DETECTION OF THE SPIDER PREDATOR, HOLOLENA NEDRA BY NAÏVE JUVENILE FIELD CRICKETS (GRYLLUS INTEGER) USING INDIRECT CUES

by

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Summary

In many species, prey detect predators using chemical cues, which may be based on the predator's previous diet. Furthermore, the predator's previous hunting strategy and diet may affect the prey's behavioural strategies to avoid predation. Juvenile Gryllid field crickets face predation by spiders, but the responses of juvenile crickets to spiders are unknown. We studied whether naïve juvenile field crickets can detect a predatory spider, *Hololena nedra*, using chemotactile cues (silk, secretions and excreta). We also studied the effect of the predator's diet on the juvenile field cricket's anti-predator behaviour. Cricket nymphs avoided the chemotactile spider cues when the spiders were previously fed with crickets, but not when spiders were not fed crickets. Our study suggests that the ability to detect the indirect cues of a potential predator may be an important factor determining the anti-predator responses of insects towards spiders.

Keywords: predator avoidance, *Gryllus integer*, spiders, chemotactile detection.

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Introduction

Chemical cues play an important role in predator-prey interactions, since animals in many species are able to recognise chemical information that is produced by a predator (*e.g.* reviews by Kats & Dill, 1998; Dicke & Grostal, 2001). Anti-predator behaviour of prey based on chemicals occurs in many aquatic (Kats & Dill, 1998) and terrestrial predator-prey systems (*e.g.* Chivers & Smith, 1998; Kats & Dill, 1998; Grostal & Dicke, 1999; Dicke & Grostal, 2001; Persons *et al.*, 2001; Barnes *et al.*, 2002; Stapley, 2003).

In terrestrial systems, predation by spiders is an important component of predation on insects, since both juvenile and adult insects form the main diet for numerous spider species (Foelix, 1996). Field crickets are vulnerable to predation, since they do not have any venomous weapons or poisonous body contents for defense. Juvenile field crickets are particularly at risk of predation because they cannot fly to escape terrestrial attacks by predators. Additionally, because of their small size, they are less able to defend themselves by kicking or biting than are adult crickets. Therefore, many animals include juvenile field crickets in their diet, including lizards, toads, mice, birds and spiders (Walker & Masaki, 1989). In the rangeland grasshopper (*Ageneotettix deorum*), another Orthopteran insect, small juvenile size is generally associated with high susceptibility to aggressive arthropod predators such as spiders (Danner & Joern, 2003). Thus, predation by spiders could be an important source of mortality for juvenile field crickets in the wild.

For young crickets, effective anti-predator responses against spider predation on the ground might include decreased mobility and avoidance of potentially dangerous sites, such as spider webs (see *e.g.* Danner & Joern, 2003). The funnel-web spider *Hololena nedra* builds a flat web with an attached funnel, often on the ground or in low vegetation where crickets normally move. Its web is relatively non-sticky, so that small crickets can escape from the web if they merely contact the edge and are not captured by the spider itself (Kortet & Hedrick, unpubl. obs.). Like many spiders, funnel-web spiders react mainly to vibrations caused by movement of the prey (Foelix, 1996) and do not easily perceive non-moving objects (Rovner, 1996). Spiders also use visual cues to locate prey animals, which may make mobile prey an easier target within the sensory range of the predator (Persons & Uetz, 1996, 1999).

Gryllid crickets can detect and even memorize certain chemical cues that are associated with their water and food (Matsumoto & Mizunami, 2000, 2002), but to our knowledge, no previous studies have examined whether these crickets can recognise chemotactile cues (silk, excreta, and secretions) from predatory spiders. However, such an ability would be highly important to juvenile crickets in the wild, where fitness is contingent upon survival to adulthood. The aim of the study was to discover whether naïve juvenile field crickets, *Gryllus integer,* are able to detect chemotactile cues (silk, excreta, and secretions) from spider predators. We conducted our study using the spider, *Hololena nedra.* These spiders are abundant in our study area, co-occur with the crickets in grassland and suburban habitats and have been observed preying upon juvenile field crickets (Kortet & Hedrick, unpubl. obs.). Since in many cases, the predator's previous diet has been shown to affect the prey's anti-predator response (Mathis & Smith, 1993; Chivers & Smith, 1998; Kats & Dill, 1998; Persons *et al.*, 2001), we also studied whether feeding juvenile *G. integer* to the spiders would affect the response of the juvenile crickets to spider cues. In addition, we weighed the crickets to see whether there was any relationship between the size of the cricket and its levels of behavioural activity, since this might affect our results.

Methods

Study animals

The naïve crickets used in this experiment ($N = 32$) were the first laboratory generation derived from wild-collected mothers from Davis, California (Yolo County, dry grasslands and suburban lawns). They were maintained at 27 ± 1°C and on a 12:12 h light/dark photoperiod with *ad libitum* food (commercial dry chicken food) and water. Experimental crickets were removed from family boxes (covered plastic containers), where they had hatched, and were separated from other members of their families at approximately the second instar. They were then kept in separate cages (small cardboard cups) in isolation for testing. We used no more than two offspring per family. After the experiments, we weighed the fresh body mass of the crickets to the nearest 0.001 g. No cricket was used in more than one experiment. The crickets we used ($N = 16$ in each experiment, mean weight $\pm$ SE $= 0.064 \pm 0.006$ g) were nymphs in their second or third instar, so their sexes could not be identified (female ovipositors were not yet visible).

The spiders we used ($N = 16$) were collected from similar habitats as the crickets (dry grasslands and suburban lawns) in Davis, California. All of the spiders were kept in cardboard cups, at the same temperature and light conditions as the crickets. Spiders were provided with *ad libitum* water, but not food, before collecting chemotactile cues for the 'non-fed-spider' treatment. These cues were collected not more than two days after the spider was caught from the wild. Each spider was used in one experiment before, and one experiment after digesting a juvenile field cricket. All of the spiders in the 'fed-with crickets' treatment were provided a juvenile cricket in their container, and silk, excreta and secretions were collected two days after the juvenile cricket was killed and at least partly consumed by the spider, by placing the spider on clean filter paper in a fresh container. No cricket remains were transferred to this clean filter paper. Thus, any cues were produced only by the spider. After collection of the silk, excreta and secretions, we weighed the fresh body mass of the spiders to the nearest 0.001 g ($N = 16$, mean weight ± SE = 0.066 ± 0.009 g). The size of the spiders was about the same as that of the experimental crickets (see above). In the wild, the spiders often seize prey equal or larger to themselves in weight (Foelix, 1996).

Collection of chemotactile cues and anti-predator tests

To collect chemotactile cues from the spiders, we placed each spider in a small (diam 37 mm) petri dish containing a filter paper disc (diam 36 mm) for 24 h (method modified from sex pheromone collection by Rantala *et al.*, 2002). To test the cricket nymph's response to chemotactile cues from a spider, we presented each nymph with two pieces of filter paper: the filter paper disk containing silk, excreta and secretions from the spider, and a control filter paper disk that had not been contacted by a spider. All filter paper disks (both treatment and control) had been cut from larger pieces of clean paper of exactly the same origin, and were handled with clean tweezers only. Treatment disks were all used within 24 hours after the removal of the spider from the disk, to prevent the possible effect of cue age on the cricket's anti-predator response (see Barnes *et al.*, 2002). The filter paper was kept inside the petri dish prior to testing to prevent possible dispersal of the chemotactile cues left by the predator.

The arena for predator avoidance trials consisted of a 15 cm diameter plastic box (L15 × W15 cm, depth 10 cm). After the 4 minutes of acclimation time that preceded the trial, a juvenile cricket was released under dim red-light illumination (25-watt red incandescent bulb, 60 cm distance) in the middle of the arena, to mimic nocturnal conditions. Each trial lasted for 8 minutes, during which time the cricket's movements were observed and recorded with the aid of a program for collecting behavioural data (AV Bio-Statistics 4.4, available at http://www.cc.jyu.fi/~ansvain/avbs/). The response of the cricket to the predator's cues was measured as the total time that the cricket spent on each filter disc and the number of times it visited each filter disc during the experiment (*cf.* Rantala *et al.*, 2002). These measures give direct estimates of the mobility of the juvenile cricket and its possible detection of spider silk, secretions and/or excreta.

Results

Experimental crickets visited the 'non-fed' spider paper a median of 1.5 times and spent 4.14 seconds there, while they visited the control filter paper a median of 2.0 times and spent 8.82 seconds there. Wilcoxon Signed-Ranks Tests revealed that this difference between the control treatment and the 'non-fed spider' treatment was not significant for either the time spent on the filter paper ($z = -1.086$, $N = 16$, $p = 0.278$) or the number of times the cricket visited the filter paper ($z = -1.425$, $N = 16$, $p = 0.154$).

Our results from fed spiders revealed a somewhat different pattern. The crickets visited the 'fed spider' filter paper a median of 1.0 times and spent

2.56 seconds there, while the respective numbers for the control paper were 4.0 times and 29.03 seconds. Here, Wilcoxon Signed-Rank Tests revealed that in the ‘fed-spider’ treatment groups, the juvenile crickets spent significantly less time on the ‘fed spider’ filter paper than the control filter paper ($z = -2.792$, $N = 16$, $p = 0.005$) and visited the ‘fed spider’ filter paper less often than the control filter paper ($z = -3.321$, $N = 16$, $p = 0.001$).

The body mass of the cricket apparently did not affect its mobility, since there was no significant correlation between cricket weight and time spent on either the treatment filter paper ($r = 0.083$, $N = 32$, $p = 0.652$) or on the control filter paper ($r = 0.144$, $N = 32$, $p = 0.433$). Moreover, there was no correlation between the body mass of the spider and the time crickets spent on its filter paper in either the ‘non-fed spider’ treatment (Pearson $r = 0.252$, $N = 16$, $p = 0.357$) or in the ‘fed-spider’ treatment (Pearson $r = 0.198$, $N = 16$, $p = 0.462$).

Discussion

Juvenile field crickets (*G. integer*) in our study were able to detect chemotactile cues left by the spider predators, but this effect was statistically significant only when these predators had previously ingested *G. integer*. Since the juvenile crickets had been reared in isolated boxes in the laboratory and had no previous experience with spiders, our results suggest that the ability of these crickets to recognise the chemotactile cues is innate. Moreover, our results suggest that it is possible that damaged field crickets may release substances that are then excreted by their predators, and that in turn alert other crickets to the increased risk of predation in the environment. However, this suggestion needs to be confirmed in further experimental studies.

Our results resemble those from another spider species, *Pardosa milvina*, which recognizes the chemotactile cues of the larger predatory spider *Hogna helluo* only when *Hogna* has been ingesting *Pardosa* (Persons *et al.*, 2001). We collected chemotactile cues over a 24-hour period. However, it is possible that confining the spider for a longer time on the filter paper would have produced a significant avoidance response in the ‘non-fed’ spider treatment also. Notably, results from our two experiments tended to be similar, with crickets spending more time on the control paper than the treatment paper in both experiments, even though the results from the ‘non-fed’ spiders were not statistically significant. A larger sample size may have revealed a statistically significant result. Moreover, it is not necessarily valid to conclude that the crickets detected the spider cues only in the 'fed' case, without a direct test between 'non-fed-spider' and 'fed-spider' groups. Finally, it is possible that some of the field-collected spiders in the 'non-fed' spider treatment had ingested crickets some time before we used them in the experiment. Even so, we obtained a significant result between the time spent on the treatment filter paper and control filter paper only after we fed spiders with crickets in the laboratory following their capture. This is a novel result.

In this study we included excreta and silk in our definition of chemotactile cues, an approach that has been used previously in similar studies (for example, Persons & Rypstra, 2000; Barnes *et al.*, 2002). This is because physical factors from silk and/or excreta may play a role in the avoidance behaviour of the cricket, in addition to chemical cues. Therefore, our results do not necessarily demonstrate that chemical cues alone are responsible for the crickets' response.

Avoidance of dangerous areas and decreased mobility should be effective methods for eluding spider predators, since many spiders detect their prey primarily through the prey's movements (Foelix, 1996; Persons & Uetz, 1999; Danner & Joern, 2003). At our study sites, both adult and juvenile crickets often reside in grassy areas near *Hololena* webs. By avoiding dangerous areas and moving less when these areas are encountered, young crickets will be less conspicuous to their predators, although their reduced mobility could incur fitness costs if it results in lower feeding rates (Danner & Joern, 2003). Avoidance of high predation-risk areas based on chemical cues has been described previously in the herbivorous spider mite, *Tetranychus urticae* (Grostal & Dicke, 1999).

In a spider predator-prey system, predator size affects the responses of prey (Persons & Rypstra, 2001), but we did not find any such effect of spider size on the activity of juvenile crickets. This might be because variation in spider size was relatively small in our experiments. In the wild, spiders hunt prey much larger than themselves (Foelix, 1996). Moreover, although the size of juvenile grasshoppers affects their anti-predator responses against lycosid spiders (Danner & Joern, 2003), we did not find any correlation between the size of juvenile crickets and their activity times. Again, this is probably because of the low variation in the size of crickets in our study.

Our experimental set-up did not allow us to directly compare the amount of time the crickets spent on the filter papers between treatments, but our data suggest that the juvenile crickets visited the control paper more often and spent more time on the control paper in the 'fed-spider' treatment than in the 'non-fed' spider treatment. This may indicate that after the detection of a dangerous cue, juvenile crickets become more active in the area they perceive as 'safe'. However, this possibility requires further investigation. Moreover, we do not know whether using a non-predatory arthropod as a second control in our experiments would alter our current results, since the crickets may avoid cues from these arthropods also.

Gryllid crickets are able to memorize certain chemical cues that are associated with the water provided in captive situations (Matsumoto & Mizunami, 2000, 2002). Therefore, it is likely that they are able to memorize the chemical cues of their potential predators as well. Such patterns have been previously described, for example, in a fish species (Mathis & Smith, 1993). If the crickets are able to memorize the chemical cues left by their predators, their anti-predator responses could be affected by their previous experiences with predators and by variation in predation risk (Lima & Bednekoff, 1999).

To conclude, chemotactile cues left by spiders affect the behaviour of juvenile *G. integer*. This suggests that indirect cues of spider predators are important in the anti-predator decisions of field crickets, and perhaps affect other insects as well. Therefore, these kinds of chemotactile cues could have important effects on predator-prey interactions, including co-evolution between *Gryllus integer* and *Hololena nedra.*

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