

## Crickets groom to avoid lethal parasitoids

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Hosts that are infected by parasitoids pay the ultimate cost: death. Here we investigate whether hosts can combat the lethal effects of parasitoids by enhancing their grooming behaviour and removing the parasitoids before they have a chance to enter the host's body. Many field cricket species are stalked and parasitized by gravid females of the parasitoid fly *Ormia ochracea*. Female parasitoids lay live first-instar larvae (planidia) on and around the cricket. Planidia burrow into the cricket host where they feed and grow. Because parasitism invariably results in host death, there should be strong selection for crickets to avoid being parasitized. We investigated whether field-caught Texas field crickets, *Gryllus texensis*, groom to avoid parasitism by *O. ochracea*. We quantified grooming behaviour of crickets when they were in the same area as *O. ochracea* adults or larvae (proximity avoidance), and following contact between the cricket and *O. ochracea* adults or larvae (contact avoidance). Crickets did not adjust grooming behaviour when in close proximity to adult gravid female *O. ochracea*, nor did they avoid planidia-laden grass. Crickets did, however, substantially increase their grooming activity when placed in an arena filled with planidia-laden grass. Furthermore, crickets that groomed more were much less likely to succumb to parasitoid infestation, compared to crickets that groomed less. Our findings suggest that grooming in *G. texensis* may function as a strong defence against parasitism by *O. ochracea*.

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Parasites can be extremely costly. They can depress insect development and maturation rates (Smith 1988), and reduce mating success, fecundity and survival (Lanciani 1975; Forbes 1991; Forbes & Baker 1991). To date, much attention has been focused on the conflict between parasites and their hosts. Research has revealed that host behaviour can influence the degree to which parasites reduce host fitness (Minchella et al. 1985; Keymer & Read 1991; Poulin 1994). For example, some hosts can avoid locations that are rich with ectoparasites (Hart 1990). Hosts can also groom to reduce the number of ectoparasites that successfully attach to them (Forbes & Baker 1990, 1991; Mooring & Hart 1995; Baker & Smith 1997; Leonard et al. 1999; Leung et al. 1999; Eckstein & Hart 2000; Mooring et al. 2000). Grooming behaviour is widely thought to function predominantly as a mechanism for ectoparasite removal in wild animals (reviewed in Hart 1990). However, aside from studies on dragonflies and honeybees (Forbes & Baker 1990, 1991; Buchler et al. 1993; Pettis & Pankiw 1998), grooming in insects has received little

attention. Furthermore, to our knowledge, grooming to avoid death by a parasitoid has never been investigated for an insect.

Here we investigate whether crickets groom to avoid certain death by an acoustically orienting parasitoid fly, *Ormia ochracea* (Diptera: Tachinidae). *Ormia ochracea* acoustically stalks and parasitizes several field cricket species throughout the southern United States and Hawaiian Islands (Cade 1975; Walker 1986; Walker & Wineriter 1991; Zuk et al. 1993; Wagner 1996; Hedrick & Kortet 2006). Female *O. ochracea* locate their cricket host by homing in on the acoustic mate attraction calls of male crickets (Sabrosky 1953; Robert et al. 1993; Mason et al. 2001). Gravid female parasitoids are larviparous, depositing their planidia (first-instar *Ormia* larvae) on and around the host cricket (Cade 1975). Planidia laid around the host cricket wave their anterior ends in the air in an attempt to make contact with a host. Once contact is established, the planidia burrow into the host's body where they feed on the cricket's fat body, and its abdominal and thoracic muscles (Adamo et al. 1995b). After 7–10 days, the parasitoid larvae purge their guts in the host, create an exit hole through the host's cuticle, and then emerge from the host (Adamo et al. 1995b). Given that parasitism invariably results in the host cricket's death (Adamo et al. 1995a; Kolluru et al. 2002; C. M. Vincent, unpublished data), crickets should be strongly selected to avoid the lethal effects of parasitism.

There are several ways in which crickets might be able to avoid or reduce the lethal costs of parasitism. First, crickets could adjust

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their acoustic signalling behaviour to reduce the probability of acoustic location by the fly. This has been found in the Hawaiian species of *Teleogryllus oceanicus*, where populations that are exposed to parasitoids alter their diel calling patterns (Zuk et al. 1993). Second, crickets could also eliminate the threat of parasitism by not producing acoustic signals. Noncalling male crickets are common in several cricket species exposed to parasitoids (Cade 1975; Zuk et al. 2006). Third, parasitized crickets could initiate an encapsulation response to kill the planidia before they have a chance to establish themselves (Vinson 1990). However, encapsulation responses are often co-opted by the parasitoid larvae and used to form a breathing tube (Vinson 1990). Fourth, parasitized crickets could also compensate for future reproductive losses by increasing their current reproductive effort (Minchella & Loverde 1981). Reproductive compensation has not been observed in this system even though it has been heavily investigated (Adamo et al. 1995a; Zuk et al. 1995; Adamo 1999; Kolluru et al. 2002; Orozco & Bertram 2004).

The fifth possible way in which crickets could avoid the costs of parasitism is for them to remove the parasitoids before they have a chance to enter their body. Adamo et al. (1995b) witnessed a gravid female *O. ochracea* briefly come into contact with a *G. texensis* male. Immediately following contact with the fly, the cricket began to groom intensely and was later found to be parasitoid free (Adamo et al. 1995b). We therefore investigated whether crickets increase their grooming behaviour in the presence of gravid female *O. ochracea* flies or when placed in planidia-laden grass. We also determined whether crickets avoided parasites when given the choice between planidia-laden and planidia-free grass. Furthermore, we examined whether the probability of becoming parasitized was correlated with the amount that an individual groomed. Our study provides the first thorough examination of whether *G. texensis* can recognize parasitoid threats and adjust their behaviour accordingly. Our study also provides the first evidence that grooming is an effective means of reducing the likelihood of parasitism in an insect.

## METHODS

Adult crickets and flies were collected nightly during 15–24 September 2008 in Buescher State Park, Bastrop County, Texas, U.S.A. Insects were brought to the Stengl 'Lost Pines' Biology Field Station, University of Texas. Male crickets appear to be most heavily parasitized by *O. ochracea* in the wild. However, we used both male and female crickets, as several authors have observed parasitized females in the wild (Walker & Wineriter 1991; Zuk et al. 1993; Adamo et al. 1995b). Furthermore, Walker & Wineriter (1991) observed equal parasitism rates between males and females, and our observations on *G. texensis* suggest that all crickets in the vicinity of a calling male are in danger of being parasitized (Vincent & Bertram, in press a). Crickets were separated by sex and housed colonially with ad libitum food (Teklad Rodent Diet 8604, Harlan Laboratories Inc., Indianapolis, IN, U.S.A.) and water.

Flies were trapped using an acoustic sound trap modelled after Walker's (1989) slit trap design. A Durabrand CD-566 compact disc player (Lennox Electronics Corporation, 35 Brunswick Avenue, Edison, NJ, U.S.A.) placed underneath the trap played the recorded call of a *G. texensis* male. The call used was recorded from a laboratory-reared male and had a frequency of 4.6 kHz. The call was broadcast through an Amplified Speaker AMX 18 (RadioShack Corporation, Fort Worth, TX, U.S.A.) at an intensity of 61 dB from 30 cm (Extech Instruments Corporation, Waltham, MA, U.S.A.). Gravid female *O. ochracea* were attracted to the broadcast and entered the trap via the slit, becoming ensnared. Captured flies were housed in a terrarium and provided with ad libitum hummingbird feed.

We defined grooming behaviour as a cricket rubbing its forelegs along its body and/or through its mouthparts. This motion typically began at the pronotum and terminated at the head or antennae. Our definition of grooming also included grooming of the hind-quarters. Crickets groomed their hindquarters using their hindlegs. This motion commenced mid-abdomen on either the ventral or the lateral side of the body and terminated at the cricket's posterior end. Our definition of grooming did not include antennal-only grooming, where the cricket draws its antennae through its mandibles, as this behaviour was rarely observed in the trials.

### *Grooming in the Presence of Gravid Ormia ochracea*

To quantify whether crickets increased their grooming behaviour in the presence of gravid female *O. ochracea*, we placed crickets in an arena and quantified the number of grooming events for one of three treatments: fly and call, call-only control, and no fly and no call control. In the fly and call treatment, we introduced two gravid female *O. ochracea* into the arena and broadcast an acoustic mate attraction signal of a male *G. texensis*. We used two flies because we have observed multiple flies acoustically locating to one playback speaker in the field. Use of multiple flies also increased the chance that at least one fly would respond behaviourally to the presence of the cricket (see below). We broadcast the male call because *O. ochracea* are unlikely to respond to a noncalling cricket (fly, walk, or touch the cricket). We included the call-only treatment to control for broadcasting the call in the fly and call treatment. We included the no fly and no call control treatment to assess baseline grooming behaviour.

When orienting to a calling cricket, *O. ochracea* engage in behaviours such as ricocheting, characterized as briefly contacting the cricket and then flying away (Adamo et al. 1995b; C. M. Vincent, personal observation), and walking, characterized as dropping to the surface from which the cricket is calling and walking to the host (C. M. Vincent, personal observation). *Ormia ochracea* also deposits planidia in the area where a cricket is calling, regardless of whether it establishes contact with the cricket (Cade 1975; C. M. Vincent, personal observation). Thus, for the fly and call treatment, we assessed whether any of these *O. ochracea* behaviours affected the cricket's grooming behaviour during the trial.

The arena was a 28 × 25 × 30 cm (length × width × height) plastic bin with a removable lid and a 12.5 × 8 cm (length × width) hole cut out in the bottom covered by a piece of mesh. On the front of the arena was a second hole measuring 12 × 8 cm (length × width). This hole had a nylon stocking glued around the perimeter to allow the experimenters to introduce and remove flies from the arena. We used the same compact disc player, pre-recorded male call and speaker as described above to trap *O. ochracea* beneath the arena. The call was broadcast at an intensity of 66 dB measured from the contact point between the speaker and the floor of the arena (1 cm). The speaker was encased in acoustic foam on all sides except for the broadcasting side to ensure that the sound broadcast only in the direction that the speaker was facing. The mesh-covered hole in the bottom of the arena was placed directly on top of the speaker. The floor of the arena was covered with a white sheet of paper to prevent crickets from being caught in the mesh and to allow easy visualization of any deposited planidia.

All trials were performed at dusk (1930–2030 hours) under a red light. Two adult crickets were randomly selected and placed into the arena. We used two crickets in each of these trials to mimic natural conditions. Two crickets also reduced the possibility that the crickets would spend most of the trial searching for the calling conspecific in the broadcast-only trials. However, searching for a calling male may have occurred in trials with two females if the females identified each other as such (Tregenza & Wedell 1997). Crickets were paired

randomly such that some trials had two individuals of the same sex while others had one male and one female. Crickets were acclimatized for 1 min. The speaker was then turned on in the call-only control and the fly and call treatment; the flies were simultaneously released into the arena in the fly and call treatment.

Grooming activity was scored for each cricket for 5 min. Because most grooming in *G. texensis* lasts for less than 5 s (C. M. Vincent, unpublished data), we scored every 5 s (or part thereof) of cricket grooming as one grooming event. Each trial was performed with new crickets and flies. In total, 54 crickets and 54 flies were used ( $N = 18$  for each treatment). A subset of trials (two fly-and-call trials; three call-only trials; five control trials) were performed with first-generation individuals in our laboratories at Carleton University, Canada.

#### *Grooming in the Presence of Planidia*

To quantify whether crickets increase their grooming behaviour in the presence of *O. ochracea* planidia, we placed crickets in an arena and quantified their grooming behaviour in the presence or absence of planidia. One cricket was used in each trial. The arena was a  $32.5 \times 19 \times 22$  cm (length  $\times$  width  $\times$  height) plastic bin covered with a piece of Plexiglas. It was divided into two equal halves by a  $2.5 \times 19 \times 22$  cm (length  $\times$  width  $\times$  height) area termed the neutral zone. The bottom of the arena was layered with grass collected from Buescher State Park. We ran two treatments: planidia-laden grass and planidia-free (control) grass. In the planidia-laden treatment, planidia were spread on the grass throughout the entire arena excluding the neutral zone ( $N = 22$ ). In the control treatment, the grass was free of planidia ( $N = 30$ ).

Planidia were collected by removing the abdomens of live gravid female *O. ochracea* and then excising their reproductive tracts using a fine pair of forceps (Vincent & Bertram, in press b). Planidia and grass were replaced after approximately every five trials. We were unable to replace the planidia and grass after each trial because it would have decimated our fly population. We instead ensured that the planidia were still alive and very active (waving their anterior ends in the air) prior to initiating each new trial.

Crickets were introduced into the arena via the neutral zone and given 1 min to acclimatize underneath a see-through plastic cup. The plastic cup was then carefully raised and the number of cricket grooming events was quantified for 5 min.

#### *Grooming and Avoidance in the Presence of Planidia*

As a follow-up to the aforementioned planidia experiment, we conducted a further experiment to determine whether crickets avoided planidia-laden areas when given a choice. In this experiment, planidia were spread on only one side of the arena ( $N = 63$ ). The planidia-laden side was alternated and the arena was visually scanned after every trial to ensure that planidia had not migrated to the opposite side of the arena.

The experiment was conducted in the same manner as the one described above. Crickets were given 1 min to acclimatize, and then released from the clear plastic cup. Grooming behaviour was then observed for 5 min. We recorded the side (planidia-free or planidia-laden) that the cricket entered first. We also recorded the time the cricket spent on the planidia-free side and on the planidia-laden side. Furthermore, we recorded the number of grooming events that occurred on each side. These data allowed us to determine whether crickets avoid areas with planidia, or whether they groom more when they spend time on planidia-laden grass. They also allowed us to quantify whether crickets were capable of distinguishing between planidia-laden and planidia-free sides of the arena and adjusting their grooming behaviour accordingly.

#### *Statistical Analyses*

Data were analysed using JMP 8 (SAS Institute Inc., Cary, NC, U.S.A.). For the gravid female fly experiment we used an ANOVA model with treatment, sex, and the treatment by sex interaction as factors to analyse whether crickets groomed more in the presence of gravid female *O. ochracea* than they did in the presence of a call-only or silent environment (controls). We also used ANOVA to test whether the presence/absence of fly ricocheting, walking, or deposition of planidia affected the number of cricket grooming events.

To analyse whether crickets groomed more in the presence of planidia-laden grass than they did in the planidia-free grass, we used an ANOVA model identical to that described above for the gravid fly analysis (treatment, sex, and treatment by sex factors).

For the planidia avoidance experiment, where crickets were permitted to travel freely between the planidia-free side and the planidia-laden sides of the arena, data for time spent on each side were converted to proportions and arcsine transformed to meet assumptions of normality. We used ANOVA models with side, sex, and side by sex interactions as factors to analyse whether crickets spent more time on the planidia-free side than on the planidia-laden side, or whether crickets groomed more when they were on the planidia-laden side than on the planidia-free side. We used a regression analysis to determine the relationship between time spent on the planidia-laden side and the number of grooming events observed during the 5 min trial. We included sex and the interaction between sex and time on the planidia-laden side as factors in the regression model. We used an ANOVA to determine whether crickets that were subsequently found to be parasitized differed from unparasitized crickets in their grooming behaviour.

There was no apparent difference between the data collected for the field-caught and first-generation animals, so we combined the two data sets (statistical tests could not be completed because too few experiments were conducted using first-generation animals).

## RESULTS

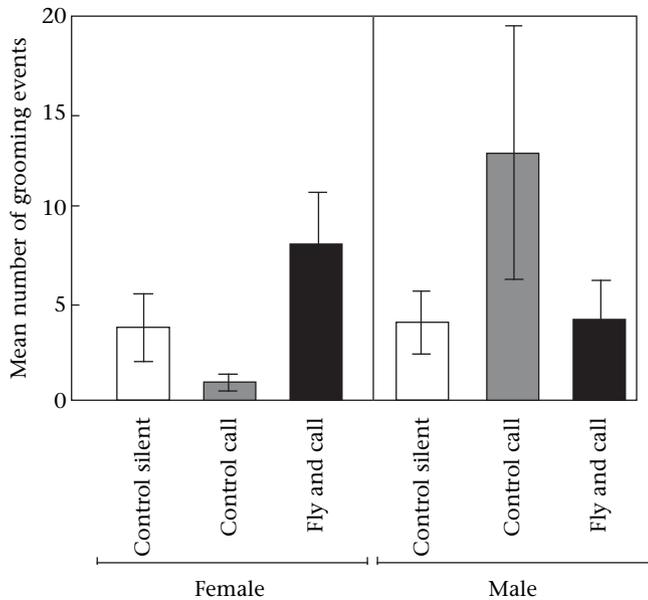
#### *Grooming in the Presence of Gravid *Ormia ochracea**

The number of grooming events observed in the gravid female fly experiment was not dependent on either parasitoid treatment (fly and call, call only, or silent control) or sex (overall model: ANOVA:  $F_{48,5} = 2.8266$ ,  $P = 0.0257$ ,  $R^2_{\text{adj}} = 0.1470$ ; treatment:  $F_{48,2} = 0.8775$ ,  $P = 0.4224$ ; sex:  $F_{48,1} = 2.0881$ ,  $P = 0.1549$ ). There was, however, a significant treatment by sex interaction (treatment  $\times$  sex:  $F_{48,2} = 5.8937$ ,  $P = 0.0051$ ). Female crickets groomed most in the presence of gravid female flies, while male crickets groomed most in the call-only treatment (Fig. 1).

Neither fly ricocheting (ANOVA:  $F_{16,1} = 0.23$ ,  $P = 0.64$ ), fly walking ( $F_{16,1} = 0.0005$ ,  $P = 0.98$ ), nor the deposition of planidia ( $F_{16,1} = 1.2$ ,  $P = 0.29$ ) affected cricket grooming. Power analyses revealed that the sample sizes needed to detect an effect of fly ricocheting, fly walking, and/or planidia deposition ranged from 307 to 13 857. There was no correlation between the number of grooming events for crickets that shared the arena during their trials (Pearson correlation:  $r_{26} = -0.13$ ,  $P = 0.53$ ).

#### *Grooming in the Presence of Planidia*

Cricket grooming behaviour was significantly influenced by the presence of planidia (overall model: ANOVA:  $F_{47,3} = 4.0017$ ,  $R^2_{\text{adj}} = 0.1526$ ,  $P = 0.0128$ ). Crickets groomed significantly more when they were on the planidia-laden grass ( $\sim 200\%$ ) than when they were on the planidia-free grass (number of grooming events for planidia-laden grass:  $9.86 \pm 5.77/5$  min observation period;

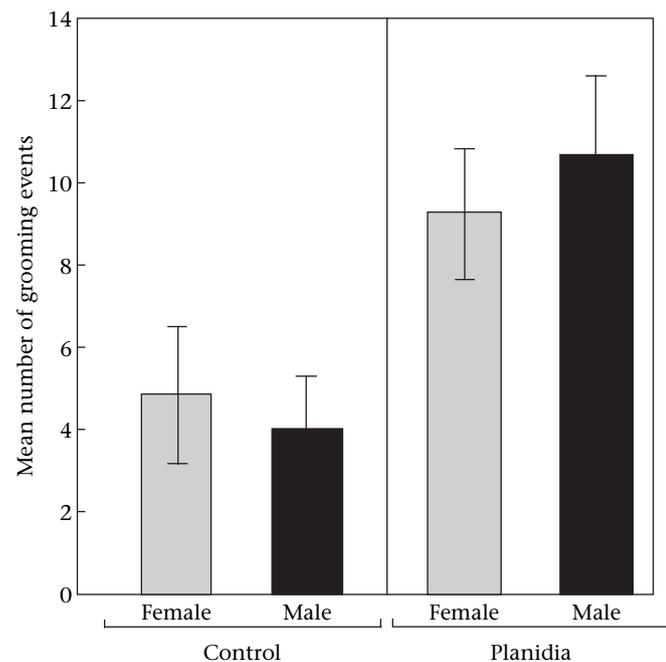


**Figure 1.** Mean  $\pm$  SE number of grooming events by *G. texensis* males and females when exposed to different levels of parasitoid threat by gravid adult female *O. ochracea*.  $N = 18$  for each treatment (control silent, control call, fly and call).

planidia-free grass:  $4.4 \pm 0.995/5$  min observation period; Fig. 2; treatment:  $F_{47,1} = 11.6682$ ,  $P = 0.0013$ ). There were no sex effects (sex:  $F_{47,1} = 0.8789$ ,  $P = 0.8789$ ). Likewise, there was no significant interaction between sex and treatment (treatment  $\times$  sex:  $F_{47,1} = 0.4704$ ,  $P = 0.4962$ ).

#### Grooming and Avoidance of Planidia

Crickets did not appear to be capable of detecting the presence of planidia and avoiding the area (overall model ANOVA:  $F_{122,3} = 0.4931$ ,



**Figure 2.** Mean  $\pm$  SE number of grooming events by *G. texensis* when placed in an arena with planidia-free grass (control treatment,  $N = 29$ ) or planidia-laden grass (all planidia treatments,  $N = 22$ ).

$R^2_{\text{adj}} < 0.0001$ ,  $P = 0.6877$ ). For the half-planidia treatment, there was no significant difference between the number of individuals that first approached the planidia-laden and planidia-free sides (males: control/planidia = 28/30; females: control/planidia = 28/22; Table 1). Furthermore, time spent on either side was not explained by treatment (planidia-laden versus planidia-free side:  $F_{122,1} = 0.4917$ ,  $P = 0.4845$ ), sex ( $F_{122,1} = 0.8990$ ,  $P = 0.3449$ ), or the interaction between treatment and sex ( $F_{122,1} = 0.1241$ ,  $P = 0.7275$ ). Number of grooming events was also not explained by treatment ( $F_{72,1} = 0.0145$ ,  $P = 0.9044$ ), sex ( $F_{71,1} = 0.0145$ ,  $P = 0.9044$ ), or the interaction between treatment and sex ( $F_{72,1} = 0.2911$ ,  $P = 0.5912$ ). The number of grooming events was, however, dependent on the amount of time spent on the planidia-laden side. Males and females that spent more time on the planidia laden side groomed significantly more often throughout the entire experiment than those that spent less time with the planidia (multiple regression:  $F_{58,3} = 3.3226$ ,  $R^2_{\text{adj}} = 0.1025$ ,  $P = 0.0258$ ; parameter estimates: time:  $t_{58} = 3.01$ ,  $P = 0.0038$ ; sex:  $t_{58} = -0.34$ ,  $P = 0.7321$ ; time  $\times$  sex:  $t_{58} = 0.18$ ,  $P = 0.8559$ ; Fig. 3). As expected, time spent on the control side did not influence the number of grooming events for males or females (multiple regression:  $F_{58,3} = 0.9726$ ,  $R^2_{\text{adj}} = -0.0014$ ,  $P = 0.4119$ ; time:  $t_{58} = 1.11$ ,  $P = 0.2719$ ; sex:  $t_{58} = -0.56$ ,  $P = 0.5786$ ; time  $\times$  sex:  $t_{58} = 1.31$ ,  $P = 0.1937$ ).

#### Efficacy of Grooming

Of the 63 crickets used in the half-planidia treatment, 48 were heavily exposed to parasitoids as they moved through planidia-laden grass for more than 1 min; the other 15 either did not approach the planidia-laden grass or spent very little time in it. Of the 48 individuals that were heavily exposed to planidia, only 12/48 (25%) died as a result of parasitism (six males and six females). We completed a post hoc analysis on the parasitized versus unparasitized crickets and found that crickets that did not die as a result of parasitism groomed almost three times as often as crickets that died from parasitoid infection (mean  $\pm$  SE number of grooming events: unparasitized:  $11.3 \pm 2.21$ ; parasitized:  $3.68 \pm 2.05$ ; ANOVA:  $F_{46,1} = 5.03$ ,  $P = 0.029$ ; Fig. 4).

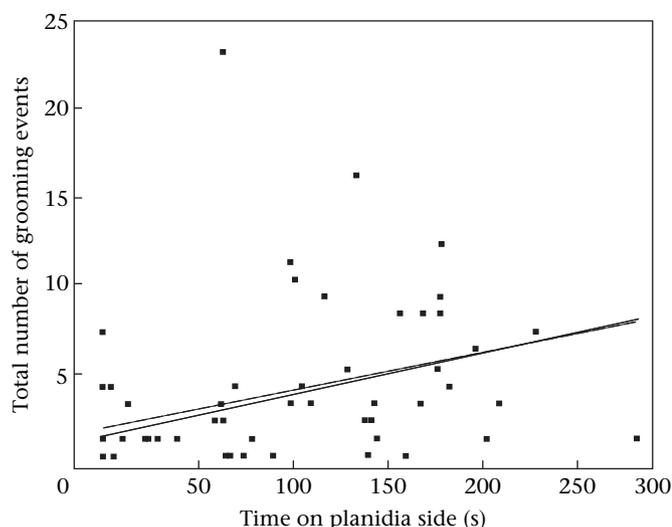
#### DISCUSSION

Giorgi et al. (2001) suggested that grooming can be costly, both with respect to energy expenditure and time. Baker & Smith (1997) further suggested that grooming may make the groomer more conspicuous to predators. If cricket grooming is costly, crickets should be selected to groom only when the threat of parasitism is high. If this were the case, male crickets might be expected to groom more than females, because of their elevated risk of parasitism by these acoustically orienting parasitoid flies. Our results revealed that crickets of both sexes dramatically increased their grooming behaviour when placed in an arena filled with planidia-laden grass compared to when they were placed in an arena with planidia-free grass. Furthermore, we found that crickets that died as a result of parasitism groomed significantly less than those that did not become parasitized. Our findings suggest that both male

**Table 1**

Cricket avoidance and grooming behaviour when given the option of 'escaping' planidia by moving to the planidia-free side of the arena

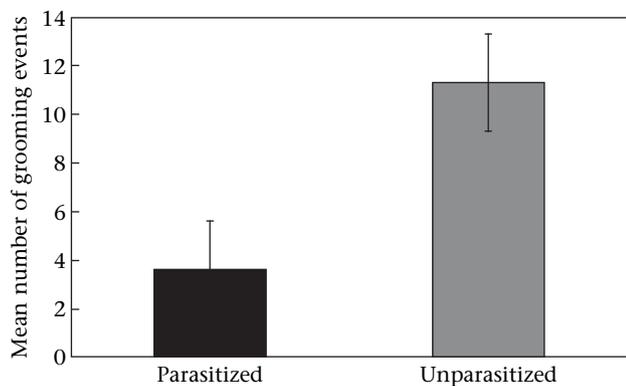
	Planidia-laden	Planidia-free	$t$	$P$
First side approached ( $N$ )	26	28	$2 \times 10^{-4}$	0.87
Mean time on side (s)	84	93	0.58	0.56
Number of grooming events	11	13	0.08	0.93
Grooming rate (grooms/min)	1.7	1.6	0.68	0.93



**Figure 3.** Relationship between time spent on the planidia-laden side of the arena and number of grooming events observed for male (solid line) and female (dashed line) *G. texensis*.

and female crickets are capable of detecting the presence of parasitoid *O. ochracea* larvae when they come in contact with them, and that grooming is an effective way of reducing their risks of parasitism and certain death.

Many organisms are capable of detecting olfactory cues associated with a potential threat and adjusting their behaviour accordingly (Chivers et al. 1996; Koperski 1998; Amo et al. 2008; King et al. 2008). Amo et al. (2008), for example, found that blue tit, *Cyanistes caeruleus*, parents delayed entry into their nestboxes when the scent of a predator was placed in their nestboxes. If crickets were capable of detecting the parasitoid planidia through olfactory or visual cues, we would have expected the crickets to approach the planidia-free side first in the half-planidia treatment, and spend more time in the planidia-free side than the planidia-laden side. However, when crickets were placed in an arena where one side had planidia-laden grass and the other side had planidia-free grass, they approached both sides with equal probability. Crickets also did not spend less time in the planidia-laden side compared to the planidia-free side of the arena. These results suggest that crickets are incapable of detecting planidia from a distance. It is unlikely that crickets could have visually detected the parasitoid planidia, because planidia are extremely small and cricket vision is generally



**Figure 4.** Mean  $\pm$  SE number of grooming events by *G. texensis* that entered the planidia-laden side of the arena and were presumed to have contacted planidia (parasitized,  $N = 48$ , of which 12 died as a result of parasitism) and those that did not become parasitized ( $N = 36$ ).

poor. Crickets are, however, known to detect potential predators using olfactory cues. Storm & Lima (2008) found that the fall field cricket, *Gryllus bimaculatus*, could recognize the odour of a predatory wolf spider when placed on a sheet of filter paper containing the spider's scent. This finding suggests that the cricket chemosensory system is predominantly contact based (Otte & Cade 1976). This would make olfactory detection of parasitoid planidia difficult from a distance. If crickets are capable of detecting planidia through olfaction, the size of the arena may have made it difficult to localize the chemical cues emitted by the planidia. A larger arena where crickets could more effectively leave the focal area and potentially experience distinct chemical environments could resolve this ambiguity.

If crickets cannot detect planidia from afar through olfactory or visual cues, but can detect them once physical contact is established, we would have expected crickets to spend less time in contact with planidia on the planidia-laden side and to groom more when they were on that side. However, crickets did not spend less time on the planidia-laden side than on the planidia-free side of the arena. Furthermore, crickets did not increase their number of grooming events or their grooming rate while on the planidia laden side, compared to the planidia-free side. In retrospect, it would have been surprising if crickets spent less time in contact with the planidia-laden grass because this is not the way that crickets typically come into contact with *O. ochracea* larvae. Usually the flies detect singing male crickets and deposit the larvae on and around them (Cade 1975). Thus, crickets have likely not been selected to detect and walk away from planidia-laden areas.

The number of grooming events that occurred was, however, strongly and positively correlated with the amount of time each cricket spent on the planidia-laden side. Crickets that spent more time on the planidia-laden grass groomed more often. Crickets groom while they walk (C. M. Vincent, personal observation), and crickets in the present study typically walked throughout both sides of the arena, repeatedly coming into contact with planidia. This continuous exposure to planidia probably resulted in our observation of indiscriminate levels of grooming behaviour between the planidia-laden and planidia-free sides.

The results of our broadcast experiment (Fig. 1) showed that male crickets increased grooming when the call of a conspecific was broadcast, while female crickets only increased grooming in the presence of a gravid female fly and a broadcasting male. Although this finding implies that males associate a calling male with an increased risk of parasitism, if this were true, we would have expected males in the broadcast-fly trials to also show an increase in grooming events, but they did not. Thus, further research into the acuity of male and female responses to broadcasting males and the threat of parasitism is required to understand the potential significance of our results.

Grooming as an antiparasite behaviour is largely accepted, but few studies have formally investigated the efficacy of this behaviour in reducing the costs of parasitism (reviewed in Hart 1990). Our study is the first to investigate the efficacy of grooming in insects. Our post experiment analysis revealed that the crickets that died as a result of parasitism groomed significantly less often than crickets that did not die. Our results suggest that grooming is an extremely effective way for a cricket to rid itself of *O. ochracea* larvae. Our data, however, do not preclude the possibility that planidia never successfully attached to the cricket, or that crickets that groomed more also had a stronger immunological response to the invading parasitoid. Therefore, a controlled study where crickets are exposed to a set number of parasites and grooming behaviour and immunological responses are quantified will be necessary to confirm that grooming is the cause of failed parasite establishment. This controlled study should also contain

a treatment where individuals are prevented from grooming altogether to determine whether this nongrooming group experiences a higher incidence of parasitism.

### Conclusion

Crickets increased grooming activity when placed in an arena filled with planidia-laden grass, and crickets that died from parasitism groomed significantly less than those that did not succumb to parasitism. These results suggest that grooming is an effective strategy to remove *O. ochracea* planidia from the cricket body. They also suggest that *G. texensis* are capable of detecting *O. ochracea* and can change their behaviour in an attempt to avoid parasitism by *O. ochracea*. This detection appears to be driven by cuticular stimulation rather than olfactory or visual signals, as crickets did not dramatically adjust their grooming behaviour in the presence of gravid female *O. ochracea*, and, when given the choice, did not avoid planidia-laden grass.

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