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# Possible Role of Polarized Light Information in Spatial Recognition in the Cricket *Gryllus bimaculatus*

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**Many insects are able to use skylight e-vector patterns to deduce their heading direction. Crickets have been well known to orient themselves to certain e-vector orientations to keep their walking direction. However, it is still unknown if crickets are able to utilize polarized light information for spatial recognition. Using an experimental paradigm similar to the Morris water maze for rodents, here we examine the possibility that the cricket *Gryllus bimaculatus* can utilize polarized light information to find the target place. Crickets were placed in a round arena with a heated floor, a portion of which was cooled, and a cross-shaped e-vector pattern was presented from the top of the arena so that the cricket could find the cool spot by walking along the e-vector direction. When the arrangement of the e-vector pattern and the cool spot were fixed throughout the experiments, the time and the walking distance to find the cool spot were significantly decreased with increasing trials, but not when the e-vector pattern was rotated between each trial. Moreover, a model selection indicated that the visual stimulus contributed to the decrease in time and distance. To investigate the cricket's exploration patterns in the arena, a test trial in which the whole floor was uniformly heated was performed before and after the training trials. In the test trial, the crickets trained with the positionally fixed e-vector pattern showed wall-following behavior for a significantly longer time than those untrained and those trained with random e-vector patterns.**

**Key words:** insect, navigation, spatial recognition, polarization vision, e-vector

## INTRODUCTION

Many insect species have a remarkable ability of navigating to reach their intended destinations, which has been a strikingly intriguing topic for biologists for a long time (Collett et al., 2013). The celestial polarization pattern is an important source of information for insects to deduce their heading orientation (for review, see Wehner and Labhart, 2006). Sunlight is scattered by air molecules and is partially linearly polarized, and thus particular electric vectors (e-vectors) dominate in the light from any point in the sky. As a consequence, a polarization pattern of concentric circles around the sun is created (Strutt, 1871). A number of behavioral studies in central place foragers such as ants (Wehner and Müller, 2006) and bees (von Frisch, 1949; Evangelista et al., 2014) have shown that these insects are able to exploit polarized skylight for foraging navigation. These species detect the e-vector orientation from the sky and deduce their moving direction relative to the sun azimuth. For example, moving in a direction perpendicular to the e-vector means that they are heading directly towards (or away from) the sun (Evangelista et al., 2014). By monitoring overhead e-vector orientation, insects can accumulate changes of traveling directions from a starting point, and conduct path integration (Wehner and Labhart, 2006). In addition to ants and bees, many other insects, such as monarch butterflies (Reppert et al., 2004) and dung beetles (el Jundi et al., 2014), have also been reported to use polarized skylight as compass information during navigation and orientation.

Crickets (*Gryllus* sp.) have been intensively used for studying insect polarization vision at both the behavioral and neuronal levels. The specific region of the compound eye that detects the e-vector of the polarized skylight, called the dorsal rim area (DRA), has blue-sensitive photoreceptors that project their axons to the lamina or medulla in the optic lobe (Labhart et al., 1984; Blum and Labhart, 2000). In the medulla, a group of first-order polarization-sensitive neurons, called POL1 neurons, has been identified (Labhart, 1988; Labhart et al., 2001). At the higher center of the brain, another type of polarization-sensitive neurons, known as the tangential neurons in the central complex, potentially encode compass information (Sakura et al., 2007). Behaviorally, it is known that the crickets walking on a treadmill ball exhibit spontaneous 'polarotaxis', i.e., they tend to orient themselves parallel to a certain e-vector direction, in

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response to a slowly rotating polarized light stimulus from above (Brunner and Labhart, 1987; Henze and Labhart, 2007). Field behavioral experiments using *Gryllus campestris*, which often have their own burrows in the natural habitat (Beugnon and Campan, 1989; Rodríguez-Muñoz et al., 2010), have shown that their inbound path to the burrow after foraging is seriously affected when a polarizer sheet is presented overhead (Beugnon and Campan, 1989). However, it is still unclear whether they are able to memorize a certain place using polarized light information because of a lack of an adequate experimental paradigm to test this ability. The place-learning paradigm for insects devised by Mizunami et al. (1998) gives us a great possibility to explore this. In this paradigm, which is a modification of the classic water maze used for rodents (Morris, 1981), the heated floor (instead of water for rodents) of the arena motivates the insect to escape to the cool spot (instead of a platform for rodents). Thus far, the place-learning capability based on visual landmarks on the wall has been found in cockroaches (Mizunami et al., 1998), crickets (Wessnitzer et al., 2008), and fruit flies (Ofstad et al., 2011) using this paradigm. A major advantage of this paradigm is that it allows to control the visual stimulus presented to the test subject and to examine the types of visual features used for place recognition. To examine crickets' ability to utilize polarized light information, we modified this paradigm by replacing landmarks with overhead polarized light stimuli. The polarized light cue was designed so that crickets could find a target place if they walked along a certain e-vector direction.

## MATERIALS AND METHODS

### Animals

Adult male crickets *Gryllus bimaculatus*, at least 1 week after their final molt, were used for the experiments. They were obtained from a laboratory colony, which was kept in a 12 h:12 h light-dark cycle at 28°C. Before the experiments, each cricket was isolated in a 100 mL glass beaker supplied with water and kept overnight under the same light and temperature conditions as before isolation to familiarize it with the solitary condition and to avoid any unknown effects of social interactions.

### Experimental setup

In a previous study, Wessnitzer et al. (2008) suggested that the cricket may use subtle visual information from the non-uniform illumination for space recognition, even when in the absence of obvious visual cues, such as landmarks, in the arena. To eliminate as much as possible potentially useful visual information, we constructed a square illumination panel with a white LED array (84 cm × 91 cm) (Fig. 1A). Each LED was arranged on an aluminum plate in a 3 cm × 3 cm grid pattern. To reduce the non-uniformity of the light, a white acrylic plate was put under the aluminum plate. At the center of the acrylic plate, there was a small round hole (φ = 2 cm) for video recording (see below). The whole setup, including the LED illumination and the arena (see below), was placed in a dark room so that the cricket in the arena could only receive the light from the LED illumination.

The experimental circular arena (φ = 40 cm) was designed according to a previous study (Wessnitzer et al., 2008; Fig. 1A). It

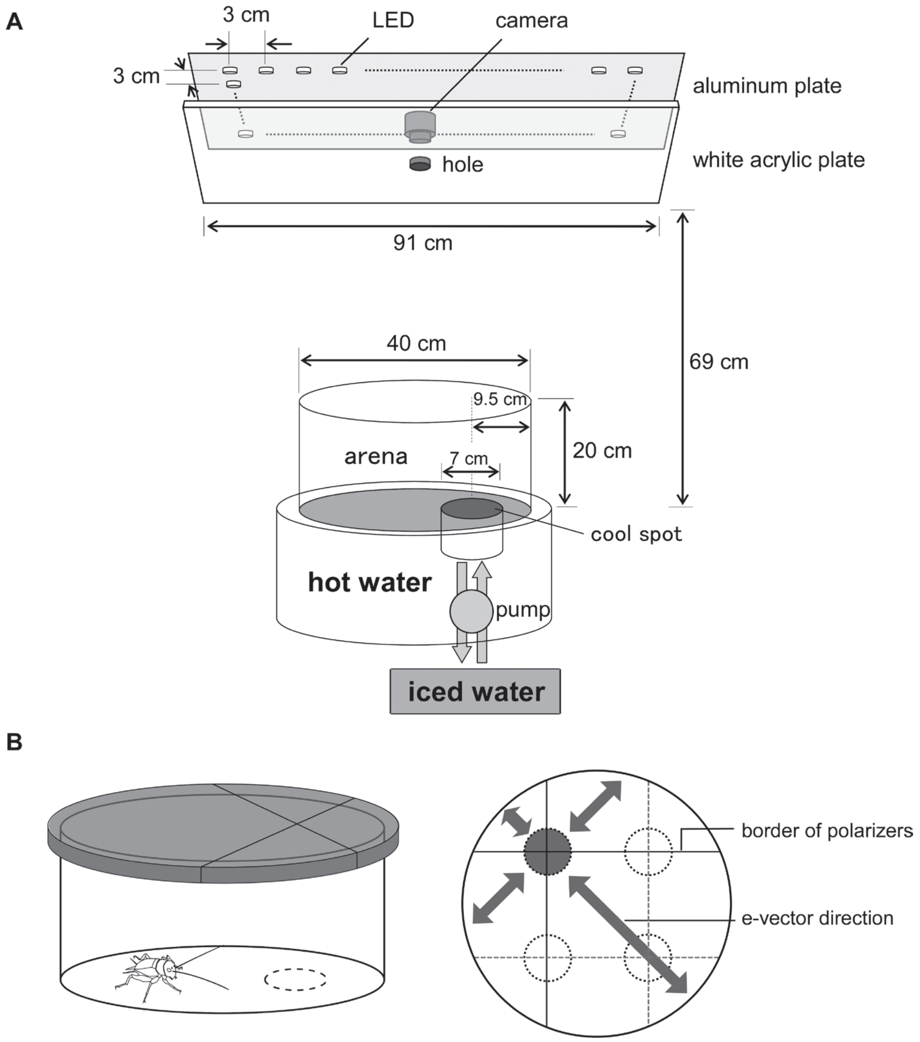

**Fig. 1.** Experimental arena and visual cues. **(A)** Schematic representation of the setup. The arena was illuminated by an overhead LED array light panel. The arena floor was heated by hot water. Only a part of the floor was cooled using iced water (cool spot). **(B)** Left: The polarizer (gray) was placed on the arena, and polarized light was delivered to the cricket. Right: Top view of the arena and the polarized light pattern. Arrows and dotted circles indicate the e-vector directions and the possible positions of the cool spot, respectively. Gray circle: cool spot location under the fixed-position condition. Open circles: cool spot locations under the random-position condition. Boundaries between nine pieces of linear sheet polarizer are indicated by dotted lines and solid lines. The solid lines indicate the boundaries where the e-vector directions changed. Note that the fixed-position polarization pattern was designed so the cricket could find the cool spot when walking along the e-vector direction.

had an acrylic white wall (height = 20 cm) and an aluminum floor heated by hot water from underneath, which forced the cricket to search for the cool spot in the arena. The temperature of the hot water was kept constant by an electrical hot plate (EC-7050, AS ONE, Japan). A part of the floor (φ = 7 cm) was locally cooled by circulating iced water using a pump (EWP-01, AS ONE, Japan), where the cricket could escape from the heat (cool spot). The location of the cool spot could not be visually identified from the floor surface, and thus the cricket had to use other cues from the surroundings to find it. The temperature of the heated floor, the cool spot, and the wall was approximately 53, 21, and 40°C, respectively. The temperature distribution of the arena floor was measured using an infrared thermography camera (F30, Nippon Avionics, Japan; see Supplementary Figure S1).

## Visual cues

As a visual cue to search for the cool spot location, an overhead asymmetrical cross-shaped e-vector pattern was presented to the cricket by putting a polarizer on the top of the arena. The polarizer was composed of nine pieces of linear sheet polarizer (Fig. 1B). Boundaries between pieces were symmetrically arranged in order to prevent the possibility that these boundaries might provide the cricket with locational information of the cool spot. The e-vector pattern was designed so that the cricket could find the cool spot by walking along the e-vector direction (i.e., orienting to the e-vector parallel to the body axis), with the center of the cross-shaped e-vector pattern and the cool spot adjusted to each other (Fig. 1B). The relative position of the visual cues and the cool spot was fixed across all trials (fixed-position), whereas in the control experiments, the relative position was randomly changed in each trial by rotating the cues 90, 180, or 270° (random-position). Under this condition, the e-vector pattern was not considered a reliable source of information for the cricket to deduce the cool spot location. The receptive field of the DRAs, the polarized light detectors in the compound eyes, is widely spreading upward (> 60°; Labhart et al., 1984), and therefore the cricket walking in the arena was able to sense the presented e-vector information using the photoreceptors in the DRAs. As a dark control, an acrylic sheet lined with red cellophane was used instead of the polarizer so that only the dim red light could slant into the arena. The spectral property in each visual condition was measured by an MK350S spectrometer (UPRtek, Taiwan; see Supplementary Figure S2). The spectral range of the polarized light fully covered the spectral sensitivity of the blue-sensitive photoreceptors in the DRA of the cricket (Labhart et al., 1984). Although the spectral range of the red light partly overlapped with the spectral sensitivity of the non-DRA green-sensitive photoreceptors in the cricket, they showed low sensitivity (see Zufall et al., 1989).

## General experimental procedure

### *Training trial*

Before the first training trial, each cricket was placed on the cool spot, which was covered with an upturned plastic beaker, and was allowed to stay there for 3 min for familiarization with the experimental arena (Fig. 2). After being placed in the glass beaker for 1.5 min, the cricket was gently put in the arena at a random location in the semicircular side opposite to the cool spot. A releasing point was 14.3–26.1 cm apart from the cool spot. Because the distance from the releasing point to the cool spot was not correlated with the walking time/distance to find the cool spot (see Supplementary Figure S3), we did not pay any particular attention to the releasing point for further analysis. Immediately after the cricket was placed in the arena, the polarizer was put on the arena and the training trial began. The walking behavior of the cricket in the arena was monitored until the cricket found the cool spot. We defined that the cricket found the cool spot when it stayed there for at least 10 s. To avoid heat-shock, the cricket was taken away from the arena if it failed to find the cool spot within a 5-min period. At the end of the trial, the cricket was again placed at the cool spot for 3 min by covering it with an upturned plastic beaker to allow the cricket to see the overhead e-vector pattern and facilitate the cricket's spatial recognition process. For each cricket, the training trial was repeated five times with 1.5 min intervals between trials (Fig. 2). During the intervals, the cricket was placed in a glass beaker, and the arena floor and wall were wiped with pure ethanol to remove possible chemical cues. We rotated the arena before every trial to eliminate the effect of the non-uniformity of light on learning performance. The crickets that could not find the cool spot during 5 min at the fifth trial were regarded as 'bad performers' and were excluded from the subsequent analysis. The total percentage of the bad performers throughout the study was 4.8% (none out of 33 crickets for fixed-condition, one out of 30 for random-condition, and three out of 21 for dark condition).

### *Test trial*

To confirm the effect of the training trials on the cricket's exploration pattern, a test trial was conducted 1.5 min after the first familiarization or 1.5 min after the fifth trial, using the arena with the uniformly heated floor (i.e., no cool spot) (Fig. 2). The cricket was gently put on the exact opposite side of the cool spot, the visual stimulus used for the training trial was presented again, and the exploratory behavior of the cricket was observed for 3 min.

## Data analysis

The walking behavior of the cricket in the arena was recorded by a USB camera (CMS-V37SV, Sanwa Supply, Japan) set above the center of the arena (Fig. 1A). X-y coordinates of the cricket's trajectory (spatial resolution: 1.8 mm/pixel) in each trial were extracted at 21 Hz (with e-vector pattern) or 25 Hz (with red light) in sampling rate by using an open-source tracking software (UMAtracker; Yamanaka and Takeuchi, 2018).

To evaluate the performance of each cricket in the trials, the time and walking distance to find the cool spot in each trial were calculated and statistically analyzed using Steel's multiple comparison tests. For further statistical comparisons, we constructed linear mixed model (LMM) for the differences in walking time and distance between the first and the second/third trials based on the full Bayesian method using STAN by *rstan* package (Stan Development Team, 2020) of R. In each model, the visual stimulus conditions (fixed- or random-) and trial numbers (second or third) were set as explanatory variables, and each individual was set as a random effect (Table 1). Each model was run on three chains with 5000 iterations with the first 1000 iterations as warm-up. Convergence

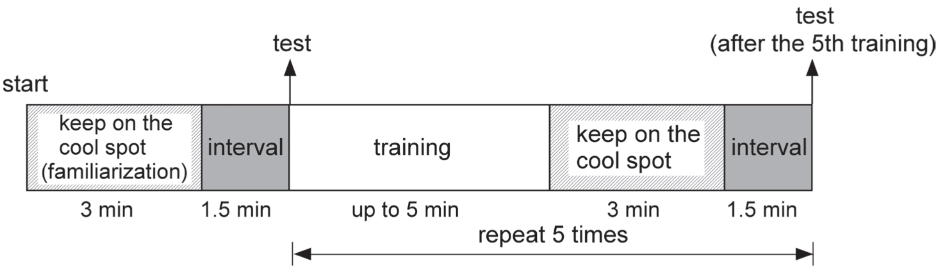

**Fig. 2.** Experimental procedure. Each cricket was trained five times with a 1.5 min interval between trials. The walking time and the distance to find the cool spot were recorded. The test trial was conducted either after the first familiarization or after the fifth training trial.

was achieved for all models, with r-hat (potential scale reduction factor) values < 1.1. Model selection was made using Watanabe-Akaike information criterion (WAIC; Watanabe, 2010).

In the test trial, the walking trajectories were compared between the crickets trained with fixed-position cues and those trained with random-position cues. As mentioned in a previous study, the cricket in the arena often shows wall-following behavior, and this may affect their performance to locate the target (Wessnitzer et al., 2008). Hence, we classified the trajectories into two categories: wall-following and non-wall-following. We defined wall-following when the cricket walked within 3 cm from the wall, and non-wall-following when it walked in the rest of the area. The total times spent for wall-following and non-wall-following within the 3-min trial were calculated and statistically analyzed using Steel-Dwass test for a multiple comparison or Mann-Whitney *U*-test for a paired comparison.

## RESULTS

Under our experimental conditions, individual crickets showed some basic features of walking behavior in the heated arena regardless of the presented visual cues. They typically showed rapid straight movements interrupted by many brief stops. In most cases, they slightly changed their heading directions after these stops. Once they ran into the wall, they often started to walk along the wall (i.e., wall-following), which was due to their thigmotactic nature. During such wall-following behavior, they sometimes stayed in the same place for a while facing and scratching the wall, likely in an attempt to escape from the heated floor. When they walked into the cool spot, they usually stopped and stayed there for a long time, but sometimes they did not stay, but rather walked past the cool spot (for an example, see Fig. 3, trial 4).

First, the crickets were trained in the arena with the overhead asymmetrical cross-shaped e-vector pattern—the center of which was always adjusted at the center of the cool spot (fixed-position). Figure 3 shows examples of walking trajectories in the five consecutive trials from one cricket trained with the fixed-position e-vector pattern. When the fixed-position e-vector pattern was presented from the top of the arena, the cricket showed a shorter walking trajectory as the trial was repeated. The time to find the cool spot significantly decreased from the first to the third and to the fifth trial in the crickets trained with the fixed-position e-vector pattern (Fig. 4A; $P < 0.05$, Steel's test). Walking distances before finding the cool spot also significantly decreased from the first to the third and to the fourth trial (Fig. 4B; $P < 0.01$, Steel's test). In the control experiments using random-position cues, we could not find any significant differences between the first and any other trials in terms of time and distance, although both showed a slightly decreasing trend (Fig. 4; $P > 0.05$, Steel's test). In addition, we also performed another control

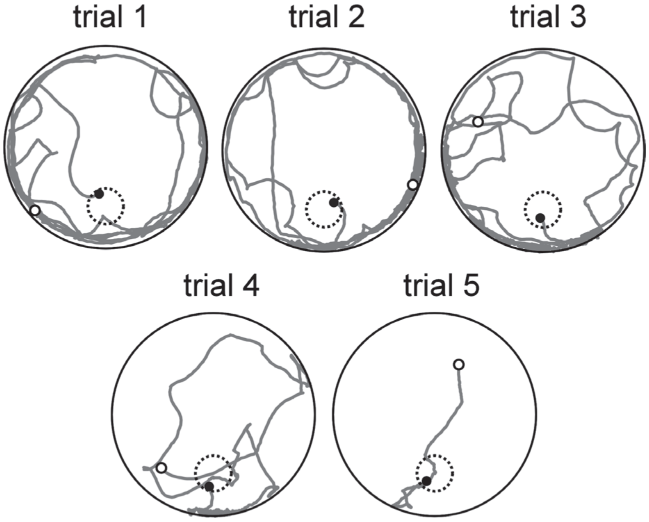

**Fig. 3.** Walking trajectories in the training trials. Examples of walking trajectories in the arena from one individual trained with the fixed-position e-vector pattern in each trial. Dotted circles indicate the position of the cool spot. Open and filled small circles indicate the start and end points, respectively.

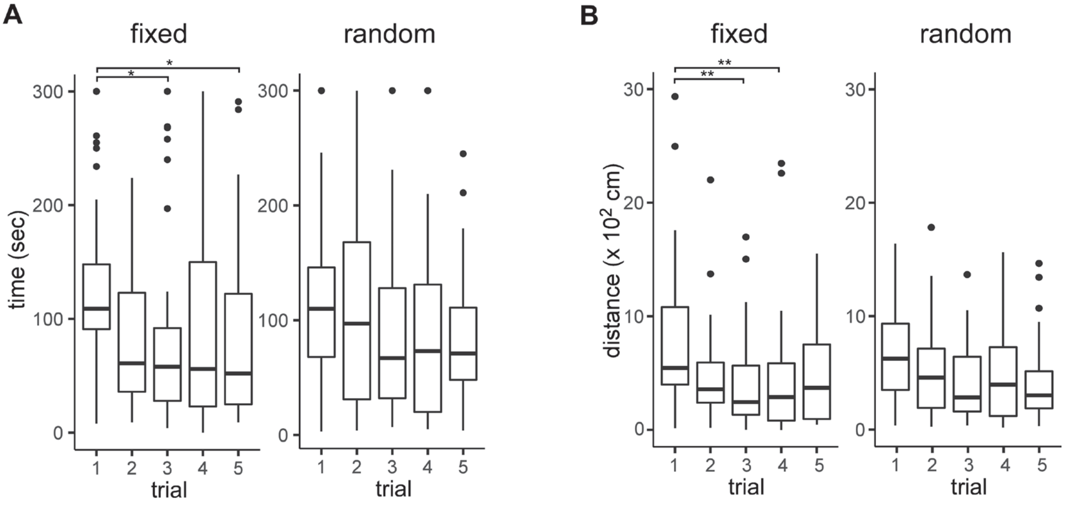

**Fig. 4.** Changes in walking time and distance during training trials. Boxplots with median values for the time needed **(A)** and the distance traveled **(B)** by the crickets to find the cool spot after being trained with fixed- (left, $n = 33$) or random-position (right, $n = 29$) e-vector patterns. The edges of the box indicate the higher and lower quartile, respectively. Whiskers indicate the higher and lower 95% confidence intervals. Dots are outliers. * $P < 0.05$, ** $P < 0.01$; Steel's test.

experiment in which only a dim red light was used. Under this condition, the cricket in the arena hardly received any visual information from the surroundings. Similar to the experiments using the random-position pattern, there was no significant change between the first and any other trials in terms of time and distance (Fig. 5; $P > 0.05$, Steel's test).

Both walking time and distance showed the first significant decrease at the third trial under the fixed-position condition. We therefore calculated decrements in time and distance from the first trial to the second and the third trials to compare them between the fixed- and random-position conditions (see Supplementary Figure S4). The decrement values under the fixed-position condition tended to be distributed more broadly than those under the random-position condition and the median was always lower under the fixed-position condition compared with that under the random-position condition (see Supplementary Figure S4). WAICs of LMMs for the change in time and distance showed that the best model was constructed with visual stimulus conditions as explanatory variables and each individual as a random effect (Table 1, model 3 and 7; WAIC = 1471.8 and 1875.1), suggesting that the presenting polarized light pattern at least partly affected the crickets' behavioral performance. The best model did not include trial numbers as an explanatory variable, which seemed to reflect that the decreases of walking time and distance were almost fully achieved during the second trial (Table 1, see Supplementary Figure S4). Taking these results together, we concluded that the crickets were able to utilize the presented visual stimulus, possibly the e-vector pattern, to locate the cool spot.

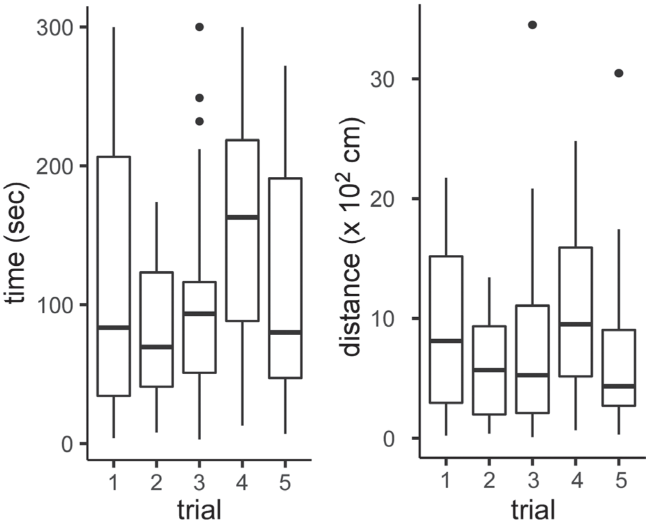

**Fig. 5.** Changes in walking time and distance under dark conditions. Boxplots with median values for the time needed (left) and the distance traveled (right) by the cricket to find the cool spot in the dark control condition ($n$ = 18). The edges of the box indicate the higher and lower quartile, respectively. Whiskers indicate the higher and lower 95% confidence intervals. Dots are outliers.

To confirm whether the crickets after the training trials search for the cool spot location based on the e-vector pattern, we conducted the test trial after the first familiarization (i.e., naive) or after the 5th trial, in which the walking pattern of the cricket was observed in an arena without a cool spot. Typical examples of walking trajectories in the test trial are shown in Fig. 6. Surprisingly, the crickets did not show apparent searching behavior around the cool spot location nor clear heading preferences along the e-vector

**Table 1.** WAICs of LMMs for the difference in walking time and distance with different parameter sets.

| model | response variable | explanatory variable | random effect | WAIC |
|---|---|---|---|---|
| 1 | difference in walking time | trial, stimulus condition | individual | 1473.1 |
| 2 | difference in walking time | trial | individual | 1476.0 |
| 3 | difference in walking time | stimulus condition | individual | 1471.8 |
| 4 | difference in walking time | – | individual | 1474.1 |
| 5 | difference in walking distance | trial, stimulus condition | individual | 1875.9 |
| 6 | difference in walking distance | trial | individual | 1876.5 |
| 7 | difference in walking distance | stimulus condition | individual | 1875.1 |
| 8 | difference in walking distance | – | individual | 1876.2 |

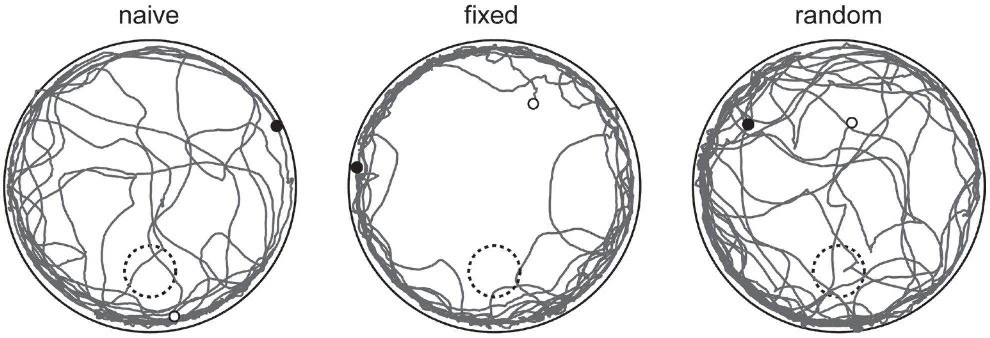

**Fig. 6.** Walking trajectories in the test trials. Examples of walking trajectories in the arena of one individual after the first familiarization (naive) and two individuals trained with fixed- (fixed) or random-position (random) e-vector pattern after the fifth training trial. Dotted circles indicate the position of the cool spot. Open and filled small circles show the start and end points, respectively. Note that the crickets trained with the fixed-position pattern showed a longer wall-following behavior than the others.

orientation even after the fifth trial with fixed-position cues. Nevertheless, we found a clear difference in walking trajectories between the crickets trained with the fixed-position cues and the random-position cues. Only the crickets trained with the fixed-position pattern showed low probability of staying in the central area of the arena (see Supplementary Figure S5, middle). For quantitative analysis, we measured the total time each cricket spent displaying non-wall-following behavior (i.e., more than 3 cm away from the wall) in the test trial. The crickets with the fixed-pattern exhibited significantly shorter time for non-wall-following behavior than either those trained with the random-position pattern or those after the first familiarization (Fig. 7; $P < 0.05$, Steel-Dwass test). On the other hand, total time of non-wall-following behavior was not different between before and after the training when the crickets trained with dark control condition (Fig. 8; $P > 0.05$, Mann-Whitney *U*-test). This result suggests that the availability of the e-vector cue in the training procedure changes the cricket's exploration pattern in the arena.

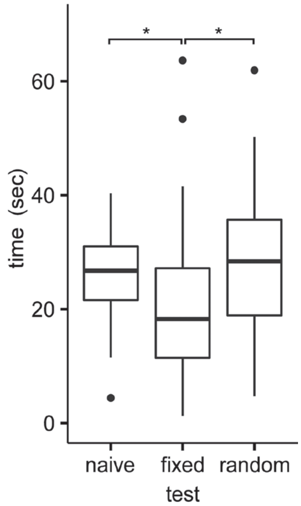

**Fig. 7.** Total time for non-wall-following behavior in the test trial before and after the training. Boxplots with median values for the total walking time when the cricket was over 3 cm apart from the arena wall. The test trial was conducted either after the first familiarization ($n = 30$ for naive) or after the fifth training trial ($n = 33$ for fixed-position; $n = 29$ for random-position). The edges of the box indicate the higher and lower quartile, respectively. Dots are outliers. Whiskers indicate the higher and lower 95% confidence intervals. * $P < 0.05$; Steel-Dwass test.

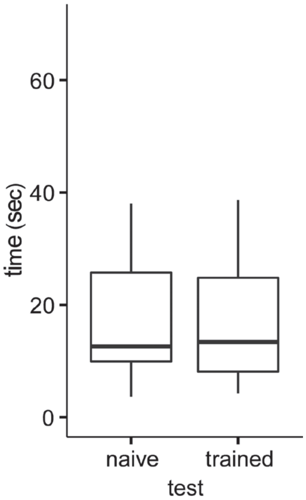

**Fig. 8.** Total time for non-wall-following behavior in the test trial before (naive) and after the fifth training trial (trained) under dark conditions. Boxplots with median values for the total walking time when the cricket was over 3 cm apart from the arena wall. The test trial was conducted either after the first familiarization ($n = 15$) or after the fifth training trial ($n = 18$). The edges of the box indicate the higher and lower quartile, respectively. Whiskers indicate the higher and lower 95% confidence intervals.

## DISCUSSION

The experimental paradigm for insects using a heated arena in this study, called the Tennessee Williams paradigm, was originally established in experiments using the cockroach *Peliplaneta americana*, in which the mushroom body in the brain has been shown to play a crucial role for place memory formation based on landmarks presented on the arena wall (Mizunami et al., 1998). Thereafter, the same paradigm was used for testing the place memory ability based on landmarks in the cricket *Gryllus bimaculatus* (Wessnitzer et al., 2008) and the fruit fly *Drosophila melanogaster* (Foucaud et al., 2010; Ofstad et al., 2011). In our paradigm, the cricket would find the cool spot by walking along the e-vector direction (i.e., orienting to the e-vector parallel to the body axis) or associating the cross-shaped e-vector pattern with the position of the cool spot. However, we could not find any indications for such kinds of learning from the trajectories after the training (Figs. 3 and 6), even though some crickets showed relatively straight paths to the cool spot (for example, Fig. 3, trial 5). Instead, the crickets showed intensive wall-following behavior (Figs. 6 and 7). The possible reason for this was probably the difference in properties as the navigational cue between the landmarks and polarized light information. The polarization pattern in the sky is, unlike with the local landmarks, a global visual cue for the moving animals and should be always stable regardless of the walking distance. In our experiment, the cricket was put in a finite space (i.e., small arena) and the overhead e-vector orientation drastically changed as the cricket walked in the arena (Fig. 1B). Under such a situation, crickets might hardly show orientation to the e-vector direction because they often reached the wall of the arena immediately after they left the wall. In fact, polarotaxis of the crickets has been observed only under slowly rotating polarized light in the infinite space (i.e., on the treadmill ball) (Brunner and Labhart, 1987; Henze and Labhart, 2007). In addition, it might be also quite hard for them to associate the typical cross-shaped pattern with the cool spot simply because such a pattern was quite unnatural for them.

In spite of such an unnatural visual condition, the crickets trained with the reliable polarized light cue improved their performance along the training trials. Under the fixed-position condition, walking time decreased significantly from the first to the third and to the fifth trial and walking distance decreased significantly from the first to the third and to the fourth trial (Fig. 4), even though the walking times/distances ranged widely among individuals, similarly to the previous study with *G. bimaculatus* using landmarks (Wessnitzer et al., 2008). These individual differences were prominent in the later trials, the fourth and the fifth trials, probably resulting from fatigue and/or heat stress that were accumulated during repeated trials, and this might be a reason why the significant decrease was found only in some pairs. To eliminate such an effect, we performed the model selection using only the data from the first to the third trial and found the best model including visual stimulus conditions as an explanatory variable (Table 1). This suggests that the availability of the polarized light pattern during the trials affected the cricket's behavior. It is, however, still not fully conclusive whether they recognized the cross-shaped e-vector pattern as a polarized light cue, as we expected, or just as a simple light intensity cue. Further experiments will be necessary to answer this question.

What made the cricket's performance improve during training trials? We found that the crickets trained with the fixed-position cue explored less in the central area of the arena compared with the untrained crickets, whereas the crickets trained with the random-position cue showed similar exploring time as the untrained crickets (Fig. 7), i.e., fixed-conditioned crickets explored less uniformly within the arena. These results indicate that the training with the reliable e-vector cue changed their exploration pattern. Although it is difficult to clarify the exact causality for this behavioral change in our paradigm, our best interpretation so far is that establishment of spatial recognition using the consistent visual environment is likely to cause this behavioral change. With such spatial recognition, the crickets were not required to explore the whole arena to find the target. As a consequence, their thigmotaxis (the wall-following behavior) might be enhanced. It has been reported that, in the Texas field cricket, *Gryllus texensis*, the amount of thigmotactic behavior was negatively correlated with the spatial cognitive performance (Doria et al., 2019). Less thigmotaxis in the untrained and the random-trained crickets in our experiment might reflect that they were attempting to recognize the arena space. There is also a possibility that the wall-following behavior was an escape from the heat for the crickets. Although it was still much hotter than the cool spot, the wall was cooler than the floor (see Supplementary Figure S1). Therefore, the area close to the wall might be regarded as a safer zone for the crickets. In the previous studies with the Tennessee Williams paradigm, intensive wall-following behavior was also observed both in the cricket and the fruit fly (Wessnitzer et al., 2008; Foucaud et al., 2010) whereas it was not in another experiment for the fruit fly using a heated ring as a border of the arena (Ofstad et al., 2011). In spite of the possibility that the wall might have acted as a safety area, the significant decrease in non-wall-following was found only in the crickets trained under the fixed-condition and this means that their wall-following behavior reflected not only escape from the heat but also their spatial recognition status.

Navigational behavior mediated by polarization vision in insects has been intensively studied in the context of foraging behavior in ants and bees (von Frisch, 1949; Wehner, 1994; Wehner and Müller, 2006; Kraft et al., 2011; Lebhardt et al., 2012). It is well known that ants and bees deduce their heading direction using skylight polarization, and that they rely on this information when moving back and forth between the food location and the nest. Some non-central place foragers, such as the cricket *Gryllus campestris* (Brunner and Labhart, 1987; Henze and Labhart, 2007), the house fly *Musca domestica* (von Philipsborn and Labhart, 1990), the locust *Schistocerca gregaria* (Mappes and Homberg, 2004), and the fruit fly *Drosophila* (Weir and Dickinson, 2012), have also been reported to use polarized light for orientation. In these species, clear polarotactic walk or flight, in which they keep their body parallel to certain e-vector orientations, has been observed, indicating that they do use e-vector information to move straight. However, it is still unknown whether these insects use e-vector information to find their destinations. In this study, we showed the possibility that the cricket *G. bimaculatus* could utilize polarized light information to find the target. To our knowledge, this is the first report to show a change in an exploration pattern depending on the availability of polarized light information in solitary insects.

E-vector detection in insects is mediated by a group of specialized ommatidia located in the most dorsal part of the compound eye (the DRA), in which the photoreceptors are monochromatic and highly sensitive to polarization (for reviews, see Labhart and Meyer, 1999; Wehner and Labhart, 2006). Electrophysiological studies using several insect species, including the cricket *G. bimaculatus*, have shown that e-vector information is encoded in the central complex, which plays a role as an internal compass (Sakura et al., 2007; Heinze and Homberg, 2007, 2009; Heinze and Reppert, 2011). Moreover, in bees, it has been shown that information regarding moving distance and direction could be integrated in the central complex (Stone et al., 2017), strongly suggesting that place memory exists in this brain region. The next step would be to elucidate the neural mechanisms underlying insect navigation, which includes clarifying how place memory is formed based on visual cues, such as polarized light, how it is stored, and how it controls navigational behavior. Compared with other eusocial insects, the cricket as a model organism offers the advantage of a relatively easy recording of neural activities in the brain (Zorović and Hedwig, 2013) and has an excellent spatial recognition ability based on visual cues.

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## COMPETING INTERESTS

The authors have no competing interests to declare.

## AUTHOR CONTRIBUTIONS

NM and MS designed the study. NM performed all the behavioral experiments. NM, RO and MS analyzed the data and wrote the manuscript.

## SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs200081)

**Supplementary Figure S1.** Temperature distribution of the surface of the arena floor.

**Supplementary Figure S2.** Spectral property of the light stimulus.

**Supplementary Figure S3.** Relationships between the distance from the release point to the cool spot and time to reach to the cool spot.

**Supplementary Figure S4.** Differences in walking time and distance between the first and the second/third trial.

**Supplementary Figure S5.** Heatmaps for the crickets' location in the test trials.

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