



## Semi-automatic behavior analysis using robot/insect mixed society and video tracking

Rodrigo da Silva Guerra<sup>a,\*</sup>, Hitoshi Aonuma<sup>b,1</sup>, Koh Hosoda<sup>a,c,2</sup>, Minoru Asada<sup>a,c,3</sup>

<sup>a</sup> JST ERATO Asada Synergistic Intelligence Project, Osaka, Japan

<sup>b</sup> Research Institute for Electronic Science, Hokkaido University, Sapporo 060-0812, Japan

<sup>c</sup> Department of Adaptive Machine Systems, Grad. School of Eng., Osaka University, Osaka, Japan

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### ABSTRACT

This paper proposes a novel robot/insect mixed society setup which enhances the possibilities for insect behavioral research and can be used as a powerful tool for interdisciplinary studies on insect behavior. Micro-robots are equipped with decoys so as to allow a controlled dynamic interaction with crickets, *Gryllus bimaculatus*. A camera records the interaction and the video is later processed for the automatic tracking of each encounter between cricket and robot. A novelty of our method lies in using the robots as tools for the controlled evoking of specific insect behaviors rather than trying to build an insect-like robot. The possibility for performing controlled repeatable movements allows the stimulation of certain insect behaviors that are usually difficult to trigger using insects alone, allowing consistent behavioral research. A set of experiments were performed in order to validate the proposed setup. We also demonstrate the use of our setup for stimulating agonistic behavior during an electromyography recording session.

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### 1. Introduction

This paper presents a method of using micro-robots for the behavioral study of crickets. Robotics has been increasingly used for the validation of behavior models of animals ranging all the way from insects (Webb, 2006) to human infants (Asada et al., 2009). In particular, with respect to insect/robot interactions, Gaustrais et al. (2004) used autonomous insect-like robots as a complementary tool for the study of the robustness of cockroach aggregation behavior against high interindividual variability. They explored the dynamics of mixed societies where one or more individuals were modified to test whether there is only a gradual change on the collective level or whether non-linear changes would occur. Later Halloy et al. (2007) showed how autonomous miniature robots

modeled to mimic cockroach behavior can affect the aggregation behavior of real cockroaches. A key difference between our approach and that of Gaustrais et al. (2004) and Halloy et al. (2007) is that we take a more analytic rather than synthetic approach, that is, we do not attempt to build a robot that faithfully mimics an insect's behavior. This allows us to be less restrictive on the constraints on the robot and setup, shifting the focus toward the studied subject itself. We believe the use of small miniature robots in such a multidisciplinary setup is a powerful tool for general and systematic investigation of insect behavior. We also describe an algorithm capable of automatically parsing and classifying agents encounters through video tracking using an overhead camera.

In order to validate our proposed setup we performed a series of experiments focusing on how subordinate and dominant crickets behave after an agonistic dispute is settled.

#### 1.1. Typical setups found in the literature

The current state of behavior research of cricket (and other insects in general) can be classified into three main categories of experimental setup: (1) *one shot*, (2) *treadmill* and (3) *free moving*.

In the *one shot* setup, the insect, arena and other apparatus are repeatedly reset into a given fixed initial condition and some stimulus is presented in a controlled fashion. Typically, the analysis is focused on the behavior that immediately follows, thus allowing carefully controlled investigations once the experimenter knows how to trigger the desired behavior. For instance, Tauber and Camhi

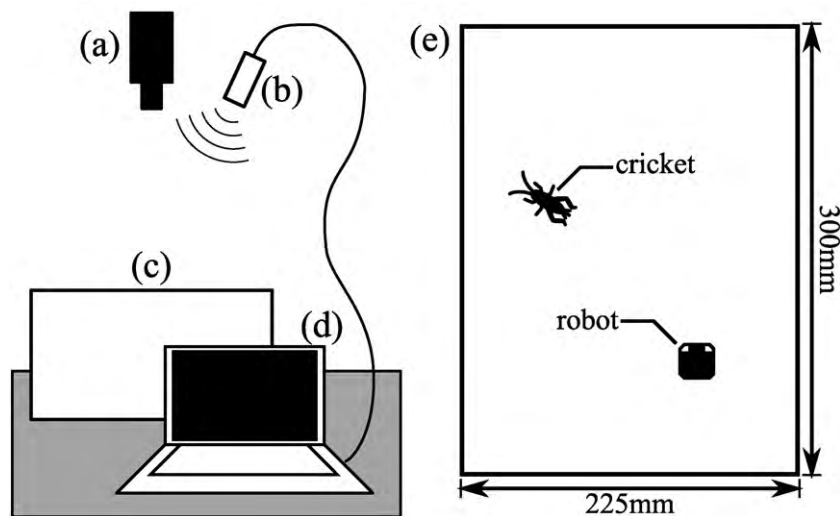
\* Corresponding author at: JST ERATO Asada Project, FRC1-4F, Osaka Univ., 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6876 8884; fax: +81 6 6876 8994.

E-mail addresses: [tioguerra@gmail.com](mailto:tioguerra@gmail.com) (R.d.S. Guerra), [aon@es.hokudai.ac.jp](mailto:aon@es.hokudai.ac.jp) (H. Aonuma), [hosoda@ams.eng.osaka-u.ac.jp](mailto:hosoda@ams.eng.osaka-u.ac.jp) (K. Hosoda), [asada@ams.eng.osaka-u.ac.jp](mailto:asada@ams.eng.osaka-u.ac.jp) (M. Asada).

<sup>1</sup> Tel.: +81 11 706 3832; fax: +81 11 706 3832.

<sup>2</sup> JST ERATO Asada Synergistic Intelligence Project, Dept. of Adaptive Machine Systems, Osaka Univ., M4-202, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6876 8884; fax: +81 6 6876 8994.

<sup>3</sup> JST ERATO Asada Synergistic Intelligence Project, Dept. of Adaptive Machine Systems, Osaka Univ., FRC1-4F, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6876 8884; fax: +81 6 6876 8994.



**Fig. 1.** Sketch of the bench setup used for the experiments depicting (a) camera, (b) infrared-transmitter, (c) arena (area inside which all the experiments were performed), and (d) computer. A computer program played a pre-programmed pattern of commands that were sent to the robots using the infrared transmitter. The camera was used for recording all trials for later tracking analysis. (e) The arena layout.

(1995) and Baba and Shimozawa (1997) use this kind of setup with the aid of video analysis for studying wind-evoked escape behavior in crickets. Another example is the use of a system of individual choice chambers (August, 1971). In the *one shot* setup it is also common to use stationary artifacts as cues (Adamo and Hoy, 1995; Tachon et al., 1999; Nagamoto et al., 2005).

The *treadmill* setup (typically a free-rotating styrofoam ball) is popular for long course tracking. Some examples are studies on phonotaxis Doherty (1985), research on audio and visual stimuli influences on course control (Bohm et al., 1991), and the study of insects' reactions to mechanical stimulation of hindwings (Hiraguchi and Yamaguchi, 2000). More recently, optical mouse sensors have been employed for more accurate measurements (Lott et al., 2007). This setup is a great solution for tracking the trajectory of a single individual in a very controlled environment. Here again the experimenter must typically have a fairly accurate idea of how to trigger the behavior to be investigated. This system can monitor only one individual at a time and insects are usually constrained so they cannot jump, flip-over or accelerate abruptly.

Relying on rigid controlled conditions prevents the unfolding complexity typical of longer interactions. The *free moving* setup is one where insects are left inside an arena while their behavior is observed, often in a more qualitative way, but also with the aid of cameras as tracking devices. This type of setup is ideal for reducing the amount of prior assumptions and for investigating social behaviors. It allows the experimenter to observe the behaviors as they arise from the stochastic complexity of the interactions among the individuals. Unfortunately, so far this could only be done exclusively with real insects, in which case the biologist can observe an animal's behavior, but cannot control it.

The novelty of our proposed method, in comparison with more classical behavioral studies, is that our setup allows the experimenter to actively produce movements which are controlled and repeatable. Along with other cues, the mechanical stimuli may have a significant influence on the likelihood of triggering crickets' behavior as if they were interacting with conspecifics. Small attachments can be added to this robot in order to convey behavioral cues such as chemical attachments, textures or other tactile cues like antennae, visual lures, etc., in a non-stationary way. Additionally our setup brings the possibility of programmatically modulating the robot's movement in real time according to some hypothetical model as a function of cricket's movements. This allows the

researcher to test different hypotheses regarding the influence of opponents' behavior on insects' choices.

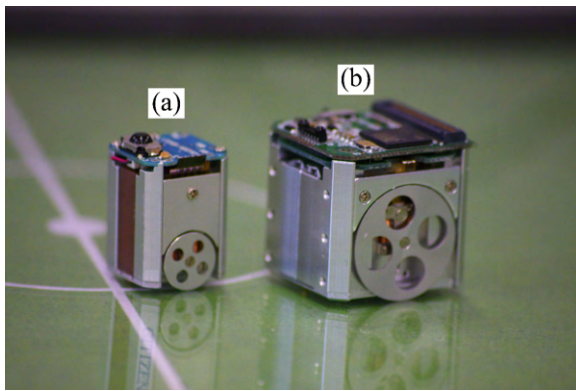
## 2. Materials and methods

The experiment setup is illustrated in Fig. 1. A laptop equipped with an infrared transmitter was used for controlling a robot's movement while an overhead camera recorded each trial. The resulting footage was later processed for the tracking of both robots and crickets. The arena was a rectangular area of dimensions 300 mm  $\times$  225 mm delimited by walls of 150 mm of height, separating it from the external environment of the lab.

### 2.1. Cricket

Male crickets of the species *Gryllus bimaculatus* DeGeer were used for this study. The male and female cuticular substances on the body surfaces of crickets evoke different behaviors in male crickets (Nagamoto et al., 2005). When male crickets come across a female and perceive its cuticular substance, they start courtship behavior for mating. On the other hand, when they come across a male, they show a stereotyped sequenced aggressive behavior, often involving aggressive stridulation and violent battles (Alexander, 1961). If the opponent cricket does not give up attacking, they open their mandibles and grapple with each other. As a result of fighting, they establish a dominant–subordinate relationship. The subordinates then actively avoid the victorious cricket for a certain period of time (Adamo and Hoy, 1995; Aonuma et al., 2004). After several hours from the initial fight, if the subordinate once again comes into contact with the dominant one, they start fighting again. Many works in the literature report and discuss these behaviors; however, we still do not have good understanding of the neuronal mechanisms underlying them. One reason for this is the technical challenge faced by researchers.

Adult sexually mature male crickets that were between 8 and 21 days after their imaginal molt were used in this study. To avoid the effect of copulation on the agonistic behavior, crickets were individually housed in transparent containers for at least 24 h before the experiment, where they could potentially see, hear and smell conspecifics but could not get involved any kind of tactile interaction. The animals were reared in plastic cases (800 mm  $\times$  450 mm  $\times$  200 mm) on a 14 h:10 h light and dark cycle



**Fig. 2.** Close-up picture of the two micro-robot versions: (a) the version used in the experiments described in this paper, and (b) the newer version available for future use. The newer version has more powerful embedded microcontroller and longer battery life at the expense of an increased body size. The faster processor allows installation of embodied devices such as speakers and microphones for production and detection of chirping.

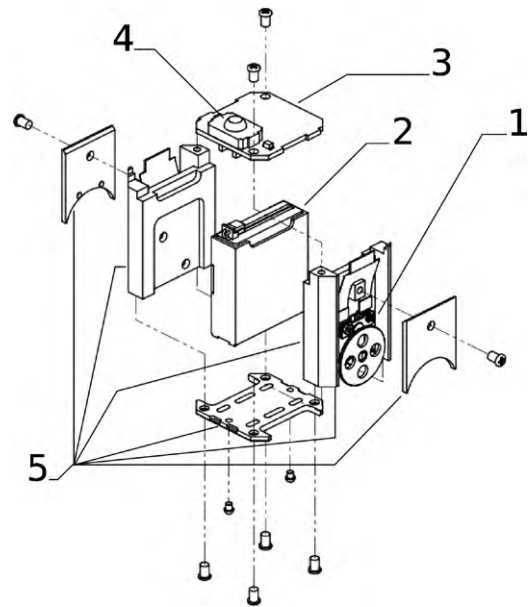
at  $28 \pm 1^\circ\text{C}$ ,  $75 \pm 2\%$  humidity. They were fed a diet of insect food pellet (Oriental Yeast Co., Tokyo, Japan), chopped carrot and water *ad libitum*.

## 2.2. Robot

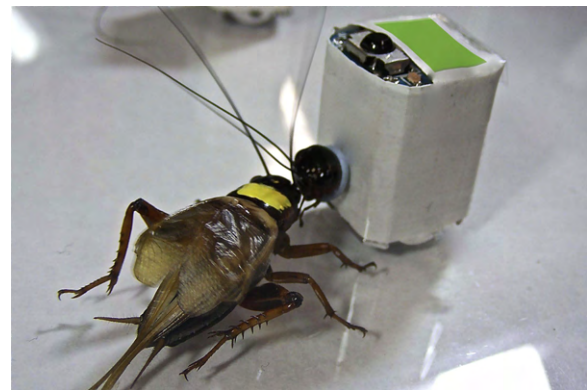
The micro-robot employed in the experiments is shown in Fig. 2a. Originally designed for an educational robotic competition (Guerra et al., 2007a,b) these robots have a size comparable to that of a typical cricket. The robot has dimensions of  $18\text{ mm} \times 18\text{ mm} \times 22\text{ mm}$  and is driven by two differential wheels. It has no sensors except for an infrared receiver used for receiving commands encoded into pulses of infrared light. The robot is capable of moving on smooth 2D surfaces at speeds comparable to those typical of a cricket when it is wandering, but it cannot perform very fast movements or jump. The robot is, therefore, able to deliver any kind of behavioral cue that fits these constraints. Examples are chemical, tactile, visual and acoustic cues. Movements can be controlled in both open or closed-loop, with or without real-time feedback from the insect's position. To allow closed-loop control, an external camera gives realtime feedback of the robot's position and orientation (Guerra et al., 2007a). Therefore the robot can be programmed to approach or avoid the crickets. It can also be programmed to face the crickets and produce alternate lateral movements in order to vaguely mimic certain interactions such as antennae fencing.

The main robot parts are (numbers according to Fig. 3):

1. **Motor:** Customized from a wristwatch motor unit for higher torque, this micro-stepper motor was originally designed for adjusting auto-focus in miniature camera/lens mechanisms such as those included in mobile phones.
2. **Battery:** Miniature one-cell rechargeable 3.7 V lithium ion polymer battery with capacity of 65 mAh.
3. **Control board:** Based on the Microchip 8bit PIC18 family of microcontrollers, each robot comes equipped with a PIC18LF1220 which features 4kb of re-programmable flash memory.
4. **Infrared sensor:** An infrared sensor is used in order to listen to commands from the PC. The sensor operates at the 40 kHz bandwidth modulation (same of most home-appliance remote controls).
5. **Body:** Micro-machined in aluminum using high precision CNC machines.



**Fig. 3.** Exploded view of the robot revealing its components.



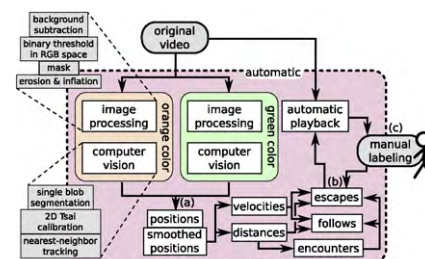
**Fig. 4.** Close-up picture of a male cricket interacting with a robot equipped with the decoy of another male cricket's head.

## 2.3. Tracking and data processing

For the automated tracking of moving insects some versatile and complete commercial solutions exist (Noldus et al., 2001). In this work the authors opted for using the open source SwisTrack (Lochmatter et al., 2008) accompanied by custom Python scripts.

Robot and crickets were marked with different colors (as depicted in Fig. 4).

Fig. 5 depicts the video tracking process. We start with the raw video files recorded from the batch of trials (one file per



**Fig. 5.** Block diagram describing the video processing.



trial). The tracking was performed in two passes, one for each agent color, and consisted of image processing and computer vision steps.

The image processing was performed in 4 steps:

1. background subtraction;
2. RGB binary threshold operation for segmentation of the color of each agent;
3. masking out of unnecessary areas;
4. inflation and erosion to cluster back together areas disjoint by noise.

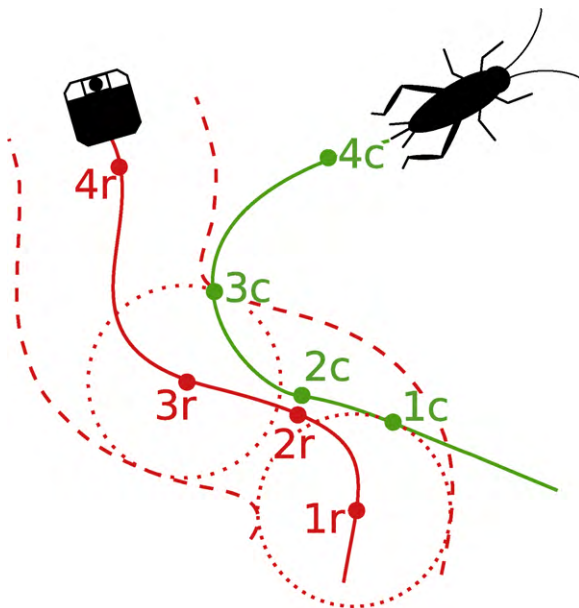
After that, three computer vision steps were performed:

1. localization of blob centroids;
2. 2D calibration using method by Tsai (1986);
3. tracking using nearest neighbors.

At the end of the video processing we had two lists of 2D coordinates representing the positions of both agents at each frame of video (Fig. 5a).

A smoothing operator with a window of 30 frames (roughly 1 s) was applied to the raw coordinates. Velocities and relative distances between the agents were computed.

All *encounters*, *escapes* and *follows* were detected as explained in Section 2.4. These encounters were automatically catalogued with the time window and respective video file of the trial where it occurred (Fig. 5b). With the aid of this information we were able to develop an interface that could playback the exact segment of video of each encounter. Each of these segments of video was played-back and watched by a human operator that hit keystrokes assigning labels to indicate qualitative features of the respective encounter (Fig. 5c). This allowed us to assess information not captured by the tracking algorithm such as chirping, biting, antennae fencing, mandible flare, among others.



**Fig. 6.** Illustration of the tracking algorithm. Paths 1r–4r and 1c–4c show the hypothetical trajectory of a robot and a cricket, respectively. The dotted circles highlight the instants that distance between the agents crossed a minimum threshold (represented with reference to the robot by the dashed curves).

## 2.4. Parsing of encounters

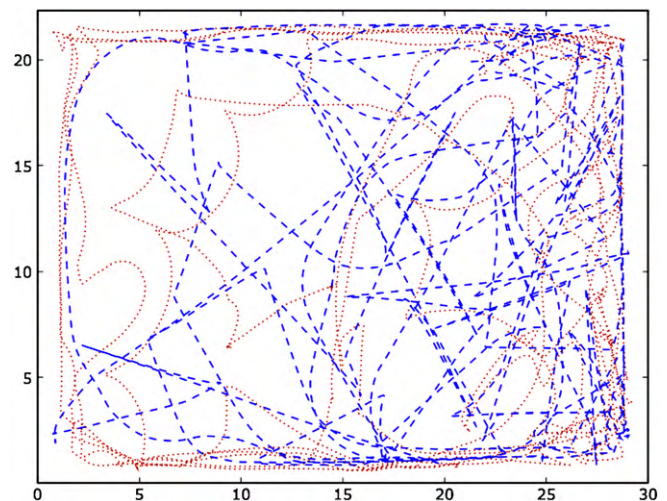
Fig. 6 illustrates the smoothed trajectories of robot and cricket in a hypothetical encounter. When robot and cricket are, respectively, at positions 1r and 1c their distances cross a minimum threshold of 40 mm (represented by the radius of the dotted circles), the beginning of an encounter is marked. They continue to move closer to each other until they reach their minimum distance at points 2r and 2c. That represents the beginning of an *encounter interval* and the frame at which that happens is labelled as a *touch frame*. Their distance then increases again to a value greater than the 40 mm threshold when at points 3r and 3c. This marks the end of an *encounter interval*. After that, the cricket and robot continue moving until the cricket's velocity eventually decreases below a velocity threshold of 2 mm/s (represented at the position 4c in Fig. 6). The frame at which that happens is called *escape frame*. A *follow* is an *encounter interval* which lasted at least 2 s and where the robot moved at least 20 mm.

## 2.5. Electromyogram recording

Electromyograms (EMG) of mandible muscles were obtained using two varnish-coated copper wires (17  $\mu\text{m}$  diameter) inserted proximally into the mandible muscle. Large amplitude mandible muscle potentials were recorded in free moving male crickets. EMG signals were picked up using an amplifier (Bioelectric Amplifier AZ-2100; Nihon Koden, Tokyo, Japan) and transferred to a computer using a Power1401 data acquisition system and Spike 2 (version 5.05) software (both Cambridge Electronic Design, Cambridge, UK). The male cricket from which the EMGs were recorded were placed in an experimental arena (120 mm  $\times$  120 mm  $\times$  100 mm). Another male cricket or a robot with or without a cricket head attached was then placed in the arena in which the behavior could be observed and the activities of mandible muscles could be recorded.

## 3. Results

The results described here demonstrate the use of our setup in practice. In our experiments we used our setup to automatically detect following and escaping behaviors of subordinate and dominant crickets after they encountered another agent (robot or cricket).



**Fig. 7.** Sample of trajectories showing a typical interaction of a cricket (dotted track) with a robot (dashed track). Notice how the cricket tends to follow the edges along the walls of the arena.

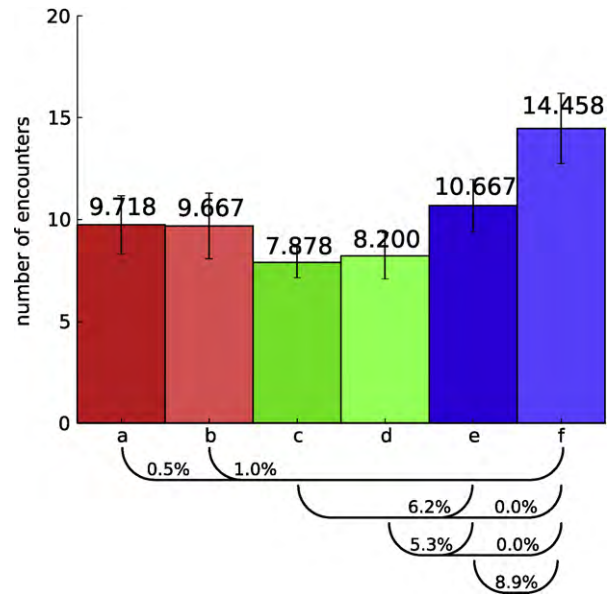
**Table 1**  
Six tested groups.

	Dominant cricket	Subordinate cricket
Robot with head attached	Group a, 38 trials, 361 encounters	Group b, 23 trials, 217 encounters
Plain robot	Group c, 40 trials, 313 encounters	Group d, 29 trials, 255 encounters
New male cricket	Group e, 19 trials, 202 encounters	Group f, 22 trials, 304 encounters

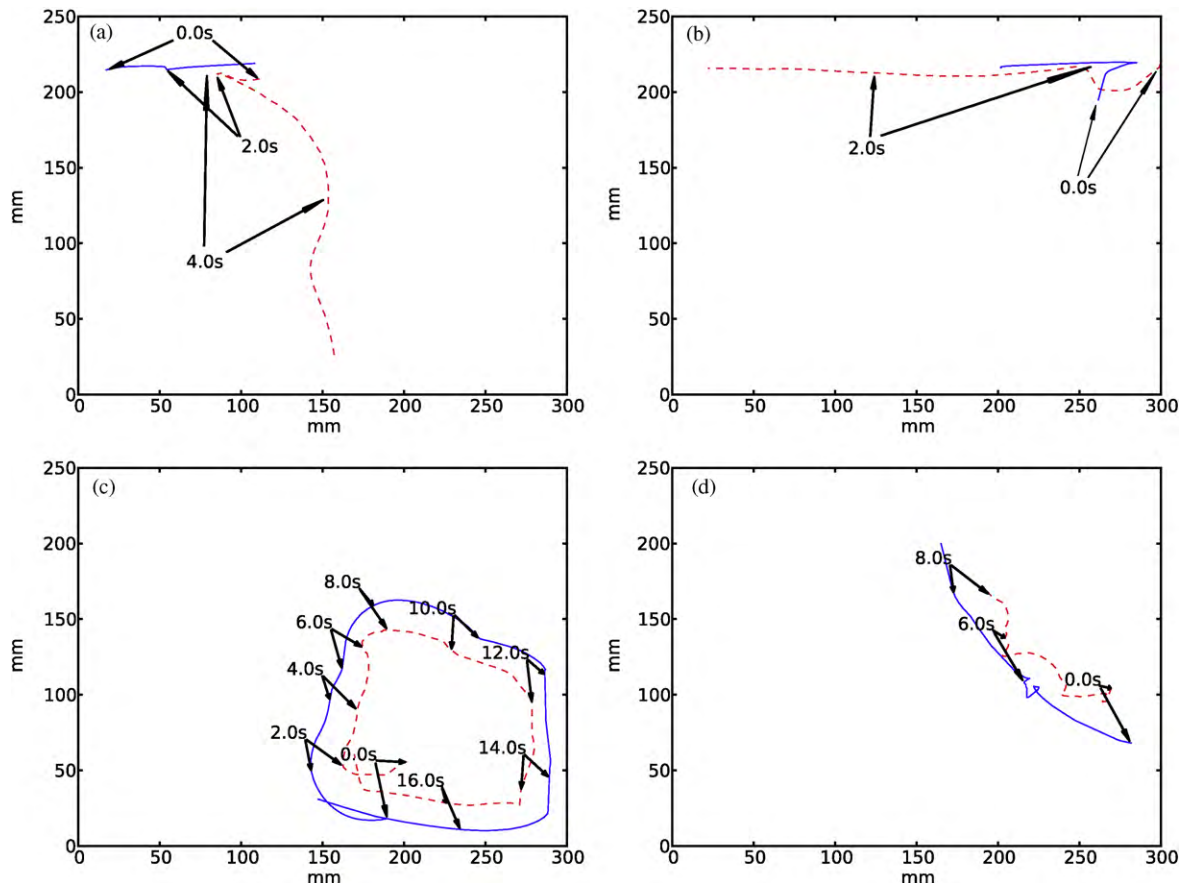
In each trial two new male crickets were left to freely interact with each other in the arena for 5 min or longer until a dominant/subordinate relationship could be clearly observed, and then depending on the group being studied either subordinate or dominant was gently removed and a third agent added in its place. This third agent was one of the following:

1. Robot covered with paper and equipped with a living male cricket head including prothorax.
2. Robot covered with paper and no attachments.
3. Another male cricket (naïve, kept isolated for 1 night).

When designing the movements of the robot the goal was not to reproduce insect motion or mimic any formally defined path pattern but rather to move the robot so as to explore the arena and encounter the crickets as much as possible. We were interested in observing the feasibility and general impact of adding artificial motion as opposed to not having any motion at all, like in the more classical studies. A sequence of moves was handcrafted with the help of a gamepad so as to allow the robot to stochastically cover a



**Fig. 8.** Average number of encounters automatically detected using our tracking algorithm for each group. The height of each bar represents the mean for that group and the lines on top show SEM. The alphabet letters label the respective group as seen in Table 1. The curved lines with percent values show level of significance, that is, the chance of the two indicated data sets being samples of a same population, only displayed for cases where  $p \leq 10\%$  (computed from the MWW *U*-test assuming normal distribution, two-tailed).



**Fig. 9.** Typical results from the automatic tracking script: (a) and (b) show examples of automatically detected cricket escapes, (c) and (d) show examples of automatically detected follows.

wide portion of the territory of the arena. This fixed pre-recorded sequence consisted of repeated forward and backward movements intertwined with a few turns and spins. The sequence was played repeatedly in a loop, with a few seconds of randomly generated spins of the wheels before each run. Fig. 7 shows the typical trajectories in a robot/cricket trial. In every batch of trials, crickets spent roughly 80% of the time wandering along the edge of the walls of the arena (less than 2.5 cm from the wall edge). This behavior is probably due to thigmotaxis, as confirmed, for instance, by Jeanson et al. (2003). They built a robust statistical model of cockroach wall-following behavior. According to their model, at the edges of a circular arena animals exhibit a linear movement mode with constant probability per unit time of leaving the edge and entering the central zone. Once in the central zone, the animals were assumed to move according to a diffusive random walk.

Trials were separated in three kinds depending on the type of agent added last to the arena, then each of these kinds was further divided in two groups according to whether the subordinate or the dominant was the cricket left in the arena after the dominance dispute. For easy reference throughout the rest of this paper the six groups were consistently labeled with the alphabet letters from *a* to *f* as described in Table 1. Fig. 8 shows the number of encounters automatically detected by our system. For all 15 two-group permutations the Mann–Whitney *U*-test (MWW *U*-test) was performed in order to access the statistical significance. The MWW *U*-test results where  $p \leq 10\%$  are depicted at the bottom of each graph. The null-hypothesis for this test is that the data of two given groups are samples from the same population. The approximate probabilities associated with those *U*-values found were computed assuming a two-tailed normal distribution. Fig. 9 shows some samples of automatically detected escapes and follows.

### 3.1. EMG recording from free moving crickets

In most cases, during aggressive disputes, the male spreads its mandibles, rushing to the opponent and then biting its body parts. Fine copper wires were inserted into a male cricket in order to record mandible muscle EMG data during such events. The male being recorded was placed in the arena and allowed to move freely for 10 min before each experiment started ( $n=5$ ). Then an opponent that could be (a) another male cricket, (b) a plain robot or (c) a robot with a living cricket head attached was placed in the arena. Larger amplitude EMG signals could be recorded when the subject, while interacting with another male cricket, opened its mandibles and attacked the opponent with biting (Fig. 10, upper trace). When the subject whose EMG signals were being recorded eventually won the fight, it chased the opponent while displaying typical aggressive stridulation. The activity of the mandible muscle decreased when the fight was settled and during chasing. When the plain robot without attached cues was placed in the arena instead of another male cricket, large amplitude EMG signals could not be observed since the subject did not appear interested in the robot and did not attack. When the experiment was repeated with a robot with a male cricket head attached, just after the robot moved, the male cricket turned towards it and started aggressive behavior. When it opened the mandibles, large amplitude EMG signals were once again recorded (Fig. 10, lower trace). When the robot retreated, the activities of the mandible muscle decreased accordingly. The cricket continued to attack the robot until the robot retreated, thus allowing us to control the duration of the fight.

## 4. Discussion

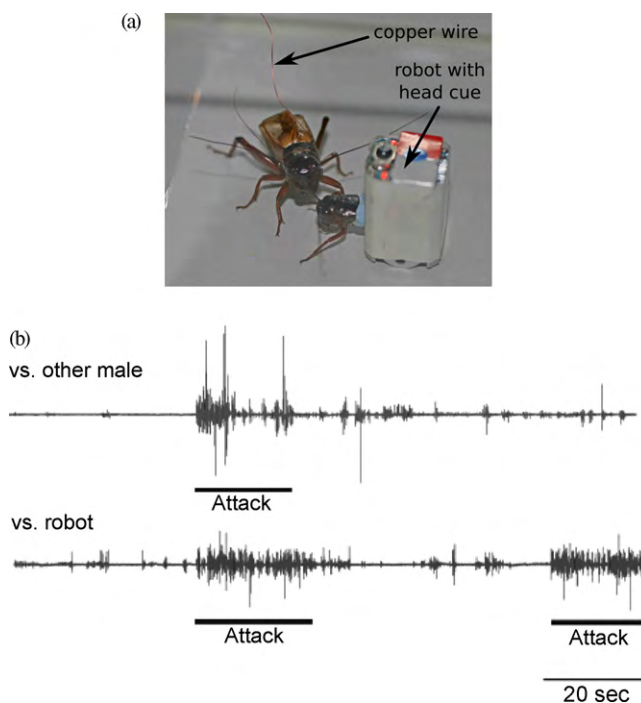
A behavioral research framework based on the multidisciplinary mixing of micro-robots and insects has been presented. Our setup allows us to trigger specific insect behaviors, enticed by controlled stimulus cueing assisted by the use of micro-robots, triggering, for instance, courtship or agonistic behavior.

Okada et al. (1999) have showed how electrophysiological recording using copper wire can aid the study of neuronal activities in a free moving insect. However, if we focus on the agonistic behavior or mating behavior in insect, there is always an opponent animal, complicating the experiment. We have here demonstrated how we can aid the investigation of the neuronal function underlying the behavior of the subject by controlling an artificial opponent. We believe the combination of using micro-robots and electrophysiological recording of neural activities of the brain can give us a better understanding of the neuronal mechanisms underlying certain social behaviors.

This work can also be greatly extended by adopting the newer version of the micro-robot (shown in Fig. 2b) which would allow the use of local processing and the attachment of embedded devices such as speaker and microphone. Using this newer system we can control the environment of experimental setups. We can, for example, examine the responses of female crickets to the calling, courtship or aggressive songs. In most of experiments working on the behavior of the female, researchers fixed animals on the treadmills or fixed the source of the sound (Hedrick et al., 2007 for example). Our system, however, allows the researcher to use free moving animals and also dynamically change the source of song.

## Acknowledgments

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**Fig. 10.** (a) Picture showing the male cricket of which the EMG signals were being recorded when it attacked a robot with living head attached. (b) The upper trace shows mandible muscle activities when the subject started aggressive behavior toward a conspecific opponent (indicated by a bar). The large amplitude EMG signals lasted until the opponent retreated, settling the fight. The lower trace shows the mandible activities when the cricket attacked the robot with a living male head cue attached. Similar to the conspecific case, large amplitude EMG signals were recorded when the subject attacked its artificial opponent. The activities decayed when the robot retreated.

Citizen Watch Co. for helping in the design and construction of such tiny robots.

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