Paper:

Behavior Change of Crickets in a Robot-Mixed Society

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This paper presents a study on cricket behavior using an interdisciplinary robot/insect mixed society setup. Field crickets of the species *Gryllus bimaculatus* were allowed to interact with micro-robots equipped with decoys. This allows the stimulation of insect behaviors that are usually difficult to bring out evoke insects alone, allowing consistent behavioral research. We performed a set of experiments focused on the comparative study of the behavior of dominant and subordinate male crickets after a dominance dispute is settled. From these experiments we were able to collect evidence on the differences between subordinate and dominant behavior towards different decoys.

Keywords: mixed-society, cricket, agonistic behaviour

1. Introduction

In biology, studying animal social behavior requires the observation of elaborate uncontrollable agents interacting with each other and with the environment, resulting in a very complex system. We propose a setup that allows consistent controllability and repeatability in insect behavior studies. Recently it has been shown how miniature robots can help with the study of the social behavior of cockroaches [1]. In an attempt inspired by that work, we used micro-robots for the behavioral study of field crickets. In contrast to [1] we do not attempt to faithfully mimic insect behavior. Instead we use the miniature robot for controlled stimulation of specific cues not necessarily constrained to those found in real insects. We believe such a multidisciplinary setup is a powerful tool for general and systematic investigation of insect behavior.

Male crickets of the species *Gryllus bimaculatus* DeGeer were used in this study. It has been shown that male crickets show different behaviors depending on whether they were in contact with a male or a female [2]. The cuticular substances on the body surface of a female introduce courtship behavior in males. On the other hand, when male crickets encounter each other and perceive opponent cuticular substances they engage in agonistic behavior involving aggressive stridulation and violent battles, from which a single winner is normally unambiguously defined while the losers flee. This aggressive behavior consists of a very stereotyped sequence of actions. The battle starts out slowly and escalates into a fierce struggle [3]. If an opponent cricket does not give up attacking, they increase aggressiveness to open their mandibles and grapple with each other. As a result of fighting, they establish a dominant-subordinate relationship. The subordinate then actively avoids the victorious cricket for a certain period of time [4, 5]. Although several works report these cricket behaviors, no one has a good understanding of the neuronal mechanisms underlying them. One reason for this is the technical challenge faced by researchers.

Experiments have been performed with crickets [4] where different stimulus cues were isolated in different modalities in order to build ethograms and better understand the mechanism underlying the regulation of the agonistic behavior.

Following a similar principle, probed different sensory cues, but we also extended the possibilities allowing the use of dynamic mechanical movements thanks to the introduction of a miniature robot. Usually biologists can observe an animal's behaviors, but can't control them. By playing back the same sequence of movements in each trial and comparing results from trials that used robots, with and without attached artifacts, we can study the influence of the different stimuli in an agent executing controlled behavior. This allowed us to successfully modulate the behavior of male crickets through the addition of different cues to the robot.

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 × 225 mm delimited by opaque acrylic walls of 150 mm of height, separating it from the external environment of the lab.

The experiments were designed to focus on how individual crickets behaved after a dominance dispute when exposed to a new external agent – a robot with a decoy

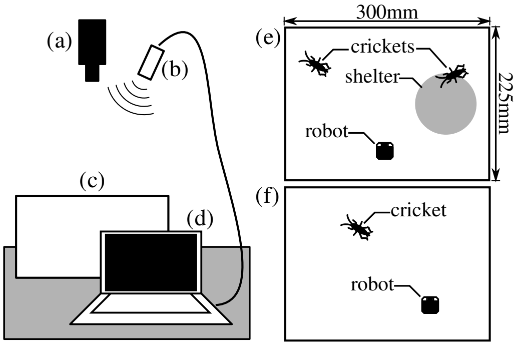


Fig. 1. Sketch of the experiment setup depicting (a) camera, (b) infrared-transmitter, (c) arena, and (d) computer. On the right a top view of (e) the arena layout used in preliminary tests, and (f) the arena layout used in the main study.

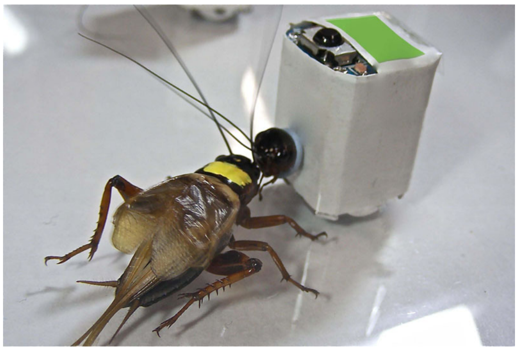


Fig. 2. Close-up picture of a male cricket interacting with a robot equipped with the decoy of another male cricket's head.

(**Fig. 2**). Trials were performed looking for subtle changes in the way subordinate and dominant agents might differ in their following or escaping behaviors and how these crickets react to the different kinds of decoys. **Fig. 3** illustrates some of the decoys used in our preliminary studies. Later a more exhaustive study was performed that focused on the living head decoy, which included an insect head and prothorax, as illustrated in **Fig. 3(f)**. Attaching a living head to a robot allows the control of the movement of the agent while keeping not only pheromones and shape silhouette but maintaining also a repertoire of cricket behaviors such as antenna fencing, mandible flare, biting, and stepping reflex on the frontal leg pair.

2.1. Cricket

The animals were reared in plastic cases (800 × 450 × 200 mm) on a 14h : 10h light and dark cycle at 28 ± 1°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*. 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 hours before the experiment, where they could potentially see, hear and smell conspecifics but could not get involved any kind of tactile interaction.

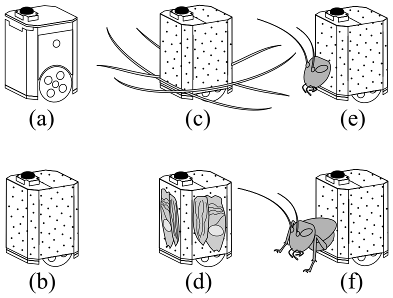


Fig. 3. Different types of decoys tried in the preliminary studies. (a) just robot, (b) robot wrapped in paper (c) robot wrapped in paper with acrylic wires; (d) robot wrapped in paper with forewings of male crickets; (e) robot wrapped in paper with a dead male cricket head attached; (f) robot wrapped in paper with a living head of a male cricket (cut after prothorax).

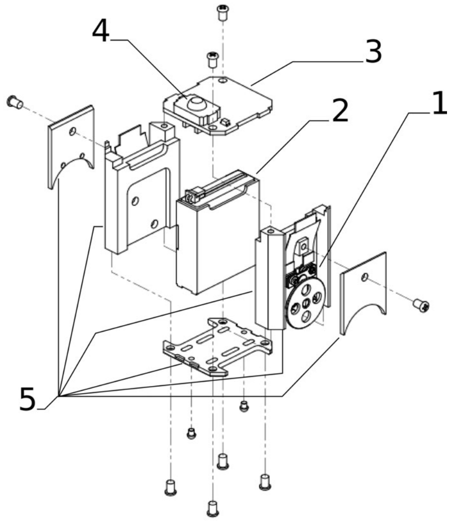


Fig. 4. Exploded view of the robot revealing its components.

2.2. Robot

The micro-robot employed in the experiments is shown in **Fig. 2**. Originally designed for an educational robotic competition [6, 7] these robots have a size comparable to that of typical crickets. The robot has dimensions of $18 \times 18 \times 22$ mm and is driven by two differential wheels, has no sensors except for an infrared receiver used for receiving commands encoded into pulses of infrared light. Commands were pre-recorded in a computer and played in a loop causing the robot to move without the use of any feedback. A short series of small random movements was performed before each repetition in order to disturb the trajectory thus avoiding systematic preference towards specific paths.

The main robot parts are (numbers according to **Fig. 4**):

1. Motor – customized from 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 – currently based on the microchip 8 bit PIC18 family of microcontrollers, each robot comes equipped with a PIC18LF1220 which features 4 kb of re-programmable flash memory;
4. IR sensor – an IR 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.

2.3. Tracking and Data Processing

For the automated tracking of moving insects some versatile and complete commercial solutions exist [8]. In this work the authors opted for SwisTrack, a simple open source solution [9]. Initially, in the preliminary studies image processing was performed in 4 steps:

1. background subtraction;
2. binary threshold operation;
3. masking out of unnecessary areas;
4. inflation & 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 Tsai's method [10];
3. tracking using nearest neighbors.

Robot and crickets were marked with different colors in order to allow automatic tracking (as depicted in **Fig. 2**). A calibrated three channel RGB binary threshold operation was used for segmentation of the color of each of the agents. The tracking was performed in two passes, one for each agent color. This solution was proven very robust and the processing of the whole batch of videos could be completely automatized through the use of computer scripting.

The resulting tracking data was then post-processed in Python starting from the computation of lower level features (such as relative distances between agents, absolute and relative velocities, velocity cross-correlations), building all the way up to more qualitative concepts related to the interaction between the involved agents (such as number of times they encountered each other, encounter durations, etc.).

3. Results

The few results here described serve to validate and demonstrate the use of our setup in practice. As a sample experiment we tested a simple hypothesis: that subordinate crickets are more sensitive to external stimuli than dominants. Since subordinate crickets flee and avoid engaging in dominance disputes we hypothesized that these individuals would show lower tolerance for engaging in physical interactions. To test this hypothesis we decided to measure how subordinate and dominant crickets behaved regarding their following and escaping behaviors when they encountered another agent.

In each trial two new male crickets were left to freely interact with each other inside the arena for five minutes or longer until a dominant/subordinate relationship could be clearly observed, and then depending on the group being studied either subordinate or dominant would be 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 (naive, kept isolated for 1 night).

When designing the movements of the robot the goal was not necessarily to 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. A sequence of moves was handcrafted with the help of a gamepad so as to allow the robot to stochastically cover a 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. 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).

For data analysis, first, all trials were separated into three different kinds depending on the type of agent added last to the arena. Then each of these three kinds was further divided in two groups according to whether the subordinate or the dominant was the cricket left inside 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**.

For every trial the raw trajectories from the recorded videos were tracked and a smoothing operator with a window of 30 frames (roughly 1 second) was applied. The resulting smoothed trajectories were then used for finding all disjoint intervals in which the distance between the two studied agents was less than 40 mm. Those were called *encounters*. For each of those encounters, escape and follow behaviors were computed as follows:

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

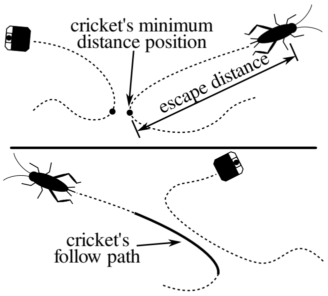
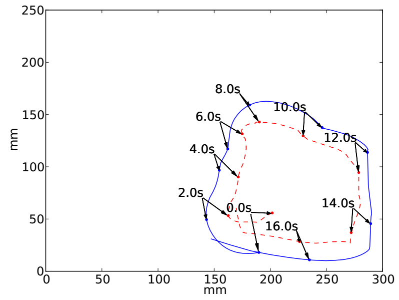


Fig. 5. Illustration demonstrating the metrics used in the results. Top: escape distance. Bottom: follow path.

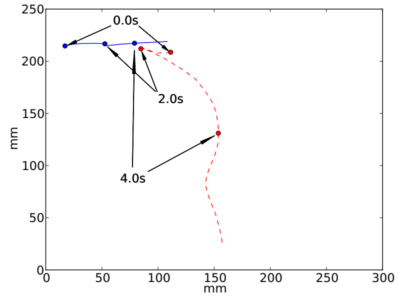
1. *Escape distance*: For each encounter the frame of minimum distance between the two agents was marked as *touch frame*. After each touch frame, the first frame in which the distance between both agents was more than 45 mm and the velocity of the cricket crossed below the lower threshold of 2 mm/s was marked as *escape frame*. The *escape distance* was computed as the distance between the position of the cricket at the touch frame and its position at the escape frame.
2. *Follow duration* and *follow length*: If the duration of the encounter was longer than 2 s and the displacement of the robot during that encounter was more than 20 mm this was considered a *follow*. The duration and path length were then respectively labeled as *follow duration* and *follow length*.

Note that item 1. above was computed for all encounters while item 2. was computed only for those where the conditions held, i.e., by definition all encounters terminated in an escape (encounter terminated by robot or cricket or both), but not all encounters had a follow. The metrics *escape distance* and *follow length* are illustrated in **Fig. 5**. **Fig. 6** shows typical trajectories in a robot/cricket trial.

Statistics of the results were calculated by mixing together data of the different trials of same group, thus yielding for each group the combined collection of data from all trials. For all 15 two-group permutations the Mann-Whitney U-test (MWW U-test) was performed in order to access the statistical significance. The null-hypothesis for this test is that the data of two given groups are samples from a same population. The approximate probabilities associated with those U-values found were computed assuming a two-tailed normal distribution.



(a) Sample follow



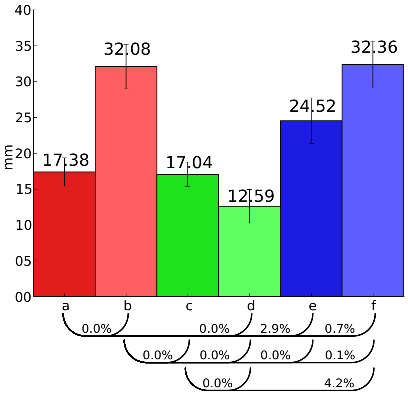
(b) Sample escape

Fig. 6. Sample of trajectories of cricket (dashed line) and robot (solid line): (a) during a follow and (b) during a escape.

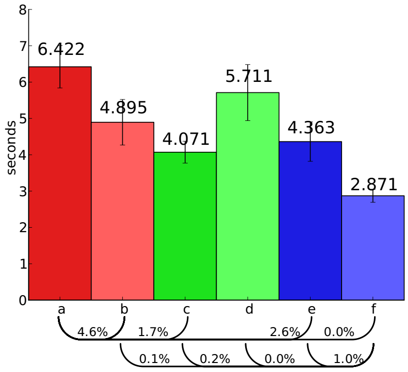
Results are plotted in the graphs shown in **Fig. 7**. The MWW U-test results for the cases where $p \leq 10\%$ are depicted at the bottom of each graph. These graphs show evidence that when interacting with other crickets as well as when interacting with a robot with cricket head attached subordinates had:

1. longer escape distances than dominants;
2. shorter follow path lengths than dominants;
3. shorter follow durations than dominants.

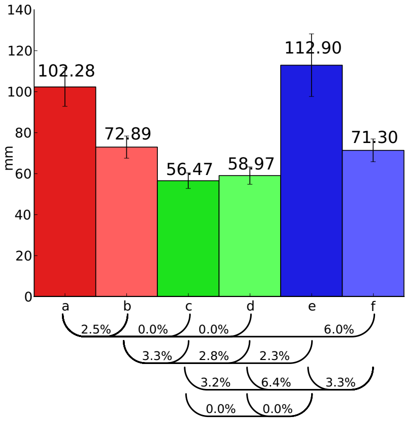
Interestingly the same observations did not hold when crickets interacted with plain robots. This suggests that subordinate crickets are indeed more sensitive than dominants when they encounter a robot with a cricket head attached or when they encounter another cricket. On the other hand they seem not to regard the plain robot in the same way. When dominant and subordinate crickets encountered plain robots their following and escaping behavior did not seem to change significantly. This indicates that subordinates only change their sensitivity towards conspecifics, probably discriminated by cuticular pheromones and cues other than movement alone.



(a) Escape distance



(b) Follow duration



(c) Follow path length

Fig. 7. (a) shows the average cricket's escape distances, (b) shows follow durations and (c) shows the followed path length. The height of each candle bar represents the mean over all encounters of that group and the lines on top show SEM. Alphabet letters label the respective group as seen in **Table 1**. The curved lines with percent values show level of significance for cases where $p \leq 10$ % (computed from the MWW U-test assuming normal distribution, two-tailed).

4. Discussion

A behavioral research framework based on the multidisciplinary mixing of micro-robots and insects has been presented. Our setup has the potential of allowing 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.

From the results we managed to find some evidence that subordinate crickets are indeed more sensitive, but only toward selective cues. This shows how the mixed society setup can be used for probing crickets' behavior toward different cues.

It has been shown how electrophysiological recording using copper wire can aid the study of neuronal activities in a free moving insect [11]. For future work we plan to combine the use of micro-robots and electrophysiological recording of neural activities on the brain. This will help us understand how animals receive, integrate and process information about the environment, and how to use this to produce motor signals, thus giving us a better understanding of the neuronal mechanisms underlying certain behaviors.

The authors believe the presented setup is a great aid for the investigation of social adaptive mechanisms in insects and toward the construction of animal/robot societies.

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- The Japan Society of Mechanical Engineers (JSME)
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