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BEHAVIOURAL RESPONSES OF THE TIGER BEETLE LARVA TO MOVING OBJECTS: ROLE OF BINOCULAR AND MONOCULAR VISION

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Summary

The larva of the tiger beetle *Cicindela chinensis* is an ambushing hunter with a body length of 15–22 mm that lives in a tunnel in the ground. It ambushes prey, keeping its head horizontal at the opening of the tunnel. When prey approaches the tunnel, the larva jumps to snap at it. When an object moves beyond its jumping range (approximately 15 mm), however, the larva quickly withdraws deep into the tunnel. These responses are mediated by two of six pairs of stemmata. How does the larva judge the hunting range using such a simple visual system? A previous study suggested that both binocular and monocular vision are used for distance estimation. Range estimation by binocular vision was further confirmed in the present behavioural observations: larvae jumped towards objects beyond the normal hunting range when virtual images of such distant objects were formed close to the larva using prisms or a narrow window. A possible mechanism involved in range estimation by monocular vision was also examined in behavioural experiments. The depth of the image in the retina appears to play a role in distance estimation because a larva with one functional stemma, the other stemmata being occluded, changed its response to a very distant object from an escape to a predatory jump when a concave lens was placed above its head. Two alternative ideas, based on optical and morphological data, are proposed to explain this behavioural change by the one-stemma larvae. First, as for myopic people, the larva might see clearly only objects that are close. Second, an infinitely distant object might produce a focused image only on the central part of the retina, whereas an object within hunting range (<15 mm) might do so on surrounding regions of the retina. The latter idea implies that the region of the retina at which the larva perceives a clear image is concerned with which type of behaviour is released, a predatory jump or an escape. We conclude that visual information about hunting range in the tiger beetle larva is extracted both peripherally by the spatial pattern of image clarity and centrally by binocular vision.

Key words: vision, stemma, larval eye, stereopsis, tiger beetle, *Cicindela chinensis*, insect.

Introduction

To judge the distance of an object is one of the most important functions of vision. Most vertebrates, including humans, estimate distance (depth) in multiple ways (e.g. from stereopsis, from feedback from focusing of the lens and from visual experience). It is also clear that some insects must also accurately estimate distance to objects, because flying insects rarely collide with obstacles. Since an insect possesses eyes with a fixed dioptric apparatus and an immobile retina and has an extremely small brain, it is unlikely that the insect measures the distance to the object using the same mechanisms as those used by vertebrates.

One of the likely mechanisms for distance perception in insects is motion parallax, in which the distance to an object is encoded by the movement velocity of its retinal image caused by self-motion of the animal: the image of a close object is displaced faster than that of a distant one. Behavioural experiments on flight control in flying insects, such as flies and bees, and on the head-swaying of stationary insects before movement all support a role for motion parallax in distance measurements by insects (e.g. Wallace, 1959; Collett, 1978; Egelhaaf, 1985; Horridge, 1986; Srinivasan et al., 1989; Kern and Varju, 1998). The participation of binocular vision has also been reported in gauging the distance to prey in the praying mantis (Rossel, 1983; Rossel, 1986), but seems to be less common for insects in general, perhaps because the interocular separation is extremely small in insects compared with that in vertebrates. Some other mechanisms have also been proposed for depth perception in insects, but these mechanisms have not been fully explained (for a review, see Schwind, 1989).

Mizutani and Toh (Mizutani and Toh, 1998) reported that larvae of tiger beetles *Cicindela chinensis* could visually discriminate objects within their hunting range, which is less than 10–15 mm, from those beyond that range, and they proposed two mechanisms for such range perception. The tiger

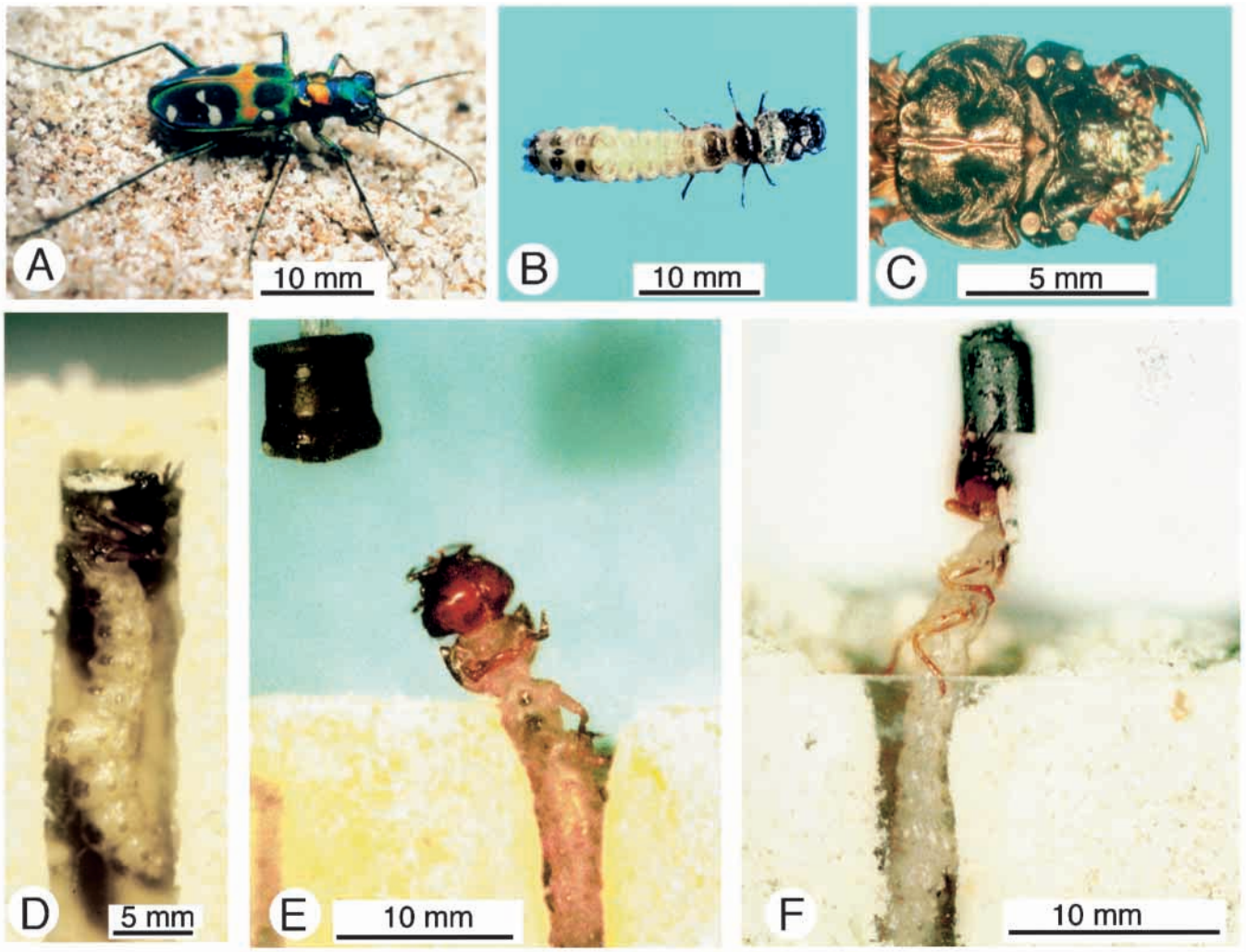

Fig. 1. (A) A tiger beetle, *Cicindela chinensis*. (B) A larva of *C. chinensis*. (C) A dorsal view of the head and prothorax of the larva. Note the two pairs of large stemmata. (D) A larva in the ambushing posture in its tunnel. The larva was released into a narrow space between a pair of glass sheets. The space was filled with soil, in which the larva dug its tunnel. (E,F) Larvae jumping towards an object moving horizontally above their head. The object is a short rubber rod. (E) Ventral view, (F) lateral view.

beetle is a running predator (Fig. 1A), but its larva lives in a tunnel in the ground (Fig. 1B–D) and ambushes prey animals, keeping the dorsal surface of its head at the level of the mouth of the tunnel. When an appropriate animal approaches the tunnel, the larva jumps to snap at it (Friederichs, 1931; Hori, 1982; Gilbert, 1989). Unfortunately, neither ambushing larvae nor jumping larvae, as shown in Fig. 1, can be observed in the field because the larvae escape deep into their tunnel before an observer can approach them. Visually guided predatory and escape behaviour can be observed, however, in the laboratory (Fig. 1E,F).

In our experimental arrangement, larvae jumped towards small objects moving at a height of 10 mm, but showed escape behaviour when the size of the object was increased. In response to moving objects at a height of 50 mm, they never showed a predatory jump, instead always performing an escape response. Even if objects subtended the same angle (e.g. 20–30 °) as viewed from the larva, the larvae only jumped in response to objects at a height of 10 mm, whereas they exclusively showed an escape response to objects at a height of 50 mm. One-stemma larvae, in which three of the four large stemmata were occluded with black paint, responded to objects moving at a height of 10 mm like normal larvae, but the size preference of the two responses was decreased. The one-stemma larvae tended to escape from objects moving at a height of 50 mm, the same as the normal larva, but sometimes jumped towards them. Jumping in repsonse to objects at a height of 50 mm by one-stemma larvae suggested that these larvae use binocular cues when judging the hunting range. Moreover, for such one-stemma larvae, moving objects at a height of 10 mm induced a jump more frequently and an escape response less frequently than did those at a height of 50 mm. These data suggest that the larvae also use monocular cues for the estimation of distance.

In the present study, the existence of different visual mechanisms for detecting object height was examined in detail

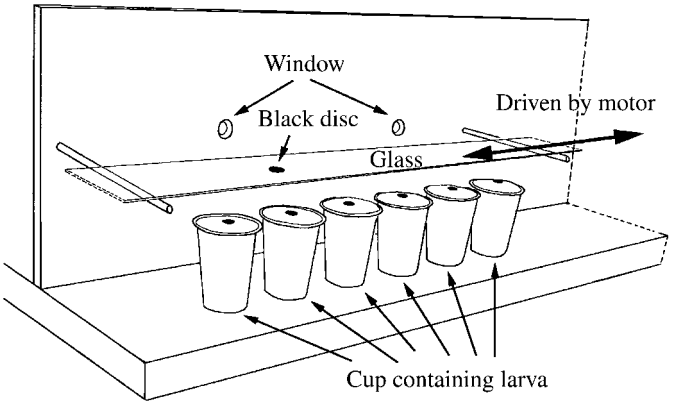

Fig. 2. Arrangement for the observation of the responses of tiger beetle larvae to moving objects. Six cups, each containing an individual larva, are arranged in this example; the number of cups varied between experiments.

by behavioural observations using an optical apparatus, and the monocular mechanism was further examined on the basis of the optics and morphology of stemmata in the tiger beetle larva.

Materials and methods

Third (final) instar larvae of the tiger beetle *Cicindela chinensis* Degeer were used throughout the present study. They were collected in the suburbs of Fukuoka, Japan. Larvae were kept individually in plastic cups filled with soil. When a larva was released onto the surface of the soil, it dug a tunnel directed along a pilot hole made in advance by the experimenter.

Behavioural observations

Between five and ten soil-filled plastic cups, each containing a single larva, were placed in a row under a glass plate (Fig. 2). The distance from the surface of the cup to the glass plate was 50 mm throughout the experiments. The glass plate was moved horizontally at a velocity of $10\,°\,s^{-1}$ (as seen by the larva) by a motor along the row of the cups. A black disc 20 mm in diameter, which subtended an angle of 22.5 ° as viewed from the larva, was attached to the glass plate so that it could be moved above the row of cups. In some experiments, two smaller discs were used, as described below. The position of each cup was adjusted so that the head of a larva was directly below the moving disc. The antero-rostral axis of the larval head was adjusted, by rotating the cup, so that it lay in the direction of movement of the disc. The responses of larvae to the moving disc were examined approximately 1 h after positioning the cups. The disc was moved either in the direction from the mouthparts to the back of the head or in the opposite direction. Larvae usually either performed a jump towards the disc or showed an escape response, although sometimes they did not respond to the moving object. Moving stimuli were presented five times with an interstimulus interval of 10 min. Since some larvae did not appear again for a long time after the preceding trial, however, the responses of such larvae were tested fewer than five times. The numbers of jump, escape and no responses were counted for each set. The responses of several sets were summed. Visual responses were examined in seven different conditions. In response to a single moving disc (1) responses of intact larvae, (2) responses of one-stemma larvae, (3, 4) responses of intact larvae and one-stemma larvae above which prisms had been placed and (5) responses of one-stemma larvae above which a concave lens had been placed were examined; in response to a pair of moving discs (6) responses of intact larvae and (7) responses of intact larvae whose visual field was partially restricted by a slit were examined. In one-stemma larvae, three of the four large stemmata, and stemmata pairs 3 and 6, were occluded by black paint.

Optical apparatus

To examine the effects of manipulation of a sight line or visual field upon the visual behaviour of the larva, the following optical equipment was used.

Twin prism

A twin prism was constructed from three coverslips: two small square coverslips (25 mm×25 mm) in contact with each other on a large coverslip (25 mm×50 mm). The small coverslips were inclined at a dihedral angle of 20 ° against the large coverslip, and the space between the small and large coverslips was filled with Canada balsam. Since the refractive indices of the coverslip and Canada balsam were approximately equal (1.5), this hand-made prism functioned like a glass twin prism with each individual prism meeting the other at its acute edge. The twin prism was placed horizontally 2–3 mm above the larva's head with the acute edges aligned with the antero-rostral axis of the head.

Lens

A uni-concave lens was placed 2–3 mm above the head of the larva. The lens was 1 cm in diameter and 3 mm thick, with a focal length of 20 mm.

Slit

To partially restrict the visual field of a larva, a white shading plate with a single narrow window (slit) was placed 10 mm above its head (Fig. 3). The slit was 6 mm in width and oriented in the median plane of the head. Two black discs, 10 mm in diameter and 10 mm apart, were attached to the glass plate 50 mm above the larva's head symmetrically with respect to the median plane of the larval head. The window was designed so that each stemma could view the contralateral disc, but could not view the ipsilateral one.

Histology

The size, visual field and orientation of the large stemmata were measured in semi-thin plastic-embedded sections. Larval heads were doubly fixed in 3 % glutaraldehyde and 2 % OsO_4 and embedded in epoxy resin using conventional methods. Semi-thin sections were stained with 0.5 % Toluidine Blue.

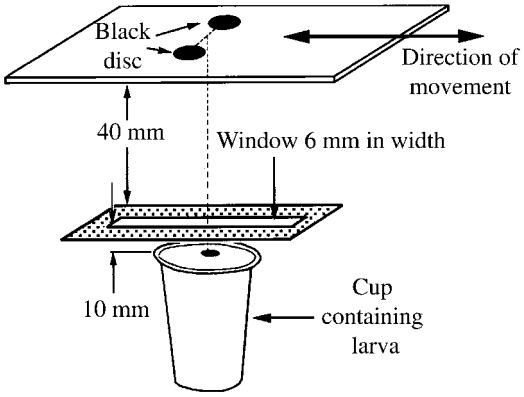

Fig. 3. Arrangement for testing the responses of a tiger beetle larva with a restricted visual field. The visual stimulus is provided by two black discs, positioned symmetrically with respect to the median plane of the larval head, and moved in an antero-rostral direction at a height of 50 mm. A plate with a narrow window is placed at a height of 10 mm above the head of the larva; the window is aligned with the antero-rostral axis of the larval head. Both sides of the plate are painted white.

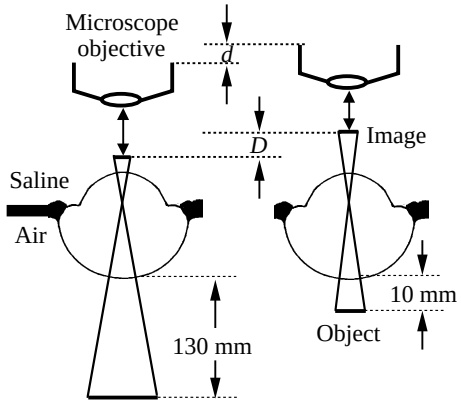

Fig. 4. Arrangement used to measure the difference in image depths for objects 10 and 130 mm from the corneal lens. The difference in position of the microscope objective for focused images of the two objects is expressed as *d*. Since the images are in saline, the real distance (*D*) to the two images was obtained by multiplying *d* by 1.33 (the refractive index of water).

The orientation and visual field of the stemmata were obtained from ray drawings. The orientation of each stemma was defined as the optical axis passing through the top of the corneal lens, and its visual field was defined as the angle formed by the rays passing through both edges of the retina (see Fig. 5A).

Measurement of the depth of the focal plane of the corneal lens

The posterior focal point was measured in an isolated corneal lens, which was inserted into a hole (0.5–1 mm in diameter) in an aluminium plate (10 mm×10 mm). The aluminium plate was positioned vertically under a microscope with the optical axis of the corneal lens aligned horizontally. A small pool was constructed from beeswax on the retinal side and filled with physiological saline (105 mmol l^{-1} NaCl, 1.5 mmol l^{-1} KCl, 0.9 mmol l^{-1} $CaCl_2$, 0.1 mmol l^{-1} NaH_2PO_4, 0.9 mmol l^{-1} Na_2HPO_4). A laser generator (GIG 5220, NEC) was placed 2 m from the microscope so that the generated beam was aligned precisely with the optical axis of the corneal lens and the light path on the retinal side could be observed under the microscope. The saline was made cloudy by adding a little skimmed milk to aid in visualization of the light path. The beam diameter was approximately 100 μm. A beam projecting to the central part of the corneal lens produced a focused image behind the lens. To measure the focal plane more precisely, a beam was projected not to the central part but to the periphery of the corneal lens by sideways displacement of the beam. Images of two light paths passing through the two peripheral edges were superimposed by computer.

Measurement of changes in image depth by changing object distances

An infinitely distant object forms its image at the posterior focal plane of the corneal lens; the closer the object approaches the lens, the deeper the image moves. The difference in image depths between objects at 10 and 130 mm was measured directly under the microscope (Fig. 4). An isolated corneal lens, set into a hole in an aluminium plate as described above, was placed external-side down under the microscope. Objects (square grids) were placed 130 and 10 mm beneath the corneal lens, and the microscope was focused on an image of the grids 130 mm below and 10 mm below. The difference *d* between the two focus levels was read from the micrometer of the microscope focus knob. Because the two focused points were in saline, the measured value of *d* was multiplied by a factor of 1.33 (the refractive index of water) to obtain the real distance *D* (Fig. 4).

Results

Stemmata and ambushing posture

The tiger beetle larva possesses six stemmata on each side of the head. They are numbered 1–6 (after Friederichs, 1931) (Fig. 5A). Stemmata 1, 2 and 6 occur on the dorsal surface of the head and they appear to look outside the tunnel at the ambushing posture, whereas stemmata 3, 4 and 5 occur on the lateral surface and thus appear to be directed towards the wall of the larva's tunnel (Mizutani and Toh, 1995). Thus, stemmata 1, 2 and 6 on either side of the head are thought to be involved in the visual behaviour of the ambushing larva. However, stemmata 6 appear to have little effect on visual behaviour because they are less organized: the cornea is not biconvex but flat, and only 30 retinular cells occur beneath the cornea (Toh and Mizutani, 1994a). In fact, we found no differences in response patterns between larvae with intact stemmata 6 and those with occluded stemmata 6 throughout the behavioural experiments with the optical apparatus (see below). Thus, the roles of stemmata 1 and 2 will be the focus of the present study.

Stemma 1 possesses a spherical corneal lens (479±19 μm in

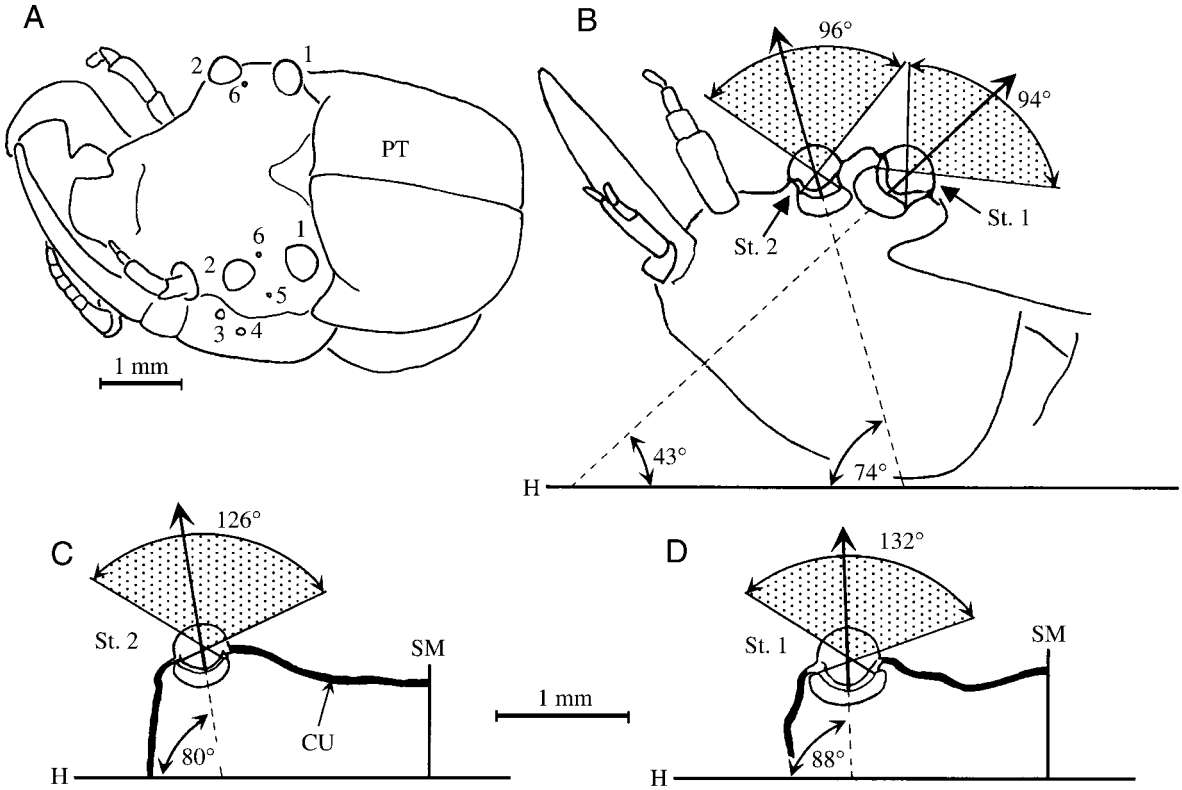

Fig. 5. (A) Diagram of the head and prothorax (PT) of a tiger beetle larva viewed from the upper left side. The six stemmata are numbered 1–6 (according to Friederichs, 1931). (B–D) Optical axes (thick line with arrow) and visual fields (stippled area) of two large stemmata measured in the sagittal plane of the head (B) and in the transverse plane (C,D). The receptive field is shown by an arc with arrows, and its angular size is given above the arc. The optical axis is extended downwards by a thin broken line to show its angle of elevation above the horizontal. CU, cuticular integument; H, horizontal plane; SM, median sagittal plane of the head; St. 1, stemma 1; St. 2, stemma 2.

diameter, mean ± S.D., N=36) and approximately 5000 retinular cells, whereas stemma 2 is smaller by a factor of approximately 0.9, with a corneal lens 415±13 μm in diameter (N=30) and approximately 4600 retinular cells (Toh and Mizutani, 1994a). Stemmata 1 and 2 are referred to hereafter as the posterior large stemma and anterior large stemma, respectively.

The optical axis and visual field were measured in sagittal and transverse sections. One example is shown in Fig. 5B–D,

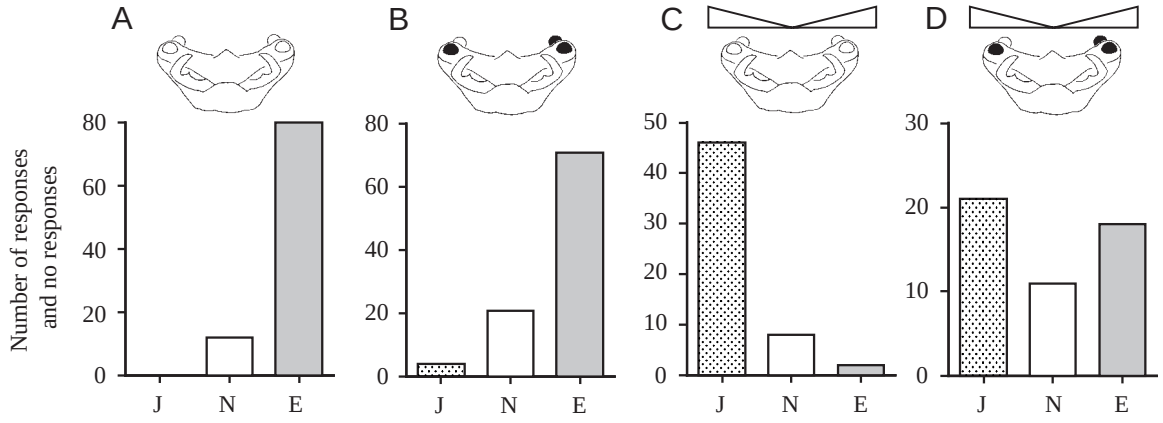

Fig. 6. (A–D) Numbers of responses (J, jump; E, escape) and of failures to respond (N) of tiger beetle larvae to targets moving 50 mm above the larva's head. Top illustrations show frontal views of a larval head with two pairs of stemmata and associated optical equipment. Occluded stemmata are filled. (A) Larvae with intact stemmata. (B) Larvae with one large stemma functional, the other stemmata being occluded by black paint. (C) Larvae with a pair of prisms in their line of sight. (D) One-stemma larvae with a pair of prisms in their line of sight. The total number of trials and the number of larvae examined varies among the four graphs: 92 trials by 25 larvae in A, 96 trials by 30 larvae in B, 56 trials by 15 larvae in C and 50 trials by 15 larvae in D.

although the values varied from specimen to specimen. The non-flat dorsal surface of the head makes it difficult to define its horizontal plane. Since the anterior and posterior large stemmata are positioned at the same level in the ambushing posture, a plane tangential to the four corneal lenses of the four large stemmata is defined here as the horizontal plane for convenience (H in Fig. 5B–D). In the example shown in Fig. 5B–D, the optical axis of the anterior large stemma is directed anteriorly at an elevation of 74° and laterally at an elevation of 80°, whereas that of the posterior large stemma is directed posteriorly at an elevation of 43° and laterally at an elevation of 88°. The receptive field is approximately 125° in the transverse direction and 100° in the antero-posterior direction in both large stemmata. Because of the asymmetrical arrangement of the retina, the receptive field was not symmetrical with respect to the optical axis. The interocular distance is approximately 4 mm between the right and left large stemmata and 1 mm between the posterior and anterior stemmata on the ipsilateral side.

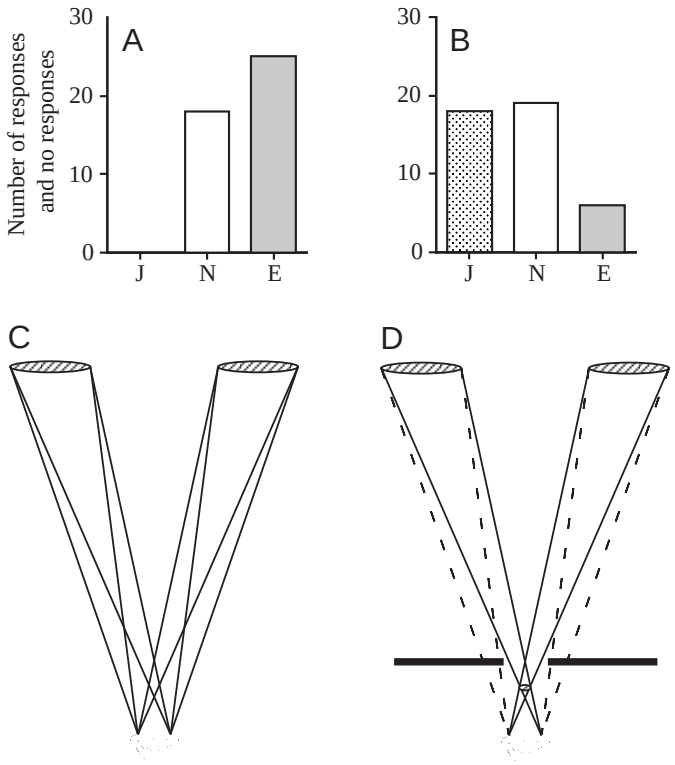

Fig. 7. (A,B) Numbers of responses (J, jump; E, escape) and of failures to respond (N) of larvae to two targets moving at a height of 50 mm above the larva's head. Two moving objects are positioned symmetrically with respect to the median plane of the larval head and moved in the antero-rostral direction. (A) Larvae viewing the two objects above their head as shown in C. (B) Larvae whose visual field is restricted by a window, as shown in D, while the two objects are moved. In D, each eye can view the contralateral object through the window, but cannot view the ipsilateral one. Forty-three larvae were used. Each larva was tested once without an obstacle, as illustrated in C, and once with the narrow window, as illustrated in D.

Responses of intact larvae and one-stemma larvae to an object moving at a height of 50 mm

When an object 20 mm in diameter was moved above the larvae at a height of 50 mm, intact larvae performed an escape response and never jumped. Since some stimuli failed to induce a response, the probability of an escape response was approximately 80% (Fig. 6A). The angle subtended by the object used was 22.5°. Mizutani and Toh (Mizutani and Toh, 1998) reported that larvae showed the maximal probability of jumping (more than 80%) in response to an object that subtended this same angle when it moved at a height of 10 mm. In response to objects of 20 mm diameter moving at a height of 50 mm, one-stemma larvae frequently showed the escape response, as in intact larvae, but they also sometimes jumped (Fig. 6B). This qualitative difference between the responses of intact and one-stemma larvae supports the participation of binocular vision in the detection of object distance.

Responses of intact larvae and one-stemma larvae viewing objects moving at a height of 50 mm through prisms

The participation of binocular vision was also supported by placing a twin prism 2–3 mm above the head of an ambushing larva. Two prisms were arranged to meet at their acute edges, which lay along the mid-sagittal plane of the larval head (upper illustration in Fig. 6C,D). For each of the paired eyes, a distant object must be seen in the direction of the zenith without the prism because the interocular distance of 4 mm is negligibly small when the object height is 50 mm. When the larva sees a distant object through the paired prisms, however, the object would appear refracted towards the median plane of the head as if it were close to the larva. Larvae covered by the twin prism frequently jumped in response to objects moving at a height of 50 mm (Fig. 6C).

However, similar experiments on one-stemma larvae did not

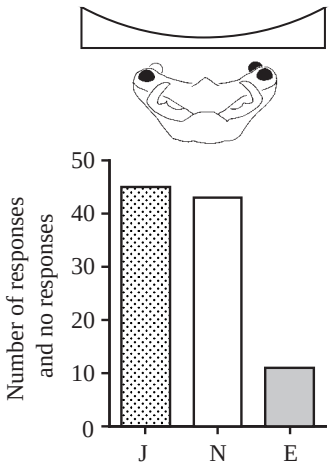

Fig. 8. Numbers of responses (J, jump; E, escape) and of failures to respond (N) of one-stemma larvae to a target moving at a height of 50 mm viewed through a concave lens as illustrated above the graph. Compare the jump frequencies with Fig. 6B. The graph is from 99 trials of 30 larvae.

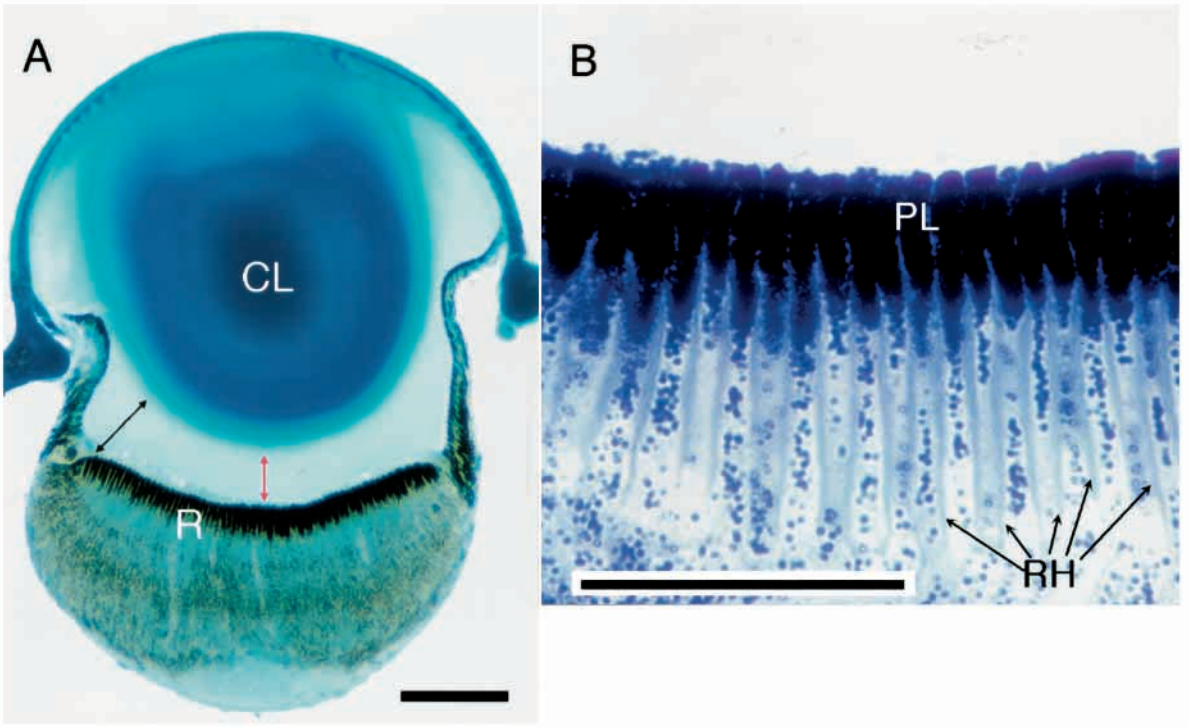

Fig. 9. Semi-thin (3 µm) mid-longitudinal sections through a large stemma stained with Toluidine Blue. (A) A posterior large stemma. Note that the distance between the internal surface of the corneal lens (CL) and the retina (R) is shorter in the central part of the retina than it is in the peripheral part (compare the length of the red and black arrows). Scale bar, 100 µm. (B) A higher magnification of the retina of a posterior large stemma. PL, pigmented layer; RH, rhabdom. Scale bar, 50 µm.

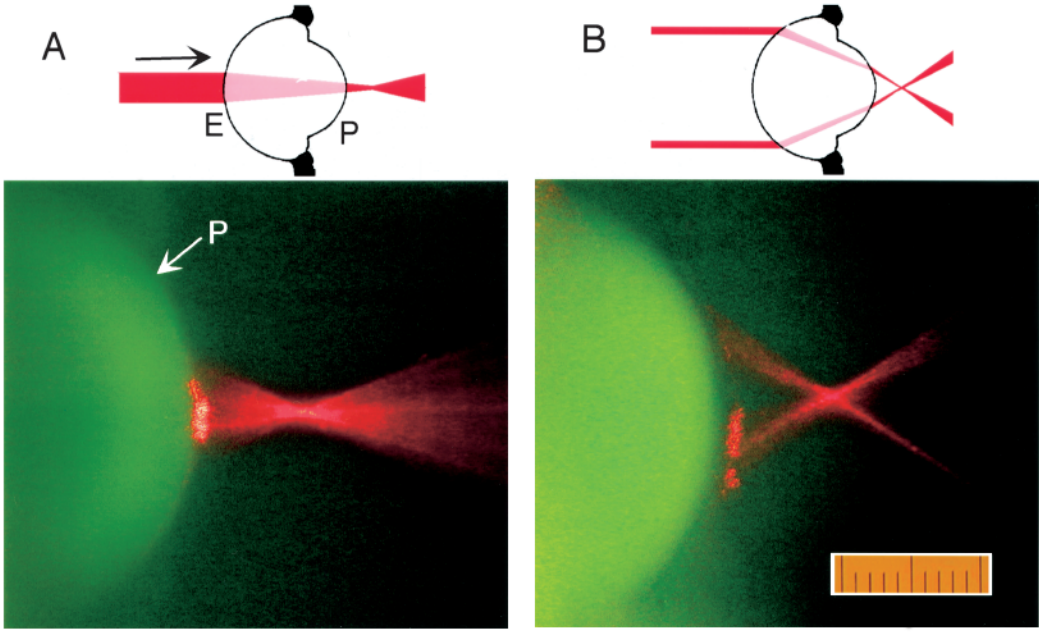

Fig. 10. Light path of a laser beam refracted by the corneal lens, as observed under the microscope. The upper diagrams show how the beam is projected onto the posterior side of the corneal lens. The arrow indicates the direction of the incident light. E, external or anterior margin of the corneal lens; P, posterior margin of the corneal lens. Scale bar, 10 µm per division.

seem to confirm a role for binocular vision. When a twin prism was placed above the one-stemma larvae, they also frequently jumped in response to moving objects at a height of 50 mm (Fig. 6D). Binocular vision is not present in one-stemma larvae because the other three large stemmata, together with stemmata pairs 3 and 6, were occluded. This disparity will be discussed below.

Responses of intact larvae to two objects moving at a height of 50 mm

The participation of binocular vision in the detection of the hunting range was further examined using a pair of moving objects. Two black discs, 10 mm in diameter and 10 mm apart, were placed symmetrically with respect to the median plane of the larval head (Fig. 7C). The responses of larvae to the two

moving objects were examined in two conditions (Fig. 7C,D). Each larva (*N*=43) was tested only twice; once for each condition. When the two discs were moved along the median plane at a height of 50 mm, larvae either showed escape responses or did not respond (Fig. 7A). However, when a plate with a single narrow window, 6 mm in width and oriented in the median plane of the head, was interposed at a height of 10 mm so that each eye could view a contralateral object through the window but could not view an ipsilateral one, the larvae frequently jumped (Fig. 7B). These results can be explained only by binocular vision: the two objects will appear as a single virtual image at a height of 10 mm when the larva sees them through the window (Fig. 7D).

Responses of one-stemma larvae viewing objects moving at a height of 50 mm through a concave lens

The results presented above indicate that binocular vision is used to judge the hunting range. However, previous behavioural experiments on one-stemma larvae suggested that the larva can partially determine object distance using monocular vision (Mizutani and Toh, 1998). We therefore considered the possibility that the depth of the retinal image may be important in detecting the hunting range. The posterior focal plane of the corneal lens may lie above the retina or, more precisely, above the effective receptive regions of the photosensitive organelles, the rhabdoms. In such an optical system, an infinitely distant object produces a blurred over-focused image on the retina; the closer the object approaches the lens, the deeper its image moves, giving the retina a clearer, better-focused image. As in myopic people, the larva may see very close objects clearly but fail to recognise distant objects, and the larva may jump only when it sees the object clearly. As for the correction of myopia in human vision by spectacles, a concave lens was placed 2–3 mm above one-stemma larvae, and their responses to moving objects at a height of 50 mm were then examined. These larvae frequently jumped in response to distant objects (Fig. 8). Since the concave lens used had a focal length of 20 mm, a virtual image of the object would be formed approximately 10 mm above the lens, which presumably released the jumping response.

Image depth of a distant object in the retina

To investigate whether the image of a distant object is really formed above the retina, the distance between the retina and the corneal lens (the lens–retina distance) was measured for the posterior large stemmata and compared with the depth of the image measured directly with a light microscope. The lens–retina distance is larger in the peripheral part of the retina than it is in the central part, because the convex interior surface of the lens faces a rather flat underlying retina (Fig. 9A). The lens–retina distance was 43–66 μm (53±8 μm, mean ± S.D., *N*=23) in the central part of the retina, and 60–100 μm (81±10 μm, *N*=23) in the peripheral part.

An image of an infinitely distant object is formed on the posterior focal plane of the corneal lens. The distance from the posterior part of the corneal lens to the posterior focal plane was measured by a laser beam generated 2 m from the corneal lens (Fig. 10). Since the anterior focal length of the corneal lens is less than 300 μm, an object 2 m away can be regarded as infinitely distant. A beam projecting to the central part of the corneal lens focuses an image behind the lens, as shown in Fig. 10A. To obtain Fig. 10B, a beam was projected to the periphery of the corneal lens by sideways displacement, and images of two light paths passing through the two peripheral edges were superimposed by computer. These experiments gave values of between 60 and 90 μm (73±8 μm, mean ± S.D., *N*=38) for the distance from the corneal lens to the posterior focal plane. Since the refractive indices of the medium between the lens and the retina and that of the retina may differ from that of water (1.33), the real distance *in vivo* may differ slightly from that obtained in the isolated corneal lens. However, this error would be small compared with the large variability in the present measurements. The image depth was also measured when the laser beam was projected at an angle of 45° with respect to the central axis of the stemma. The depth of the focused image at 45° off-axis was almost the same as that along the central axis of the corneal lens.

From the mean values given above for the morphological and optical measurements, the image of a distant object is estimated to be formed 20 μm deep in the central part of the retina. These results do not support our analogy between the stemma and human myopia, because the image of a distant object will be formed within, but not above, the retina.

Image depth of an object close to the retina

Since the predatory jump is released in response to moving objects when they are less than 15 mm from the larva, the position in the retina at which such close objects form images must be important. The depth of the image when the object distance was varied from 130 to 10 mm was estimated in the isolated corneal lens by direct measurements under the microscope, as shown in Fig. 4. Images were deeper (12.0±4.2 μm, mean ± S.D., *N*=10) for objects at 10 mm than for objects at 130 mm. If we take to 53 μm as the lens–retina distance in the central part of the retina and 81 μm for the peripheral part, the image of an object at 10 mm from the lens is estimated to be formed 32 μm and 4 μm, respectively, deep in the central and peripheral parts of the retina.

Where in the retina does a focused image form?

The morphological and optical data discussed above suggest that images of both distant and close objects may be formed at some level within the retina in the central part of the eye, although variations were large in all our data. We examined the relationship between image depth and rhabdom position. The retina consists of a palisade-like arrangement of distal processes of retinular cells (Fig. 9B). Since receptor processes contain pigment granules in their distal regions, the distal layer of the retina is heavily pigmented for a depth of 20–40 μm (Toh and Mizutani, 1994a; Toh and Mizutani, 1994b). Rhabdoms occurred between the distal processes of retinular cells for

60–100 μm in depth, but they were extremely narrow or absent in the distal-most region of the pigmented layer at depths between 10 and 20 μm. Thus, rhabdoms were extensive more than 20 μm and less than 10–below the surface of the retina. It is assumed that a distant object may form an image in focus at the distal tip of the rhabdom, whereas a close object may form an image below it: a distant and close object were estimated above to form focused images 20 μm and 32 μm, respectively, deep in the central part of the retina. A larva would need a clear image of a close object for the coming jump. However, since the lens–retina distance is larger in the peripheral part of the retina than it is in the central part, a close object off-axis may form an image just in focus at the tip of the rhabdom in the peripheral part of the retina. To summarize, distant and close objects form focused images on the rhabdom in different part of the retina, i.e. distant objects in the central part of the retina and close objects in the peripheral part. As described above, an analogy with human myopia appears unlikely, but this possibility cannot be excluded because of the large variability in morphological and optical measurements reported here.

Discussion

In the present study, the mechanisms by which the tiger beetle larva determines when its prey is within the hunting range were studied using behavioural, optical and morphological methods. In insects, the use of motion parallax in distance estimation is well known. A role for motion parallax in gauging the hunting range in tiger beetle larva was examined in several preliminary experiments. Predatory jumps and escapes were analyzed using a high-speed video camera (500 frames s^{-1}). Frame-by-frame analysis revealed that larvae move their heads slightly just before a jump. However, this head movement did not appear to be self-motion to aid motion parallax, but instead was thought to represent a preparatory movement for the coming jump because no such head movements occurred just before the escape response.

Compared with motion parallax, a role for binocular vision in distance perception in insects has been less widely reported because their narrow interocular separation would not appear to allow such triangulation. However, for short distances, binocular vision can function effectively, for example in the adjustment of foreleg extension in the praying mantis when striking at a target (Rossel, 1983; Rossel, 1986). In the tiger beetle larva, an interocular separation of 4 mm does not seem to be small compared with its hunting range of within 15 mm. In fact, the present behavioural experiments using both the twin prism and the narrow window apparatus seem to confirm the suggestion (Mizutani and Toh, 1998) that the larva uses binocular cues to determine the distance to its prey.

However, the frequent jumping of one-stemma larvae when they viewed moving objects at a height of 50 mm through a twin prism (Fig. 6D) cannot be explained by binocular vision (Fig. 6D). There are two possible explanations for such unexpected results. First, the black paint masking the stemmata might have been imperfectly applied or have become detached during the course of repeated trails. However, microscopic inspection of occluded stemmata after the behavioural experiments excluded this possibility. Second, the image of the distant object when viewed through the prism might be greatly distorted and, therefore, appear close to the larva. The larva would observe the moving object first in the anterior or posterior direction at a low elevation. Viewing such an object at a low elevation through the prism might cause image distortion. This possibility is now under investigation.

Thus, behavioural data using the twin prism do not provide conclusive evidence for the participation of binocular vision in the determination of hunting range, although larvae with intact eyes jumped more frequently when they viewed distant objects through the twin prism than did one-stemma larvae (compare Fig. 6C and Fig. 6D). However, behavioural data using two objects with a narrow window (Fig. 7) firmly support a role for binocular vision. Larvae with intact stemmata never jumped in response to two moving objects at a height of 50 mm, but they often jumped when they viewed such objects through a narrow window. These jumps must be triggered by the formation of a virtual image as shown in Fig. 7D.

How the larva uses binocular vision to determine the hunting range is not known. The large stemmata on both sides of the head are likely to be involved, because the right and left stemmata are 4 mm apart and their visual fields largely overlap. However, the anterior large stemma and the posterior large stemma on the ipsilateral side are unlikely to function together in this way because they are only 1 mm apart and their visual fields overlap to a smaller extent (Fig. 5B).

Mizutani and Toh (Mizutani and Toh, 1998) proposed an additional unknown mechanism for distance perception in the tiger beetle larva on the basis of behavioural observations of one-stemma larvae. In the present study, an increased jumping frequency in response to moving objects at a height of 50 mm in one-stemma larvae resulting from placing a concave lens over the head (compare Fig. 6B and Fig. 8) suggests that the level in the retina at which the moving objects appear as a clear image just in focus is related to the release of the jump or escape response.

We predicted that, if an image just in focus moved across the tip of the rhabdom, it would be perceived clearly with high temporal and spatial contrast, but if an out-of-focus image moved across this same region it would be blurred, with low contrast. It is not known whether movement of an image just in focus and one only 10 μm out of focus on the rhabdoms is encoded differently in follower neurons. However, this is possible because recent work suggests that some second-order visual interneurons show clear distance sensitivity at a peripheral level in the visual system of the tiger beetle larva (J.-Y. Okamura and Y. Toh, in preparation).

We investigated both differences in the lens–retina distances between the central and peripheral parts of the stemma and differences in image depths between distant and close objects. Both differences were small: less than 30 μm for the lens–retina distance and approximately 10 μm for image depths. There is no doubt that a clear image appears at some

level in the retina, but the exact level cannot be determined because of large variability in our measurements. Nevertheless, two mechanisms are proposed on the basis of the present morphological and optical measurements. The first is an analogy with human myopia, where only close objects produce clear images on the main part of the rhabdom, but distant objects form only blurred images on it. The second is that distant objects produce clear images on the main part of the rhabdom in the central part of the retina, whereas close objects produce clear images in the peripheral part of the retina. Which of these two alternatives is more likely cannot be determined from the present data.

Some of the variability in our results must be due to measurement error. The stemmata of tiger beetle larvae are extremely small compared with human eyes. The focal length of the dioptric apparatus is approximately 16.7 mm in the human eye (Land, 1981) compared with approximately 300 μm for the corneal lens of the large stemma of the tiger beetle (Y. Toh and J.-Y. Okamura, unpublished data), i.e. less than 1/56 that of the human eye. In such small eyes, the ratio between morphological and optical measurement errors inevitably increases. The optical measurement of the difference in image depths for objects 130 and 10 mm from the lens will include such errors because focused images were examined by eye under a microscope. The experimenter adjusted the microscope knob using a micrometer (1 μm per division) to obtain an image just in focus, but the resolution of the human eye is not good enough to detect changes in image focus caused by a single 1 μm movement of the microscope objective. Variation in the size of stemmata from specimen to specimen also decreased the reliability of the data in the present study. To minimize variation due to individual differences, future experiments will incorporate measurements on specific individuals: all morphological measurements, including electron microscopy, will be carried out on one of the paired stemmata, and all optical measurements will be carried out on the other (Y. Toh and J.-Y. Okamura, in preparation).

In conclusion, the tiger beetle larva determines whether an object is within its hunting range using both binocular and monocular vision. However, the role of monocular vision may be minimal in the natural habitat. During the last 10 years, we have sampled more than 2000 larvae from the wild but have never found larvae with partially damaged stemmata. Nevertheless, since the larva fixes its head a few millimetres below the surface of the ground in the ambushing posture under natural conditions, it is possible that its visual field at low elevation is monocular as a result of restriction of the visual field by the circular tunnel wall (Fig. 11). Prey such as caterpillars and pill bugs approach the tunnel on the ground. Debris from flies has been found around the opening of the tunnel; flies do not seem to be caught when they are flying over the tunnel, but instead when they approach the opening of the tunnel on the ground, perhaps attracted by larval food remains. Thus, most prey may be detected first in the monocular visual field. Preliminary experiments have shown that larvae jump in response to objects even at very low elevation (Y. Toh and J.-Y. Okamura, unpublished data), suggesting that monocular vision does play an important role in the natural habitat.

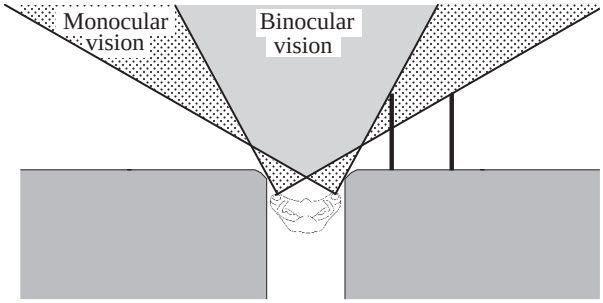

Fig. 11. Visual fields from binocular and monocular vision for a larva with its head 2 mm below the opening of its tunnel. If an object 5 mm in height approaches the tunnel opening, the larva sees it using monocular vision only, for the range between the two vertical lines.

In the present study, two mechanisms of distance perception have been proposed in the tiger beetle larva, but many questions remains unanswered. These will be addressed by future behavioural, morphological and optical studies.

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