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Unilateral range finding in diving beetle larvae

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ABSTRACT

One of the biggest challenges that predators, such as the larvae of the diving beetle *Thermonectus marmoratus* (Coleoptera: Dytiscidae), are faced with is to accurately assess the distance of their prey. Most animals derive distance information from disparities of images that are viewed from different angles, from information that is obtained from well-controlled translational movements (motion parallax) or from the image size of known objects. Using a behavioral assay we demonstrated that *T. marmoratus* larvae continue to accurately strike at artificial prey, even if none of these typical distance estimation cues are available to them. Specifically, we excluded bilateral binocular stereopsis by occlusion, confounded possible motion parallax cues with an artificially moving prey, and excluded the possibility that beetle larvae simply approached their targets based on known prey size by presenting different prey sizes. Despite these constraints, larvae consistently struck our artificial targets from a distance of ~4.5 mm. Based on these findings we conclude that *T. marmoratus* likely employ an unusual mechanism to accurately determine prey distances, possibly mediated by the object–distance-dependent activation of specific subsets of their many-tiered and peculiarly positioned photoreceptors.

KEY WORDS: Insect, Distance estimation, Predator, Vision

INTRODUCTION

The larvae of *Thermonectus marmoratus* Gray 1831 are highly successful visually guided predators hunting prey such as mosquito or midge larvae. Their heads are characterized by two complexly organized, tubular eyes (E1 and E2) on each side of their head (Maksimovic et al., 2009; Mandapaka et al., 2006). These eyes have a bifocal lens (Stowasser et al., 2010) and multiple retinas with extremely narrow vertical visual fields. While hunting, the larvae bring the prey into the visual field of the four principal eyes and then slowly approach their target while performing dorso-ventral pivoting movements to scan their frontal visual field (Buschbeck et al., 2007). At close range, scanning movements cease, and shortly after the larvae perform a ballistic strike to capture the prey. The retinas of their principal eyes consist of many tiers, an organizational feature that in principle can be used to obtain range-finding cues (Blest et al., 1981; Collett and Harkness, 1982; Nagata et al., 2012). Specifically, distance information could be derived from the differential activation of groups of photoreceptor cells that are situated at different distances behind the lens, a mechanism that has rarely been considered. We here present evidence that the larvae of

T. marmoratus are able to successfully gauge distances even when deprived of commonly known mechanisms, and thus likely employ such an unusual range-finding strategy.

Insects typically derive distance information from the image size of an object of known size, from stereopsis and from motion parallax (for a review, see Schwind, 1989). The importance of the last two mechanisms has been demonstrated in multiple species such as mantids (Kral, 2012; Poteser and Kral, 1995; Rossel, 1983), locusts (Collett, 1978; Kral and Poteser, 1997; Sobel, 1990) and dragonflies (Olberg et al., 2005). Using an experimental arena with a well-controlled artificial stimulus that confounded motion parallax cues, we first tested whether *T. marmoratus* larvae indeed are effective in gauging the distance of a target from cues other than simply image size. Specifically, we hypothesized that if that were the case, larvae should strike from distances that are independent of the size of the presented prey. In a second set of experiments, we demonstrated that larvae maintained their ability to accurately gauge object distance, even after excluding stereoptic cues through occlusion of relevant eyes on one side of their head. As in our experimental design typical mechanisms of distance perception were excluded, but larvae nevertheless were able to correctly gauge the distance of their target, we here propose that these larvae are using an unusual alternative method.

RESULTS AND DISCUSSION

We first asked whether larvae are able to accurately gauge the distance of dummy prey independently of the object size. If larvae were using the absolute image size as their primary range-finding cue, one would expect them to strike at a target of twice the size from about twice the distance. Fig. 1A, however, shows that in our experiment this was not the case: strike distance for the larger target was not significantly different from that for the smaller target, yet it was significantly shorter than twice the strike distance to the smaller target.

To test for the necessity of stereopsis, we occluded the two principal eyes and a ventral eye (see Materials and methods) on one side of the head. Fig. 1B shows that there is no significant difference in strike distance for these unilaterally blinded animals, when compared with sham-treated controls. When challenged with targets of twice the size, occluded as well as non-occluded animals tended to strike at the target from a slightly greater distance, but this difference was small and not significant. The striking distance of the eyes-occluded animals to the larger target remained significantly shorter than twice the striking distance to the smaller prey. All significance values are based on two-tailed Student's *t*-tests.

Taken together, our results suggest that larvae could accurately gauge prey distance, even in an experimental setup where commonly used range-finding cues were excluded, or at least severely limited as discussed below.

One of the most prominent distance vision cues in insects is motion parallax, which relies on translational movements that result in closer objects moving faster across a photoreceptor array than further away objects. This distance cue is particularly effective if an

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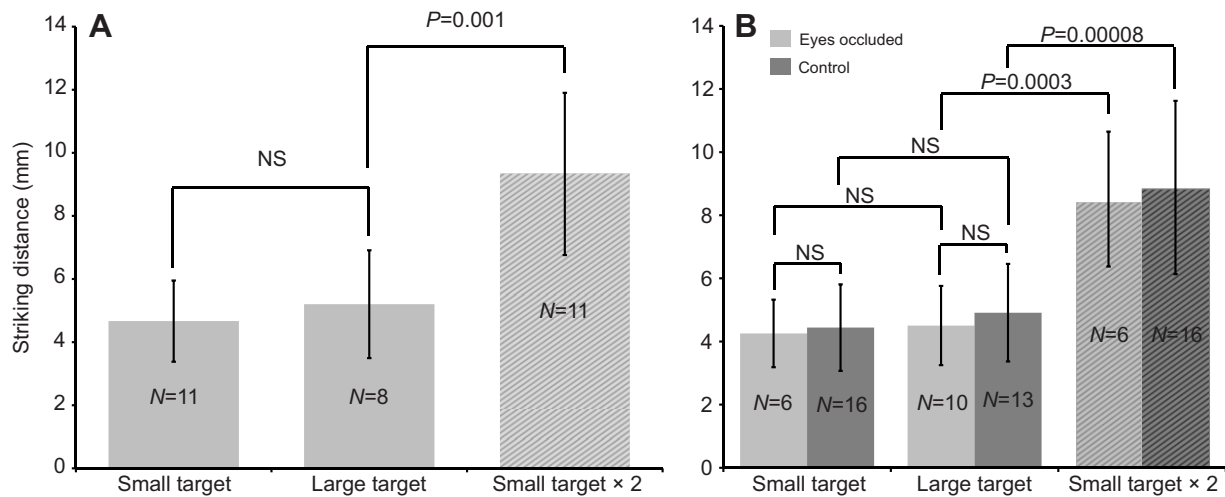§Author for correspondence (elke.buschbeck@uc.edu)

Fig. 1. Second instar larvae typically strike from ~4.5 mm, regardless of the size of the target. (A) The striking distance to the larger target is significantly smaller than two times the striking distance to the smaller target (two-tailed Student's *t*-test, *P*=0.001). (B) Similar results are observed when the principal eyes on one side of the head are occluded (two-tailed Student's *t*-test, eyes occluded *P*=0.0003, control *P*=0.00004). In both graphs, the columns on the right illustrate two times the striking distance to the smaller target (the hypothetical striking distance if larvae were to strike solely based on the angular extent of the target). In both experiments there was no significant difference between the striking distance to the larger and smaller target (two-tailed Student's *t*-test, *P*>0.05). *N* indicates the number of larvae that were tested.

animal has tight control over its own translational movements while viewing stationary objects against a structured background. This frequently is the case for peering movements (Collett, 1978; Kral, 2012). For example, a praying mantis will translate its head horizontally prior to striking a stationary target. Exaggerating their image translation can lead to systematic strike distance errors (Kral, 2012). Could *T. marmoratus* larvae therefore obtain motion parallax cues while approaching their prey? This is unlikely for the following reasons. First, the arena's background was homogeneous, minimizing information on relative movement. Second, our targets were in motion, and it is known that such motion impairs an animal's ability to use motion parallax (Schwind, 1989). This is because object movement elicits confounding translational patterns on the retina, leading to unresolvable ambiguities. Presumably for this reason even mantises, which are well known to rely on motion parallax for stationary objects, use other strategies for range finding if presented with moving objects (for a review, see Kral, 2012).

Although larvae perform dorso-ventral scanning movements while homing in on their prey (Buschbeck et al., 2007), and these movements have strong rotatory and some translatory components, there are additional reasons why these vertical movements are unlikely to have provided noteworthy motion parallax cues. First, the principal eyes of these animals have extremely narrow visual fields (see Mandapaka et al., 2006), severely limiting image resolution along the vertical axis. Specifically, their retinas extend in depth (tiering) and along the horizontal plane. However, along the vertical axis there are only dorsal and ventral photoreceptors (see Fig. 2D, inset). Therefore, within each principal eye, this organization allows each tier to detect no more than the contrast between two vertical image points at any given time. Second, the shape of our artificial prey consisted of a vertical streak, which leads to a nearly homogeneous image (with edges only at the top and bottom) during their vertical scans. Close examination of videos suggests that shortly before striking, their narrow visual fields frequently were fixated somewhere along the vertically monotonous portion of the target. Moreover, larvae frequently ceased scanning shortly prior to striking. Nevertheless, they were

able to maintain a steady distance during this time, even while the target continued to move (see supplementary material Movies 1 and 2). Horizontal movements of the larvae are also unlikely to provide motion parallax cues, because they are primarily observed at relatively large distances while the animal orients to the prey (Buschbeck et al., 2007). During prey approach, in contrast, horizontal movements tend to be limited to tracking the prey (A.S. and E.K.B., personal observation), which would not result in translational patterns on the retina and hence are unlikely to provide utilizable distance cues.

Taken together, it is highly unlikely that larvae could have obtained sufficient motion parallax cues in our setup. It remains unclear to what extent such cues play a role under natural conditions. However, frequently moving prey, a nearly homogeneous background and limited control over water perturbations may provide major constraints in that regard. It is conceivable that such constrains have driven the evolution of alternative strategies.

Another important distance cue for insects is stereopsis, which relies on systematic image differences between two eyes (Schwind, 1989). Our second experiment (Fig. 1B) demonstrated that larvae can strike from consistent distances even if eyes on one side of the head are occluded, suggesting that triangulation between the two sides of the head is not necessary. As larvae have two principal eyes on each side of the head, we also need to ask whether there could be triangulation between the dorsal and ventral eye. However, these eyes are very closely positioned and hence would gain limited depth information, even if images could be resolved sufficiently along this axis. Making some assumptions, this can be quantified by a calculation (Eqn 1) described by Collett and Harkness (Collett and Harkness, 1982).

$$\Delta'd_{\text{further}} = s^2\alpha' / (a - s\alpha')$$
$$\Delta'd_{\text{closer}} = s^2\alpha' / (a + s\alpha') \, , \tag{1}$$

Here, $\Delta'd$ is the minimum distance that an object must be from a target distance in order to be distinguishable. If the preferred strike

distance s is 4.5 mm, the interocular separation (based on our unpublished histology) a is 0.2 mm and α' (corresponding to the 1.5 deg proximal photoreceptor vertical acceptance angle that was determined from preliminary physiology) is 0.026 radians then $\Delta'd_{\text{further}}$=6.5 mm and $\Delta'd_{\text{closer}}$=1.7 mm. The preferred striking distance of 4.5 mm therefore would be indistinguishable from distances between 2.8 and 11 mm, presumably leading to frequent failures. In addition, as discussed in the previous section, our experimental design in combination with the larvae's anatomy highly limits the information that could have been obtained along the vertical axis.

As larvae were able to gauge distance, even when common distance-measuring mechanisms were not available, we have to ask what other distance cues were available to them. Given the elaborate larval eye organization, we propose that the most likely explanation

is a mechanism that relies on specific activation of photoreceptors of different retina tiers of one or both of the two principal eyes. Recently, a novel monocular distance sensor was described in a jumping spider (Nagata et al., 2012), a particularly intriguing parallel, because the principal eyes of jumping spiders are similarly tubular, and they too contain a distinctly tiered retina that is considered essential for the described distance-estimation mechanism. While the details of the mechanism need further investigation, we here propose that *T. marmoratus* are able to derive distance information unilaterally from the details of how images are focused within the retinas and retinal tiers of their principal eyes. To the best of our knowledge, a comparable mechanism has not yet been proposed for any insect.

MATERIALS AND METHODS

Thermonectus marmoratus larvae were offspring of our laboratory colony. Slightly starved second-instar larvae were transferred into the test arena and given 15 mins to acclimate prior to a 15 min test period, during which a motor-driven dummy prey, that was novel to the larvae, was moved in small horizontal circles (~1 cm diameter) at ~12 revolutions min^{-1} (Fig. 2A). The dummy prey consisted of a vertical black rod that was either 0.36 mm wide and 5 mm long, or twice those dimensions. This orientation and these dimensions are compatible with prey that these larvae naturally hunt and were accustomed to hunt prior to the experiment. Some animals were painted 1 day prior to testing with opaque nail polish (Chrome, Sally Hansen, Farmingdale, NY, USA) so that E1 to E3 were unilaterally occluded (test animals; Fig. 2B). The opaqueness of the nail polish was confirmed under the microscope. Occlusion of the ventral E3 was necessary to maintain a natural hunting position. Sham controls were painted on top of the head (Fig. 2C). Prior to application of nail polish, animals were transiently cooled on ice and head regions were dried with cotton.

The animals' hunting behavior was filmed directly and as a reflection from a 45 deg mirror. The absolute distance of the larval eyes to the strike-point on the artificial target was obtained from 3D coordinates of several points that were visible in both perspectives. Actual distances were calculated after calibration of the images to the prey size. The frame that immediately preceded the strike was used to record the position of the corners of the artificial target, as well as the position of the center of the eye cluster (Fig. 2D). The strike trajectory and strike point on the target were determined from the consecutive frame. To avoid learning effects, for each individual, only the first successful strike at the novel artificial target was included in the data analysis, though succeeding strikes yielded similar results (data not shown). After each trial it was confirmed that the larvae's eyes were still occluded.

Supplementary material
Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.092833/-/DC1

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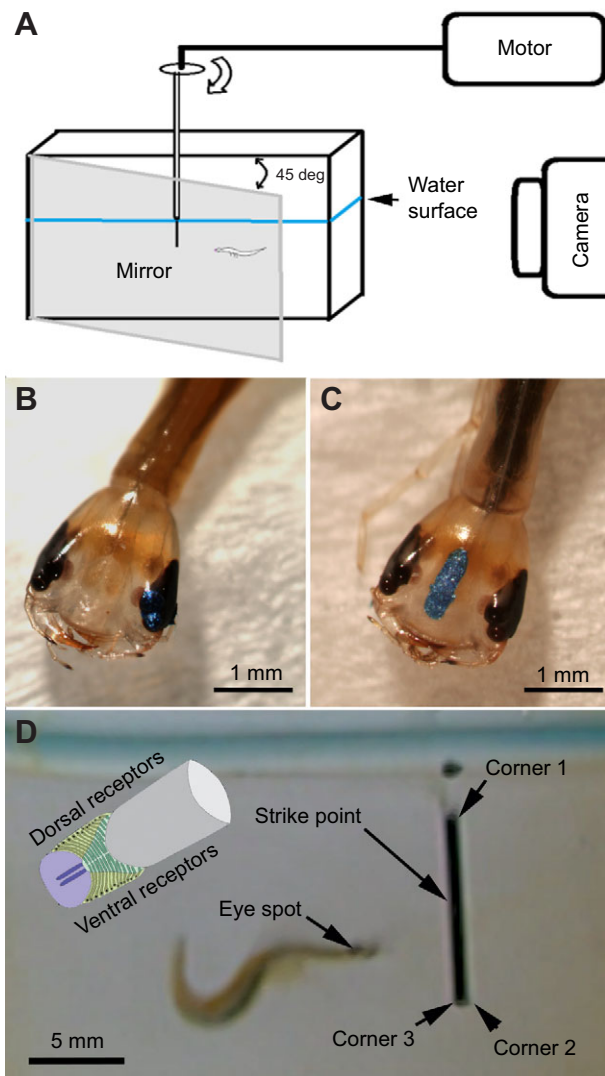
Fig. 2. Experimental set up of beetle larvae attacking an artificial target. (A) Arena. (B) To occlude the principal eyes on the left side of the head, opaque nail polish was applied. (C) Control animals were painted in the center of their heads. (D) Three-dimensional coordinates of key points (as shown) were determined from direct and mirrored video frames, and used to calculate the strike distance within the 3D space. The inset depicts a schematic diagram of a sagittal section of the principal eyes, illustrating the limited extent of the retina in the vertical direction as well as the tiering of the distal photoreceptors.

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