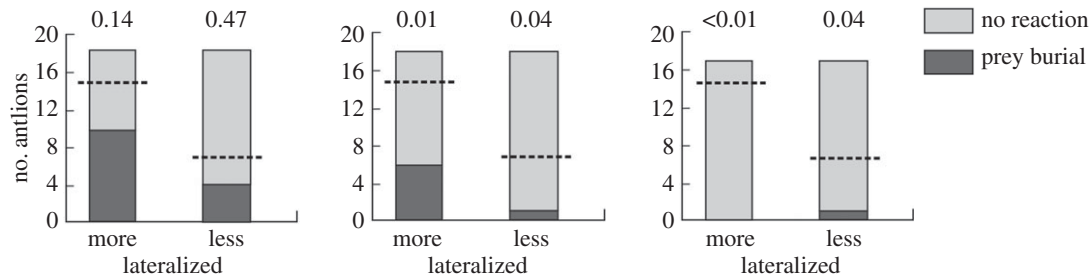
Figure 2. Memory of more and less lateralized larvae of the antlion *Myrmeleon bore* trained to associate a vibrational cue with prey disappearance. Numbers of larvae burying prey (dark bars) and not burying prey (light bars) are shown. The results obtained at 3, 6 and 9 days after the end of the experiment are shown from left to right. Broken lines indicate the result of the third/fourth test. *P*-values from FETs comparing the results from the third/fourth test with the results from each memory test are indicated above the bars.

abilities, which is consistent with the idea that brain lateralization enhances brain functions [7,8]. A few studies have tested this hypothesis (e.g. fruit flies with greater brain lateralization were demonstrated to possess superior memory [21]), and we provide novel evidence supporting it. Additionally, our results are consistent with reports showing that antlions can associate vibrational cues with various events [16–18], providing further evidence suggesting that long-standing assumptions about the limited abilities of antlions for learning are false [15].

The methods employed here (i.e. the categorization of individuals as more or less lateralized on the basis of a single behavioural measure) may be responsible for the low numbers of more lateralized individuals detected. However, if our methods are appropriate for detecting general brain lateralization in antlion larvae, then the rarity of lateralization suggests that it has high costs, unaffordable by most of the larvae [15]. Naturally, these costs need not be limited to energy constraints (e.g. costs of neural overdevelopment in one hemisphere) and may also involve behavioural costs associated with a side bias. For example, in fishes, more lateralized individuals showing eye-use asymmetry benefit in terms of performing two tasks simultaneously [22], but these fish also pay costs when they are faced with tasks requiring matched information from both eyes [23]. Thus, it would be interesting to experimentally address the issue of the costs of brain lateralization in antlion larvae. Costs aside, clearer benefits of increased brain lateralization in antlion larvae should be examined, e.g. higher foraging success rates. Such a link might be expected because learning associated with foraging has been shown to shorten the duration of the vulnerable larval stage in antlions [17].

Overall, using larval insects, we show that behavioural asymmetry stemming from brain lateralization is associated with superior cognitive performance, and this association supports the hypothesis that brain lateralization enhances individual functioning.

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Chapter IV Larval antlions show a cognitive ability/hunting efficiency trade-off connected with the level of behavioural asymmetry

SHORT COMMUNICATION

CrossMark

Larval antlions show a cognitive ability/hunting efficiency trade-off connected with the level of behavioural asymmetry

Krzysztof Miler[1] · Karolina Kuszewska[1] · Gabriela Zuber[1] · Michal Woyciechowski[1]

Abstract

Recently, antlion larvae with greater behavioural asymmetry were shown to have improved learning abilities. However, a major evolutionary question that remained unanswered was why this asymmetry does not increase in all individuals during development. Here, we show that a trade-off exists between learning ability of larvae and their hunting efficiency. Larvae with greater asymmetry learn better than those with less, but the latter are better able to sense vibrational signals used to detect prey and can capture prey more quickly. Both traits, learning ability and hunting efficiency, present obvious fitness advantages; the trade-off between them may explain why behavioural asymmetry, which presumably stems from brain lateralization, is relatively rare in natural antlion populations.

Introduction

Many animals respond differently to stimuli on one side of their body than they do to stimuli on the other. Such behavioural asymmetry presumably reflects the level of brain lateralization, i.e., brain functions that involve one hemisphere more than the other (Rogers et al. 2013). In humans and other vertebrates, the connection between the behavioural asymmetry and the brain lateralization is widely assumed (Levy 1977) and has some experimental support (Vallortigara and Rogers 2005). For instance, the increased ability to simultaneously perform two tasks (predator vigilance and food searching) has been demonstrated to be associated with high brain lateralization in chicks (Rogers et al. 2004). This connection has also been demonstrated in invertebrates, such as in fruit flies, which show superior memory when they possess highly lateralized brains (Pascual et al. 2004). More frequently, however, the level of brain lateralization

is simply inferred from the level of behavioural asymmetry. In fish, several cognitive advantages of being highly behaviourally asymmetric (and presumably having high brain lateralization) have been demonstrated (see Sovrano et al. 2005 and, more recently; Bibost and Brown 2014; Dadda et al. 2015). In invertebrates, too, behavioural asymmetry was shown to increase cognitive functioning, i.e., learning speed (see Miler et al. 2017 for an example in the predatory neuropterans, antlions), possibly reflecting the benefits of the brain lateralization.

In predatory species, the detection of stimuli that co-occur with prey encounters, such as visual cues, enable the anticipation of prey arrival, thus increasing capture success. In antlions, vibrational cues correlated with prey arrival can be learned and used to modify foraging strategy in adaptive ways (Kuszewska et al. 2016), so these organisms should be selected for more efficient learning and thus greater fitness. However, only 24% of *Myrmeleon bore* antlions originating from a single population in Poland were reported to show increased levels of behavioural asymmetry, which, as mentioned above, correlates with their enhanced cognitive performance (Miler et al. 2017). From an evolutionary perspective, this finding indicates potential major fitness costs of behavioural asymmetry in certain kinds of tasks. For example, antlions live in sandy areas and capture prey using pitfall traps (Scharf et al. 2011), and tossing sand at a prey item is a tactic that can increase capture success. However, the efficiency of

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this behaviour depends on the direction in which the sand must be thrown (Bongers and Koch 1981) and thus may differ due to individual behavioural asymmetry (i.e., side bias).

Here, we used *M. bore* antlions with higher or lower behavioural asymmetry to test the hypothesis that more-biased individuals perform better at a cognitive task but worse at hunting prey than less-biased individuals. Interestingly, behavioural asymmetry was observed previously in the context of foraging (for examples on toads see Vallortigara et al. 1998 and Robins and Rogers 2004) but not in connection to learning.

Methods

We collected 200 *M. bore* larvae from the Błędowska Desert (Poland) and assessed their preferred direction in righting behaviour in 20 trials (allowing ~ 10 min between trials) (Miler et al. 2017). In each trial, a single larva was placed inside a plastic Eppendorf tube (1.5 ml) that was then gently shaken. This resulted in the larva falling on its back, and we noted the direction (left or right) in which it righted itself. Then, we categorised the larvae into two groups: (1) more lateralized (left turns occurring in 0–5 or 15–20 trials) and (2) less lateralized (left turns occurring in 6–14 trials). Afterwards, each larva was weighed, and 24 weight-matched groups of 4 larvae each were created, with each group comprising two more and two less lateralized individuals (96 larvae in total). These larvae were individually housed in paper boxes (25 × 15 × 10 cm) that were half-filled with sand, fed a single ant prey item (live *Lasius niger* worker) and left to acclimate and build traps for 48 h. Within each group, one more lateralized and one less lateralized individual were assigned to the relevant (contingent) condition, and the other two larvae were assigned to the irrelevant (non-contingent) condition. Two groups of larvae (eight individuals) were excluded because some of the individuals within these groups failed to build traps. In total, we tested 88 larvae, 44 in the relevant and 44 in the irrelevant condition (22 more lateralized and 22 less lateralized individuals in each case).

The experiment was run in blocks, each involving 2 days of training followed by a break day. Ants were placed in antlion pits twice per training day, between 10 a.m. and 6 p.m. Larvae in the relevant condition were presented with a vibrational cue approximately 10 s before prey delivery, whereas larvae in the irrelevant condition were presented with the vibrational cue 5–10 min before or after prey arrival, thus providing no opportunity to associate the cue with the prey. Vibrational cues involved the delivery of 4.5 ml of sand through a funnel with an attached plastic pipette tip directed towards the edge of the antlion pit, and a small container (a metal pipe, 4 cm in diameter, blocked off at the bottom with a sheet of foil) below the pipette prevented additional sand from accumulating in

the box and enabled vibrations to be conducted (see Supp. Fig. 1A). Each delivery of the vibrational cue to an antlion was treated as a test. Larvae prepare for prey arrival (i.e., show mandible movement at the bottom of the pit) when they make the association with the vibrational cue. Therefore, after cue delivery but before prey delivery (as mentioned, ~10 s), preparation for hunting (i.e., reaching the learning criterion) can be easily observed. Once an individual showed mandible movement after the cue in two consecutive tests, it was marked as having reached the learning criterion. The following day, the distance at which the vibrational cue elicited the learned response in these individuals was tested (the distance test). For each larva, six distances from the edge of the antlion pit were used, in decreasing order, with a 10-min interval between the different testing distances: 15, 12, 9, 6, 3 and 0 cm. Vibrational cues were delivered at these distances as 4.5 ml of sand falling from a funnel with a plastic pipette tip into a small container below. Prey was never delivered during the distance test. Since they did not learn, the larvae in the irrelevant condition never proceeded to the distance test. Hence, in each group of four larvae, the training sessions for the two larvae in the irrelevant condition were terminated when both larvae in the relevant condition reached the learning criterion. The next day, prey capture latency was tested in the larvae from the irrelevant condition (the latency test). A circular plastic arena (11 cm in diameter) covered in Fluon (Sigma–Aldrich, Germany) was placed around the antlion pit, and a group of five live *L. niger* worker ants was introduced (see Supp. Fig. 1B). The test began when the first worker stepped into the antlion pit, and we measured the latency (in seconds) to the capture of any of the ants. The maximum test time was 3 min.

Statistical analyses were conducted in STATISTICA 13 (Tibco, Poland). The learning speed of the larvae in the relevant condition, with group (more vs. less lateralized) as a factor, was analysed using the Wilcoxon matched-pairs test (dependent variable: the number of sessions to reach the learning criterion). The maximum distance at which the vibrational cue elicited the learned response in the larvae in the relevant condition in the distance test (dependent variable: the distance at which the learned response was evident) and the latency to prey capture in the larvae in the irrelevant condition in the latency test (dependent variable: the latency to ant capture) were analysed similarly.

Results

We detected 48 highly asymmetric individuals out of a total of 200 larvae tested for bias in righting (24%). None of the larvae in the irrelevant condition "learned" the focal association, which was not surprising, as this condition was designed solely to ensure that only individuals in the relevant condition learned. The occurrence of a learned
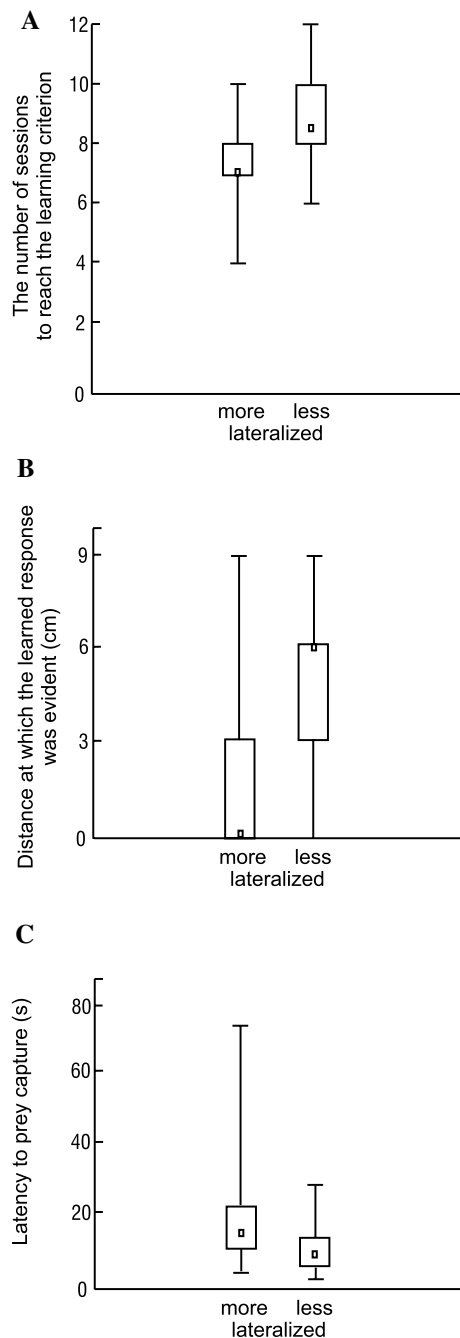
Fig. 1 Behaviour of more and less lateralized antlion larvae. **a** The number of sessions to reach the learning criterion in the relevant condition. Valid $N = 22$ pairs; $Z = 3.944$; $p < 0.001$. **b** Distance from the edge of the pitfall trap at which the learned response was evident. Valid $N = 22$ pairs; $Z = 3.506$; $p < 0.001$. **c** Latency to prey capture. Valid $N = 22$ pairs; $Z = 3.685$; $p < 0.001$. Statistics: Wilcoxon matched-pairs tests. Squares indicate medians, boxes indicate quartiles, and whiskers indicate ranges

response was significantly lower in terms of the number of sessions required to reach the learning criterion among more lateralized larvae than less lateralized ones (Fig. 1a),

indicating that the former learned more quickly than the latter. However, the occurrence of a learned response was significantly lower in terms of the maximum distance at which the response was evident among more lateralized larvae than among less lateralized ones (Fig. 1b), meaning that the more lateralized larvae showed lower sensitivity to vibrations than the less lateralized larvae. Furthermore, the latency to ant capture was significantly higher among the more lateralized larvae than among the less lateralized ones (Fig. 1c), indicating that the more lateralized individuals showed lower hunting efficiency.

Discussion

Our data show that antlion larvae that display behavioural asymmetry learn faster, but they simultaneously experience decreased vibration sensitivity and, probably as a result of this, exhibit lower hunting efficiency. These results strongly suggest that a trade-off exists between larval ability to hunt efficiently and learn quickly. In this study, we detected the same ratio (24%) of highly asymmetric individuals in a population as in the previous study reporting asymmetry in *M. bore* and utilising the same type of side-bias testing (Miler et al. 2017). This low number is much more likely to be connected with the trade-off than with the methods employed (i.e., the categorization of individuals as more or less lateralized on the basis of a single behavioural measure), as previously suggested (Miler et al. 2017).

Fitness costs have been found to be associated with asymmetry in other animals. Fish pay these costs when they are faced with tasks requiring matched information from both sides of the body (Dadda et al. 2009) and when forced to compete for resources (Chivers et al. 2017), whereas behaviourally asymmetric dogs experience problems when solving puzzles (Marshall-Pescini et al. 2013). In antlions, the costs of behavioural asymmetry in terms of lower hunting efficiency may stem from lower vibration sensitivity, at least partially connected to morphological asymmetry at the peripheral level, as was demonstrated for several species of bees in connection to their learning abilities (Anfora et al. 2011; Frasnelli and Vellortigara 2017).

Importantly, there is an alternative interpretation of the results of the distance test. Here, larvae were trained to associate the cue with the prey at the edge of their trap and then tested for the learned response at several distances from their pitfall trap in the distance test. Vibrations delivered farther away from the larvae differ in strength from the learned cue. Thus, it may be that the more lateralized larvae show less generalization (Shepard 1987; Ghirlanda and Enquist 2003). In this context, it is not a bad thing, because vibrations may occur not only due to the approaching prey but also due to various distractors. Subsequently, showing hunting readiness

to all vibrations would be unnecessary. In any case, the issue of vibration sensitivity in the more and the less lateralized groups of antlions seems worth further study as it may be quite the opposite from suggested above: if the more lateralized group perceives the learned and tested stimuli as more different (i.e., shows less generalization) than the less lateralized group, then the former should have higher vibration sensitivity. The distance test, then, leaves us no hint as for the reasons behind differences in hunting efficiency between more and less lateralized larvae.

Our results are consistent with previous reports that antlions can associate vibrations with environmental events (Guillette et al. 2009; Hollis et al. 2011; Kuszewska et al. 2016) and that those with pronounced behavioural asymmetry possess superior cognitive skills (Miler et al. 2017); here, this latter phenomenon was demonstrated with a novel task (i.e., hunting readiness as opposed to prey burial in the previous study). The hypothesis that behavioural asymmetry conveys fitness advantages, especially in a cognitive context, is gaining experimental support (Güntürkün et al. 2000; Magat and Brown 2009), but the evidence is still scarce for invertebrate species. An important step that is missing here is the demonstration of the direct connection between the behavioural asymmetry and the brain lateralization, presumably responsible for behavioural side bias (Miler et al. 2017).

Overall, we demonstrate that behavioural asymmetry is associated with superior cognitive and inferior hunting performance in larval antlions. A trade-off between these two traits might explain why brain lateralization is relatively rare in natural antlion populations.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Chapter V Moribund ants do not call for help

Moribund Ants Do Not Call for Help

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Abstract

When an antlion captures a foraging ant, the victim's nestmates may display rescue behaviour. This study tested the hypothesis that the expression of rescue behaviour depends on the life expectancy of the captured ant. This hypothesis predicts that the expression of rescue behaviour will be less frequent when the captured ant has a lower life expectancy than when it has a higher life expectancy because such a response would be adaptive at the colony level. Indeed, significant differences were found in the frequency of rescue behaviours in response to antlion victims with differing life expectancies. In agreement with prediction, victims with lower life expectancies were rescued less frequently, and those rescues had a longer latency and shorter duration. There was also a qualitative difference in the behaviour of rescuers to victims from the low and high life expectancy groups. Several explanations for these findings are proposed.

Introduction

Foraging ants are faced with a serious threat from co-occurring trap-building antlion larvae because these predators are highly specialized for capturing terrestrial invertebrates [1]. Some ant species have evolved means of avoiding antlion predation, i.e., avoiding areas where antlions form aggregations [2]. Nevertheless, in the event that an ant is captured by an antlion larva, nearby nestmates may exhibit risky rescue behaviour to save the captured ant from predation [3]. This behaviour can be displayed by one or more individuals (rescuers) and is directed towards another individual (victim) to allow the latter to free itself from a dangerous situation [4]. In the context of antlion capture, rescue behaviour is possible because the steps required for the larva to firmly grip and begin consuming the ant take some time. Rescue of the captured ant can take several forms spanning several behavioural categories, from relatively simple digging around the victim and pulling on the victim's limbs to more precise behaviours such as removing sand that is covering the victim and directly attacking the mandibles of the antlion [3,5]. When engaged in a rescue attempt, the rescuer faces a serious threat of becoming a victim itself, which is why this type of behaviour has been considered highly altruistic [4]. Although the mechanism underlying the rescue behaviour remains a mystery, it is hypothesized that it is mediated by the action of the captured ant, which releases a facilitating pheromone. Observations made by Czechowski and co-authors strongly suggest that ants in distress do emit signals that summon their nestmates [3]. These signals are colony-specific [6] and may originate from the Dufour's and poison glands, at least in some ant species [7]. Furthermore,

the fact that anesthetized ants elicit no rescue [6,7] strongly supports the notion that active pheromone release by the victim is necessary for the expression of rescue behaviour. Therefore, the behaviour of rescuers may be considered as an indicator of the behaviour of the ant in distress, at least with respect to call for help signalling.

The present study tested the hypothesis that the frequency of expression and characteristics of rescue behaviour exhibited by rescuer ants depend on the life expectancy of the victim ants. Such a relationship between rescue behaviour and life expectancy may be adaptive for two non-exclusive reasons. First, this relationship may constitute a broader social isolation syndrome of moribund ants. Specifically, dying ants serve their inclusive fitness by leaving their nests [8]. Their own perceived low life expectancy serves as a trigger to do so, lowering their chance of interaction with nestmates. It is adaptive for individuals with low life expectancy to avoid interactions, because such individuals are typically sick, and may easily transmit disease within the nest [9]. Rescue behaviour is a form of interaction between nestmates, and as with any other type of interaction, it can be avoided by moribund ants. Second, the rescue behaviour and life expectancy relationship may reflect the high colony-level cost to saving individuals with low life expectancy [10]. The "division of labour by division of risk" hypothesis states that mean worker longevity is prolonged and overall colony performance increases when safe tasks are performed by high-value workers with high life expectancy and when risky tasks are carried out by low-value workers with low life expectancy because the future benefit to the colony from soon-to-die workers is low [11–14]. Thus, in the context of rescue behaviour, investing resources into saving individuals of low value that are characterized by low life expectancy appears counter-adaptive because they are of limited use to the colony.

In agreement with both of the above explanations, it is shown here that ants more frequently rescue their nestmates and perform more intensive rescues when the imperilled individuals have a higher life expectancy than when they have a lower life expectancy. Although alternative explanations are possible, the results of this study may be interpreted as a decline in the calling for help with decreasing life expectancy in ant victims.

Materials and Methods

Approximately 500 active *Formica cinerea* foragers were hand-picked from each of the three different colonies from the same site (Błędowska Desert). Overall, 300 antlion larvae (*Myrmeleon bore*) from the same locality were also collected. In the laboratory, antlions were kept in plastic cups half filled with dry sand (7 cm in diameter, 15 cm high), while ants from each colony were kept in separate plastic boxes (25 x 17 x 10 cm), at a constant 24°C and 40–60% RH and a 12:12 L:D cycle. Ants were fed sucrose solution ad libitum, while antlions were not provided with any food; both were allowed to habituate to the setup for two days after transportation from the field. Then, on day three, in each ant colony, four groups of workers were created (50 ants each) and kept in separate plastic boxes (25 x 17 x 10 cm), while the rest (approximately 300 per colony) remained in their original plastic boxes. Two of these groups were untreated controls, and the other two were experimental groups whose life expectancy was artificially shortened by exposure to ~100% carbon dioxide for 1.5 h. The method used for the experimental manipulation has been used previously, and its efficiency in lifespan shortening in ants is well established [15,16]. One control and one experimental group were used as the sources of the captured ants during the tests, and the other two groups of workers were used to establish that the carbon dioxide treatment was successful (checked daily until all were dead). For the tests, which were conducted on the day after the carbon dioxide treatment, an antlion larvae capture bioassay was performed inside the cups in which the antlions were kept. In each test, a control forager or a forager with shortened life expectancy was dropped into the antlion

pit. Immediately after the ant was captured, a potential rescuer (one of the remaining control ants from the respective colony) was introduced into the cup but not into the pit. Each test lasted three minutes, during which it was noted whether rescue behaviour occurred, and if so, the latency to the first episode of rescue, the total duration of rescue, and the types of behavioural categories displayed by the rescuer. Four behavioural categories of rescue were used: pulling at the victim's limbs/antennae/mandibles, digging around the victim, removal of sand covering the victim, and direct attack on the antlion mandibles. The operational definitions of behavioural categories were the same as in previous studies of ant rescue behaviour [5,6], except that the definition of pulling in the present study was applied to antennae and mandibles as well as limbs. The order of testing within each colony was counterbalanced for both the control and experimental groups. All tests that lasted less than three minutes (because the victim was completely buried under the sand or was released from the grasp of the predator for some reason) were excluded. The final number of tests in each group from each colony was 30. No ants or antlions were used more than once. Data were analysed in SPSS Statistics 21 software (IBM, Warsaw, Poland). The two-tailed Fisher's Exact Test (FET) was used to detect between-group differences in the occurrence of rescue, pulling, digging, sand removal and attack on the antlion. Data on mortality, the latency to and the duration of rescue were analysed with Generalized Linear Mixed Models (GLMM) using a loglink function and Poisson error distribution. Colony was included as a random factor, while group was used as a fixed factor.

Results and Discussion

The present study tested the hypothesis that the frequency of the expression of rescue behaviour is lower when the victim ants have a lower life expectancy than when they have a higher life expectancy. Life expectancy was experimentally shortened in this study using carbon dioxide exposure; this manipulation was effective at reducing life expectancy ($F_{1,298} = 799.697$, $p < 0.0001$; Fig 1). Exposure to CO_2 is known to mimic accelerated ageing in insects [17] and may be used to study the active responses of ants to low life expectancy [8,13,15,16]. Importantly, ants of varying, unknown age and characterized by high initial variance in life expectancy were used here to establish both the untreated control and experimental (poisoned with carbon dioxide) groups of workers. As indicated by a rather high mortality in control groups, individuals with lower life expectancy were also present in the controls. This was to be expected because the ants used in this experiment were active foragers collected from the field. However, the results of the present study indicate that rescue was attempted less frequently towards ants with lower life expectancies (rescue occurred in 34 out of 90 cases in the experimental group, compared to 50 out of 90 cases in the control group; FET yielded p = 0.02). If rescue behaviour was directed towards ants with lower life expectancies, it was expressed after longer periods ($F_{1,82} = 195.672$, $p < 0.0001$) and for shorter durations ($F_{1,82} = 218.937$, $p < 0.0001$; Fig 2) than for ants with higher life expectancies. There was also a qualitative difference in the behaviour of rescuers when attempting to rescue an ant with lower life expectancy. These victims were rescued with less frequent attacks on the antlions, which was the most advanced form of rescue in the test type applied in this study (19 out of 50 cases in the control group, compared to 5 out of 34 in the experimental group; FET yielded p = 0.03; Fig 3). There were no between-group differences in the occurrence of other behavioural categories (p = 0.08 for pulling at the victim's limbs/antennae/mandibles, p = 0.82 for digging around the victim, and p = 0.57 for transport of sand covering the victim; p values from FET; Fig 3). In general, the behaviour of potential rescuers following introduction into the test cup involved quick, erratic movements within the space provided followed by contact with the victim and, ultimately, rescue behaviour.
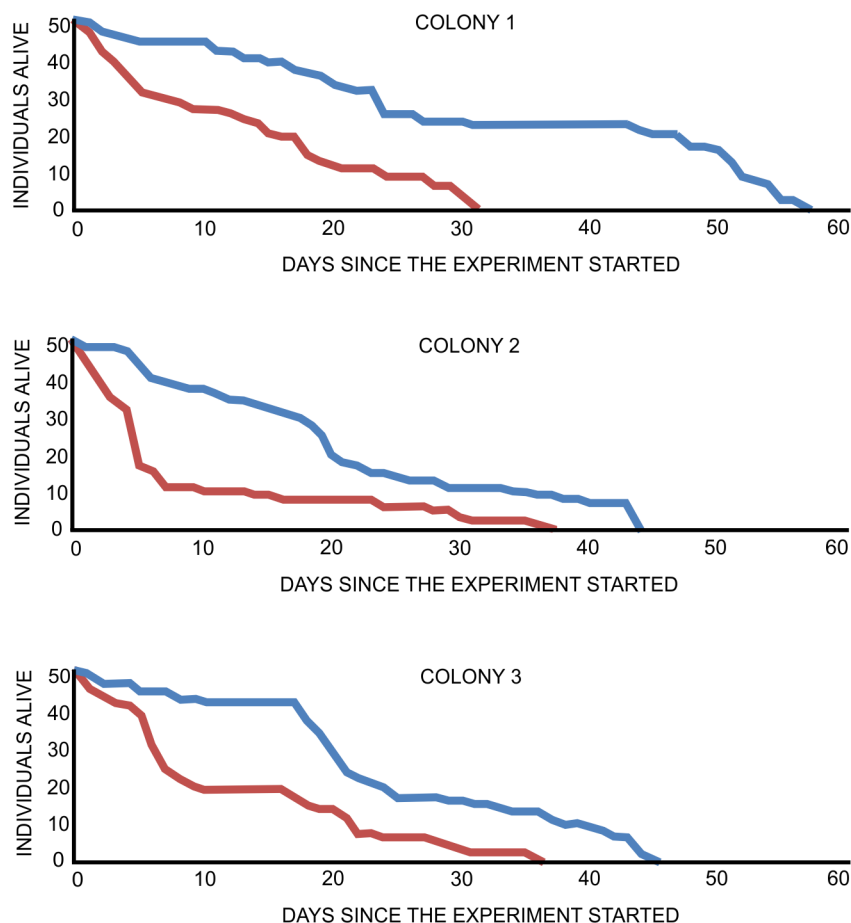
Fig 1. Mortality of *Formica cinerea* foragers in control and experimental treatments. Blue lines indicate untreated control groups. Red lines indicate groups with artificially shortened life expectancy induced by carbon dioxide exposure. Mortality data were analysed using GLMM. Significant differences were observed among groups ($F_{1,298}$ = 799.697, p < 0.0001).

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Typically, rescue behaviour was expressed in less than one minute. Also, rescue behaviour often occurred discontinuously, i.e., with repeated breaks and shifts among behavioural categories. Importantly, because tests started immediately after the introduction of potential rescuers and lasted for a fixed time period, latency and duration data were linked. Therefore, an additional GLMM analysis for duration adjusted by latency was performed, and the adjusted duration data, multiplied and rounded, was obtained from the rescue duration time divided by test time minus the latency to rescue. This analysis revealed that group was a significant factor (GLMM: $F_{1,82}$ = 60.131, p < 0.0001), meaning that lower rescue duration in an experimental group is not merely a reflection of less time being available for rescue due to higher latency. Further studies on rescue behaviour should account for this type of latency bias.

The present study showed that foragers characterized by a lower life expectancy elicited lower rescue levels than did those with a higher life expectancy. This result complements the study by Nowbahari and colleagues on the behavioural regulation of ant rescue by division of labour [7]. They showed that foragers dominated in both giving and receiving help, with nurses rescued for shorter durations and after higher latencies than foragers. This latter finding is unexpected, because nurses are generally characterized by higher life expectancies than foragers. From the perspectives of social isolation syndrome and the "division of labour by division
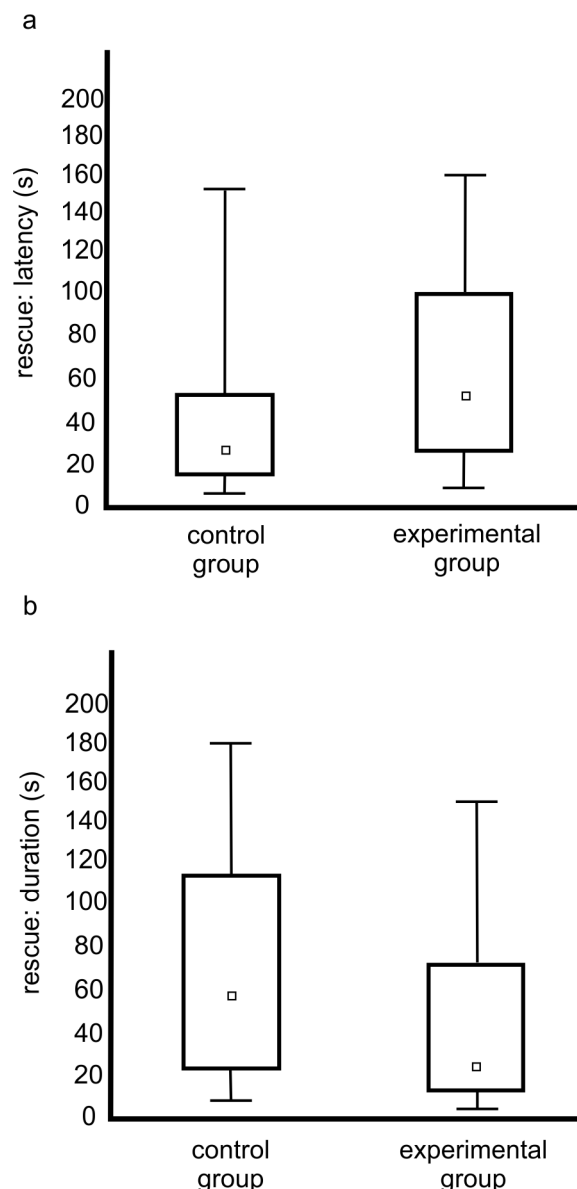
Fig 2. Between-group differences in rescue behaviour characteristics. The latency to rescue is presented in panel a) and the duration of rescue in panel b). Squares indicate the median, boxes indicate quartiles, and whiskers indicate the minimum and maximum values. Control groups were untreated, whereas experimental groups were exposed to carbon dioxide. Data were analysed using GLMM. Significant group differences were observed for both latency ($F_{1,82} = 195.672$, $p < 0.0001$) and duration ($F_{1,82} = 218.937$, $p < 0.0001$).

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of risk" hypothesis, nurses should receive higher levels of rescue than foragers: according to social isolation syndrome, nurses should be less isolated than foragers and therefore be subject to higher levels of interactions (in this case, rescue), whereas according to the "division of labour by division of risk" hypothesis, nurses are of higher value to the colony than are foragers and should therefore receive relatively higher levels of rescue effort. This contradiction between theory and experimental results may be related to worker physiology. The pheromone-based call for help by victims is considered of key importance for rescue elicitation in ants [3–7,18]. The physiological capability of workers to signal distress can be expected to change with
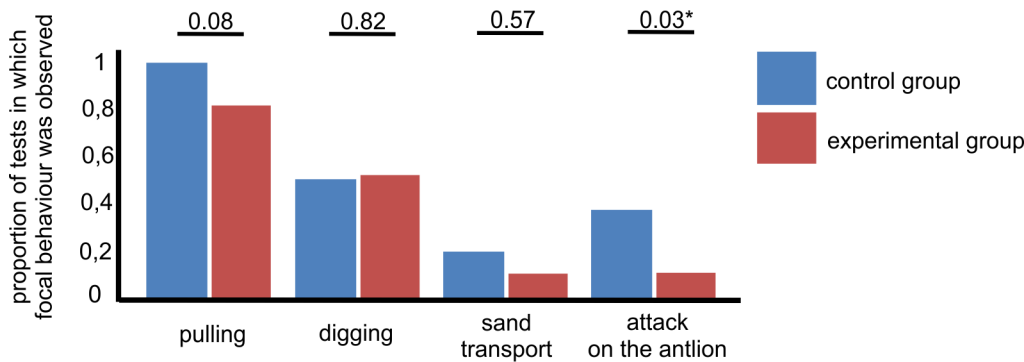
Fig 3. The proportion of tests in which rescue behavioural categories in control and experimental treatments were observed. Control groups were untreated, whereas experimental groups were exposed to carbon dioxide. Star indicates significance with p values for between-group differences indicated above the bars. The data were analysed separately for each behavioural category using Fisher's Exact Tests.

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maturation from low at the nurse stage to high at the forager stage. The ecological relevance of rescue is higher for foragers than for nurses [3,7]; therefore, foragers could be more physiologically equipped for giving and receiving rescue behaviour. Indeed, physiology and caste have been shown to be strongly interconnected in other aspects of behaviour [19].

It is plausible that the strong effect of group observed here is the result of the behaviour of the captured ants. Specifically, the between-group differences in the latency and duration of the rescue indicated that the effectiveness of initiating a rescue response declines as the life expectancy of the victim decreases. Obviously, this effect may increase colony fitness by minimizing high-cost/low-benefit behaviours. First, low life expectancy in nature indicates ill health; therefore, there is a risk of disease transmission, which may be mitigated by the social isolation of moribund ants [8]. The effect described here may be one component of a broader social isolation syndrome of dying ants. Here, it may be reflected in the reduced propensity of a dying ant to call for help when in a dangerous situation, resulting in mortality due to predation. There are likely other components of this syndrome in effect, such as food retention in the crop, that results in mortality due to the disrupted energy balance [20]. Second, moribund individuals seeking rescue may be a maladaptive behaviour from the perspective of the colony because of its cost: the effort required to save individuals with a low life expectancy is not worth the future value that they may provide [10–13]. In this case, a gradual loss of effectiveness in inducing rescue behaviour would benefit the colony because low-value individuals would not induce their nestmates to risk their own, more valuable lives. Although the present results support the predictions of both social isolation syndrome and the "division of labour by division of risk" hypothesis, additional research could provide further insight. For instance, progress may be made by studying the propensity of potential rescuers that differ in life expectancy to perform rescue behaviour.

Although this hypothesis requires further testing, ants with low life expectancy may altruistically withhold calls for help, presenting similarities to self-sacrificing [21] and waste-managing ants [22] described elsewhere. For example, in *Forelius* ants, which close their nest entrances each night from the outside, the individuals performing this task are probably near the end of their lives (i.e., have low life expectancy) and actively perform their last duty to their colony. Interestingly, the frequency of contact between these workers and their nestmates is reduced, simply because they are outside the nest, dying in solitude. Similarly, in the nests of leaf-cutting ants, only those individuals of low value to the colony and that are moribund participate in the dangerous task of waste management; their frequency of contact with nestmates is also low because waste-managing ants are isolated in specific nest compartments. Therefore,

the relationship between life expectancy and isolation appears consistent among several ant species and behaviours.

In sum, moribund foragers elicit lower levels of help from their nestmates than do foragers that are characterized by higher life expectancy. Thus, the patterns of rescue behaviour in ants are even more complex than previously thought. Additional studies on rescue behaviour expression should focus on the characteristics of distress calls, which are likely critically important in inducing help behaviours in not only ants, but also other organisms that exhibit similar behaviours.

Supporting Information

S1 File. Mortality data for the control and experimental ant groups.
(XLSX)

S2 File. Raw data gathered in rescue behaviour tests. They include information on whether a rescue occurred, and if so, the latency and the duration as well as the behavioural categories.
(XLSX)

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Author Contributions

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Chapter VI Secretions of mandibular glands are not involved in the elicitation of rescue behaviour in *Formica cinerea* ants

Insectes Sociaux

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Secretions of mandibular glands are not involved in the elicitation of rescue behaviour in *Formica cinerea* ants

K. Miler[1] · K. Kuszewska[1]

Abstract Certain ants perform rescue behaviour for other ants that require help, and the expression of rescue behaviour is hypothesized to depend on signals ("calls for help") sent by the imperilled individuals. We studied whether the mandibular glands were involved in the elicitation of rescue behaviour in *Formica cinerea* Mayr ants. In the first experiment, we determined the occurrence and characteristics of rescue behaviour directed towards nest mates with impaired mandibular gland communication. We did not observe any difference in rescue behaviour directed towards individuals who were untreated, treated with paint over the mandibles, or sham-treated with paint over the thorax. In the second experiment, we determined whether rescue behaviour would occur towards dummy ants coated with the contents of the mandibular glands. Compared with the control untreated nest mates, we found that rescue behaviour was not directed towards either the untreated dummy ants or the dummy ants covered with crushed mandibular glands. Our results indicated that the "call for help" signal does not originate from the mandibular glands. Therefore, we propose that gaster-tip glands represent a plausible alternative source of rescue-eliciting pheromone(s) for the *F. cinerea* ants examined in this study.

Keywords Mandibular gland · Pheromone signalling · Reduced communication · Rescue behaviour

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Chemical communication among nest mates plays a crucial role in the functioning of an ant colony. For example, the group predation of ponerine ants occurs via recruitment behaviour based on pheromones secreted by scouting individuals (Maschwitz and Schönegge 1977), and the queens of Pharaoh ants produce pheromones that enable their recognition by workers and special functions in the colony (Edwards and Chambers 1984). Pheromones in ants are likely involved in every aspect of their lives and ensure colony integrity (Jackson and Morgan 1993). One of the best examples of the complex social behaviour controlled by pheromones is provided by certain sand-dwelling ants, including the species in this study, *Formica cinerea* Mayr, showing rescue behaviour towards nest mates that require help (Czechowski et al. 2002). Indeed, the elicitation of rescue behaviour, specifically in *Formica*, is hypothesized to depend primarily on pheromonal signals ("calls for help") sent by the imperilled individuals (Czechowski et al. 2002). Thus, we studied whether mandibular glands were involved in the expression of rescue behaviour in *F. cinerea* ants. These glands are the most likely candidates for the source of rescue-eliciting pheromone(s) because of their involvement in the related functions, e.g., coordinating, alerting, and attracting (Attygalle and Morgan 1984; Ali and Morgan 1990). To demonstrate the potential importance of secretions from mandibular glands, we designed two experiments in which mandible-based pheromone communication was blocked between nest mates or the contents of the mandibular glands was used to provoke the expression of rescue behaviour. Our methods were similar to those used in previous studies (e.g., Hölldobler et al. 2013; Stuttard et al. 2016).

In the first experiment, the ants were tested in dyadic encounters of individuals from the same colonies. In each test, one ant required help, as it was entrapped on

the surface of the sand (namely, the entrapment bioassay, e.g., Nowbahari et al. 2009; 2012), whereas the nest mate was free. The entrapped ant was either untreated (control group), had a drop of paint applied over the mandibles (group with blocked pheromone communication via mandibular glands), or had a drop of paint applied over the thorax (sham-treated group). In each test, we noted whether the free ant performed rescue behaviour, the latency to the first episode of rescue, and the total duration of rescue. Digging around the entrapped nest mate, pulling at its limbs, transporting sand particles away from it, and biting the snare entrapping the nest mate were evaluated as the main subcategories of rescue behaviour. In the second experiment, the ants were tested in an analogous situation, but the 'trapped' ant was either untreated (first control group) or a dummy ant that was either untreated (second control group) or covered in the crushed contents of a mandibular gland (experimental group). The same type of data was collected in the second experiment, and the same subcategories of rescue behaviour were evaluated. We used a two-tailed Fisher's exact test (FET) to detect the between-group differences in the rate of occurrence of rescue behaviour and a Kruskal–Wallis ANOVA to detect the between-group differences in the latency and the duration of the behaviours (see the Supplementary Information for detailed descriptions of the materials and methods).

In the first experiment, we found that rescue behaviour occurred in 16 of 30 tests with the first untreated control group of ants, in 12 of 30 tests with the second mandible-treated group of ants, and in 11 of 30 tests with the third thorax-treated group of ants. Based on these results, the frequency of rescue behaviour occurrence among the groups was not significantly different (FET yielded nonsignificant results for each comparison). In addition, differences were not observed among these three groups in either the latency to the first episode of rescue (K–W ANOVA: $H = 0.123$, $p = 0.940$) or the total duration of rescue (K–W ANOVA: $H = 0.192$, $p = 0.908$). In the second experiment, we found that rescue behaviour occurred in 31 of 60 tests with the first untreated control group of ants, in none of the 60 tests with the third control group of dummy ants, and in 4 of 60 tests with the second gland-treated group of dummy ants. These results indicated that only the live ants in the first group elicited rescue behaviour (FET yielded a nonsignificant result for the comparison between the second and the third groups).

The data from the first experiment could be confounded by certain minute residual pheromone(s) on the body surface of trapped ants with blocked pheromone communication via the mandibular glands [i.e., these ants could discharge rescue-eliciting pheromone(s) originating from the mandibular glands before the experimental procedure, which would explain the subsequent rescue

behaviour]. However, the effects of pheromone residuals were unlikely, because in the second experiment, the contents of the mandibular glands did not elicit rescue behaviour towards the dummy ants. All four attempted rescues of the gland-treated group of dummy ants were weak and could have resulted from other substances transferred onto them during experimental procedures (Bagnères et al. 1991). Thus, our results indicated that the mandibular glands are not involved in the elicitation of rescue behaviour in *F. cinerea* ants.

The previous reports have indicated that the mandibular gland secretions of ants function at a minimum to attract conspecifics (Cammaerts et al. 1981; Howard et al. 1982) and release both alarm and digging behaviours, which are responses involved in rescue operations (Wilson 1958; McGurk et al. 1966). However, these studies involved ants from genera that were not used in the present study (*Formica*), including *Pogonomyrmex, Wasmannia*, and *Myrmica*. In addition, the mandibular glands in *Formica* workers contain low quantities of volatile materials (Bagnères et al. 1991). Therefore, other glands in *Formica* are most likely involved in rescue elicitation, such as Dufour glands, which function in communication (Löfqvist 1976; Attygalle and Morgan 1984). An alternative or complementary explanation could be that the production of CO_2 by nest mates that require help attracts other ants and releases the basic forms of rescue behaviour, alarm, and digging behaviours, as observed in *Solenopsis* ants (Hangartner 1969). Notably, stridulation may be an alternative mode of communicating for help. Indeed, stridulation is hypothesized to have evolved among the ants to alert nest mates that rescue is required, although this hypothesis has been largely rejected (Golden and Hill 2016). Moreover, stridulation as a call for help is not relevant in *Formica*, because stridulatory organs are absent in this genus (Czechowski et al. 2002). Thus, the "call for help" in our study species of *Formica* could not have involved vibroacoustic signals.

The current studies on the selected ecological and evolutionary aspects of rescue behaviour are strongly dependent on the hypothesis that individuals who require help emit "call for help" signals; however, this behaviour remains largely unknown (e.g., Nowbahari et al. 2009; Miler 2016), and uncovering the mechanism of rescue behaviours in ants is an essential component of further research. Although mandibular gland secretions did not elicit rescue behaviours in this study, they can possess such a function in other sand-dwelling ants which display rescue behaviours (Hollis and Nowbahari 2013). In *F. cinerea* ants as well as other species of this genus that display rescue behaviours, however, "gaster-tip" gland secretions should be investigated in further studies on the glandular origins of the rescue-eliciting pheromone(s).

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Chapter VII Author contributions

We hereby declare that the contribution of authors is as follows:

In Chapter III: K. Miler (70%), K. Kuszewska (20%), M. Woyciechowski (10%).

…………………………….. ……………………………..

K. Miler   K. Kuszewska

……………………………..

M. Woyciechowski

In Chapter IV: K. Miler (60%), K. Kuszewska (20%), G. Zuber (10%), M. Woyciechowski (10%).

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K. Miler   K. Kuszewska

…………………………….. ……………………………..

G. Zuber   M. Woyciechowski

In Chapter V: K. Miler (100%).

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K. Miler

In Chapter VI: K. Miler (60%), K. Kuszewska (40%).

…………………………….. ……………………………..

K. Miler   K. Kuszewska

Chapter VIII General discussion

Antlion-ant interaction system is postulated to be a good example of a coevolutionary arms race (Hollis et al. 2015, Hollis 2016). Indeed, antlions have been shown to display multiple tactics of insuring efficient ant capture, such as early detection of approaching prey (Devetak 1985, Fertin & Casas 2007, Mencinger-Vračko & Devetak 2008) and learning to anticipate prey arrival (Guillette et al. 2009, Hollis et al. 2009, Kuszewska et al. 2016), whereas ants, in addition to showing rescue, were reported to avoid places where antlions aggregate and build many traps (Gotelli 1996, Morrison 2004, Beponis et al. 2014, Hollis et al. 2017). The overreaching goal in this dissertation was to use the antlion-ant system as a model of the predator-prey interaction to address the issues of capture success in the predator and capture avoidance in the prey.

Coevolution between predator and prey is one of the most recognized examples of antagonistic relationships, often taking form of the so-called arms race between involved parties. Spectacular examples of such arms races are known. Snake predators of the genus *Thamnophis* evolved resistance to extremely deadly tetrodotoxin used as defence by prey, amphibians of the genus *Taricha* (Brodie & Brodie 1991). Moth prey of the genus *Bertholdia* broadened its defensive repertoire in the course of evolution by developing ultrasonic clicks that jam sonars used by bat predators of the genus *Eptesicus* (Corcoran et al. 2009). Both of these examples, along with many others from available literature, involve hard-wired responses of either the predator (toxin resistance) or the prey (sonar jamming). In turn, present dissertation is about contributing to our state of knowledge about potential coevolution of antlions and ants, with focus on the importance of learning abilities in the predator and secondary means of capture avoidance in the prey, both of which are highly plastic features, mostly overlooked in studies about predator-prey interactions (Hollis et al. 2015, Hollis 2016).

Trap-building antlions spend majority of their life as larvae inhabiting sands (Scharf et al. 2011). They have drawn scientific interest for a long time (Turner 1915, Druce 1923) and our knowledge about their biology is developing (Scharf & Ovadia 2006, Scharf et al. 2011).

Antlion larvae and trap-building predators in general were for a long time viewed as behaviourally fixed (Huey & Pianka 1981, Johnston 1982). Recently, new research on antlion larvae made it obvious that they possess plastic behavioural repertoire (Fertin & Casas 2007, Mencinger-Vračko & Devetak 2008), which includes learning (Guillette et al. 2009, Hollis et al. 2009, Kuszewska et al. 2016). Research presented in this dissertation demonstrated that some larvae learn better than other larvae, and these differences are connected to the pronunciation of larval brain asymmetry, providing novel evidence of the relationship between brain asymmetry and cognitive functions (Miler et al. 2017; Chapter III of this dissertation). Increased brain asymmetry results in increased behavioural asymmetry (sidedness in behaviour) (Levy 1977, Vallortigara & Rogers 2005), which may actually be used as an indicator of brain asymmetry. An indicator of increased brain asymmetry in antlions, the increased side-bias in righting, shows that only about 20% of *M. bore* larvae have strongly asymmetrical brains (Miler et al. 2017; Chapter III of this dissertation). This low number was further hypothesized to stem from the fact that increased brain asymmetry is costly. In another study, the idea that strong behavioural asymmetry bears costs on larval foraging behaviour was tested (Miler et al. 2018; Chapter IV of this dissertation). This further research is significant in terms of optimality of investment into different life strategies ("smart side-biased poorer foragers vs dumb unbiased better foragers"), and develops further our knowledge about insect asymmetries, which is poor especially in the context of cognitive skills (Pascual et al. 2004). Naturally, there still are interesting gaps in knowledge that need to be filled with further research. For instance, the stability of learned skills in antlions is of high importance: their larval life can last even three years during which they moult two times (Hollis et al. 2015, Hollis 2016). If the environment is predictable, then it is beneficial to learn environmental cues and remember them. It would be interesting to expose antlions to predictable environment (consistent cue-prey association) vs unpredictable environment (no cue-prey association) for

prolonged time (long enough for larvae to moult) to check whether cues learned at one larval stage are remembered in another larval stage. Memory persistence through moulting was never tested in antlions but other insects have been shown to display similar effects (for example, moths remember what they learned at caterpillar stage, see Blackiston et al. 2008). Also, research demonstrating that behavioural asymmetry is connected to hunting efficiency (Miler et al. 2018; Chapter IV of this dissertation) has strong implications in terms of vibration sensitivity. As it turns out, larvae trained to associate the vibrational cue with the arrival of pray show lower response levels to cues differing from those which have been learned, i.e. delivered further away from the larva. There are two alternative explanations for this phenomenon: the first is that larvae with higher asymmetry have lower vibration detection skills and therefore react only to those close to them, and the second is that larvae with higher asymmetry have higher vibration detection skills and therefore react only to those that resemble the learned cues the most. It would be very interesting to experimentally determine which of these explanations is true.

In the above part of the General discussion the focus was on the antlion side of the story, but there is also another side, the side of ants. Sand-dwelling ants co-occurring with antlions were reported to avoid places where they build many traps (Gotelli 1996, Morrison 2004, Beponis et al. 2014), which can be considered as main means of avoiding antlion predation. However, in 2002, Czechowski and his co-authors described the so-called rescue behaviour of some species of *Formica* ants in the context of antlion larva capture (Czechowski et al. 2002), i.e. that ants may rush into the trap to free the captured individual. Since then, few papers have been published regarding this phenomenon, all of them making use of the laboratory artificial entrapment bioassay which is thought to accurately reflect the antlion larva capture situation (Hollis et al. 2015, Hollis 2016). These studies (Nowbahari et al. 2009, 2012, 2016, Hollis & Nowbahari 2013a, Duhoo et al. 2017), carried out on *Cataglyphis cursor* ants, demonstrated

that rescue in ants is extremely fine-tuned behaviour. First, ants direct their help only towards relatives, which has obvious fitness benefits (Nowbahari et al. 2009). Second, rescue can be very precise, i.e. directed exactly towards the thing that holds the imperilled individual in place (Nowbahari et al. 2009). Third, foragers receive the most help and also give the most help, which is understandable when looking at this issue through the ecological lens: only foragers are exposed to the antlion larva threat as ants tending to other tasks stay inside the safe nest (Nowbahari et al. 2012). Fourth, rescue behaviour evolution occurred in sand-dwelling ants, but only in these species that were and still are under the antlion larva threat (Hollis & Nowbahari 2013). Fifth, surprisingly, new-born ants receive high levels of rescue, an effect probably related to their high value for the colony and the fact that due to their limited mobility they may be susceptible to some dangerous situations inside the nest (e.g. collapsing chambers) (Nowbahari et al. 2016). Sixth, the sequence of different behavioural categories in any rescue action is organized and designed towards better efficiency (Duhoo et al. 2017). All these conclusions are highly significant and open new research avenues. In research presented here, carried out on foragers of *Formica cinerea* ants (one of species originally described by Czechowski and co-authors, see Czechowski et al. 2002), it was investigated whether rescue behaviour aligns with colony-level fitness interests (Miler 2016; Chapter V of this dissertation). Natural selection in social insects such as ants acts not only on the individual-level, but also on the colony-level. Thus, rescue should be preferentially directed towards foragers of high value, i.e., individuals that will still work for the colony after rescue (foragers with relatively high life expectancy). There are two nonexclusive reasons for that to happen. First is the social isolation of moribund ants, which withdraw from social interactions (Heinze & Walter 2010, Bos et al. 2011). Second is the disproportionally high cost of rescuing moribund individuals compared to the benefits that result from it (Porter & Jorgensen 1981, Tofilski 2002, 2009). In both cases, moribund individuals should elicit lower levels of rescue in their nestmates (compared to

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normal individuals). Apparently it is so in *F. cinerea* (Miler 2016; Chapter V of this dissertation). Importantly, this particular study was conducted using the antlion larva capture bioassay to test rescue occurrence, and not the laboratory simulation of it (artificial entrapment bioassay).

Little research has been done regarding the mechanism of rescue behaviour in ants. The main hypothesis states that when an ant stumbles into the pit of the antlion larva and gets captured, it sends a signal ("call for help") which summons its nestmates to the site of capture and triggers rescue behaviour (Czechowski et al. 2002). If rescue is indeed elicited in this way, then in workers of *F. cinerea* the "call for help" is likely chemical (Miler & Kuszewska 2017; Chapter VI of this dissertation). In case of *F. cinerea*, it would be advantageous to determine whether gaster-tip glands (specifically, Dufour glands) are these from which "call for help" originates, which would be the first step towards identifying the compound(s) responsible for rescue occurrence and synthetizing them (Frank et al. 2017, 2018). Dufour glands are likely candidates for being the source of "call for help" signal in this species (Bagneres et al. 1991). Another work which needs to be done is to experimentally test the hypothesis that the ability to "call for help" deteriorates with decreasing life expectancy (Miler 2016; Chapter V of this dissertation). This will only be possible after the nature and the source of rescue-eliciting signal in *F. cinerea* is determined.

To sum up, present dissertation consists of four main chapters, i.e. published papers, about the interaction of antlions and ants. In these papers, it is demonstrated that 1) some antlion larvae may learn that vibrational signals predict prey occurrence and that they learn this association quicker when they display high behavioural asymmetry (Miler et al. 2017; Chapter III of this dissertation), 2) naïve antlion larvae displaying high behavioural asymmetry show lower hunting efficiency, which may explain why only low numbers of larvae are highly behaviourally asymmetric (Miler et al. 2018; Chapter IV of this dissertation), 3) ants may rescue

each other from antlion predation, but they show lower proneness towards rescue when the imperilled individuals are moribund (Miler 2016; Chapter V of this dissertation), and 4) the "call for help" signal, responsible for rescue elicitation, probably does not originate from the mandibular glands in case of the sand-dwelling *F. cinerea* ants, which co-occur with antlions. Further research is needed to deepen and expand these conclusions.

IX Acknowledgements

X References

(only Introduction and General discussion)

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