DIPLOMARBEIT

Titel der Diplomarbeit

Visual acuity in the larvae and adults of the assassin bug *Platymeris biguttatus* (Reduviidae, Heteroptera, Insecta)

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Contents

Abstract

The aim of this thesis was to examine how the visual acuity in the predatory reduviid *Platymeris biguttatus* increases throughout larval development. For this purpose, morphological examinations of the most important eye-parameters, a behavioural choice and optokinetic experiments were conducted. Eye-parameters, such as facet diameter, interommatidial angle and number of ommatidia, were investigated for all five larval stages and the adult animals. Adults and third-instar larvae were tested in the behavioural experiment but only adults in the optokinetic experiment. The behavioural choice experiment was based on the assumption that *Platymeris biguttatus*, like its reduviid relative *Triatoma infestans*, would exhibit a photonegative reaction and be attracted to a dark stimulus when exposed to bright light. The bugs were compelled to run in a V-shaped arena to either an aisle containing a dark stripe or an aisle without such a stimulus. The optokinetic response experiment was based on Bernd Hassestein's (1949) experiment. The morphological data showed that facet diameter and the number of ommatidia increase more or less continuously from one instar to the other and in apparent relation to the body size parameter tibia length. However, the interommatidial angle does not develop continuously. The number of facets increases from about 105 in the first larval stage to a mean of 880 in the adult animal. Throughout the bug's development, the facet diameter increases from 34μm to 69μm, and the interommatidial angle in the anterior part of the eye decreases from 10.4° to 3.7°. In the behavioural choice experiment, adult *P. biguttatus* chose the aisle containing the stimulus significantly more often than the aisle lacking the stimulus – down to stimulus sizes of 1.5°. A grey back panel, which was placed in the aisle lacking the stimulus, severely diminished the likelihood of the bug choosing the aisle with the stimulus. Larval animals of the 3rd instar could only be shown to react to a stimulus 15° wide. In the optokinetic response experiment adult animals showed significant reactions when exposed to gratings composed of black and white stripes as narrow as 1.5°. Particularities of the development of eye parameters and the significance of the results of the behavioural tests are discussed.

1 Introduction

Bugs of the genus *Platymeris* are skilled predators that inject a highly effective poison into their prey (Edwards 1961). *P. biguttatus* preys on other arthropods and, along with the majority of reduviines, displays a feeding behaviour that is termed "wait and grab" (Haridass et al. 1987). Edwards (1962) studied the hunting behaviour of *Platymeris rhadamantus* and observed that these bugs approach their prey in a series of short, quick movements and that their pouncing behaviour is triggered by the preys' movement. Very little movement is needed to trigger the pounce that leads to the assassin bug grabbing its prey and injecting its venom. Edwards' studies on *Platymeris rhadamantus* concur closely with my own observations on the hunting behaviour of *P. biguttatus*. These two species resemble each other and differ only in body size, *P. biguttatus* being slightly larger, and in the colour of spots on their elytra and bands on their femura. Both elytra spots and femoral bands are yellow in *P. biguttatus* and red in *P. rhadamantus*. This resemblance along with the comparison of the description of the behaviour of *P. rhadamantus* and my own observations of *P. biguttatus* led me to assume that they hunt and behave in similar ways.

The speed and precision with which *Platymeris* bugs hunt their prey is impressive. To catch prey in this manner is crucial for the survival of *Platymeris* bugs and demands a certain degree of sensual accuracy. *Platymeris biguttatus* possesses large, prominent eyes which has led to the assumption that vision is a major aspect in this hunting behaviour.

An assassin bug's eyes, along with its antennae and tibial combs, are essential for prey location, as has been shown in studies on the reduviid *Rhynocoris kumarii* (Claver and Ambrose 2001).

Studies on the importance of visual stimuli in the hunting behaviour of arthropod hunting bugs have shown that impaired vision seriously affects predatory behaviour (Awan et al. 1989, Freund and Olmsted 2000, Haridass 1985).

The eyes of heteropterans, such as *P. biguttatus*, are complex eyes of the acone apposition type, which means that each ommatidia is composed of six peripheral and two central rhabdomeres which form an open rhabdom (Deckert and Göllner-Scheiding 2003).

In compound eyes, as opposed to single-chambered eyes, the size of the eye is more of a dominant factor for the quality of vision. The reason for this is that compound eyes employ multiple optical systems - ommatidia - with multiple lenses, where each lens (in apposition eyes) forms a tiny image. The rhabdom, situated within a single ommatidium, is the sampling unit of a compound eye and the interommatidial angle ($\Delta\Phi$) determines how fine the image is sampled (Land and Nilsson 2002).

Some eye-parameters are especially important in achieving acuity and eye size is the limiting factor to these parameters. The most important eye parameters are: lens diameter, photoreceptor diameter and the angular spacing between receptors (Kirschfeld 1971, 1976, Land 1997).

The spacing of the receptors determines how finely images can be resolved. The amount of light reaching the receptors, which is dependent on lens diameter, is important because at low light levels the ability to resolve contrasts declines due to photon numbers being too low to provide statistically reliable signals (Land 1997).

Due to the wave nature of light, the resolution of small lenses is severely limited. Diffraction is the reason why lenses are not practical below a certain size, which explains why ommatidia cannot simply be made smaller in order to improve resolution (Mallock 1894). To increase resolution in a compound eye, it is necessary to increase the number of ommatidia in the eye as well as the size of the single ommatidia, which is why eye-size is such an important factor for insects (Land 1997, Mallock 1894). The positive correlation between body size and acuity has been shown in studies on mantispids (Kral et. al. 2000) and bumblebees (Spaethe and Chittka 2003).

These limitations of the apposition compound eye affect all hemipterans. Predators are more affected by these limitations than animals that pursue a different feeding-mode. Since eye-size is such an important factor in the compound eye, larvae are under even greater duress.

In hemimetabolic insects, such as bugs, eye development is especially interesting since it unravels in distinct, abrupt steps, and much more gradually than in holometabolic insects.

In holometabolic insects, such as bees, beetles, butterflies and flies, a single dramatic event metamorphosis separates the larvae from the adult animal. During metamorphosis the eyes, as well as the whole body change in a single step. Quite commonly, the larvae look nothing like the adults and are adapted to different habitats and food sources (Truman and Riddiford 1999). In contrast, the larvae of hemimetabolic insects, such as bugs, cockroaches, crickets, mantispids etc., largely resemble the adult animals and often pursue the same trades.

Regarding *Platymeris biguttatus* this means that even the youngest larvae are predatory and are confronted with the task of finding and catching suitable prey. The early larval stages of *P. biguttatus* are tiny in comparison to the adults. Adults can reach sizes up to 40mm, while larvae of the first instar are no greater than 6mm. The eyes of these larvae are accordingly smaller than those of the adults, and the decrease in size of an apposition compound eye by a

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given factor results in a decrease of the eye's resolution by the square of the same factor (Land 1997).

Nevertheless, these tiny larvae must catch prey. Furthermore, the prey they catch must be roughly their own size (Li et. al. 2010), despite the fact that it is more difficult to visually locate small prey than large prey which the adult bugs hunt. How do these larvae manage to localise their food? How do their eyes develop over time to deal with this task?

The size of an eye is limited and the two most important qualities of the eye - acuity and sensitivity - compete against each other for the available space. The number of facets and the interommatidial angle are parameters that have the greatest impact on acuity, whereas the facet diameter has great impact on the eye's sensitivity.

Larvae of hemimetabolic insects undergo a series of ecdyses to grow, and with each ecdysis the larvae develops a step closer to assuming the habitus of the imagines. Along with the overall growth of the animal, its eyes grow - not consistently, but in distinct steps with each ecdysis.

The question that arises from these circumstances, and the larvae's need to find prey, is how do these eye parameters develop from one instar to the next. Does the number of ommatidia increase more rapidly than the diameter of the facets? Do these parameters develop continuously and more or less parallel from one instar to the next or does one parameter change dramatically during the early stages of larval development while another changes at the final stages? Can a trade-off between resolution and sensitivity be identified by regarding the development of the compound eyes of *P. biguttatus*? How does the eye development affect the animals' ability to spot single objects and resolve gratings?

To find out how well *Platymeris biguttatus* sees and how its eyesight develops during its larval development, several approaches were used in the course of this thesis.

Morphological measurements of the eye and body, and behavioural experiments, a choice experiment and an optokinetic reaction experiment, were performed.

Morphological examinations were conducted on all five instars and the adult animals, the behavioural choice experiments were tested on adult animals and 3rd instar larvae only, and for the optokinetic experiment only adult animals were tested.

The behavioural experiments could not be conducted in the style of von Frisch and similar experiments, which rely on the animal's ability to learn, since bugs have not demonstrated this kind of behaviour. In addition, *Platymeris biguttatus* is a predatory animal and need not

be fed frequently, which would almost certainly make it nearly impossible to train these bugs using food rewards.

In this thesis the behavioural experiment on *Platymeris biguttatus* was a spontaneous preference test based on the assumption that the animals would seek to flee into darkness when exposed to bright surroundings without cover. The stimuli used consisted of black pieces of paper in an otherwise white arena. The aim of the behavioural choice experiment was to ascertain the smallest size of an object for it to be detected by adults and larvae. It is therefore a single-object detection experiment.

In contrast, the optokinetic experiment was applied to collect information on the maximum resolvable spatial frequency (minimum separable) of *Platymeris biguttatus*.

This method to determine an animal´s visual acuity was developed by Bernd Hassestein (1949) in his doctoral work on the beetle *Chlorophanus viridis*. The experimental set-up of the Y-maze globe allows scientists to determine an animal's maximum resolvable spatial frequency (minimum separable) in an almost non-invasive manner and has been used, adapted and applied to many animals with various stimuli since then (Kaiser 1974, Lazzari and Nunez 1989, Lott et al. 2006, Fenk and Schmid 2010).

The basis for the optokinetic test is that the composition of a grating of dark and light stripes can only be resolved reliably if there are two receptors (ommatidia) to view each cycle of the grating, one for the dark and one for the light stripe (Land 1997). This means that the spacing of the receptors in any given eye can be mathematically derived if the finest grating, which the aforementioned eye is able to detect, is known.

2 Material and Methods

2.1 Study site and animals

All morphological measurements and the behavioural choice experiments were conducted in a laboratory at the University of Vienna, Austria. The optokinetic response experiment was performed in Leverkusen, Germany.

Platymeris biguttatus belongs to the subfamily Reduviinae (Reduviidae, Heteroptera), is well known to terrarium owners and quite easy to keep. The animals are predatory and to my knowledge they accept as prey any living insect that is commonly available in pet-shops; even animals considerably larger than themselves.

The development from egg to adult requires a little more than 8 months, during which the animals undergo a series of five ecdyses. Adult animals can live up to two years after the last ecdysis (Li et al. 2010), which makes them suitable for long-term behavioural experiments. The animal´s size reaches up to 4cm and makes them quite easy to handle and observe, while the pace of the development provides sufficient time to conduct behavioural experiments on single larvae with only a remote risk of the animals going into ecdysis before the test runs are completed.

Studies on this animal are relatively scarce, but a comparatively new study on the biology of these bugs was recently conducted by Li et al. (2010), while a number of studies have been made on *P. biguttatus´s* close relative *Platymeris rhadamantus* (Edwards 1961, 1962).

Animals used in the experiments and morphological study were bought as larvae at pet fairs, ordered from a breeder in Germany or bred at the University.

For morphological measurements, whole animals and exuviae were used. Adult animals and larvae were killed, preserved in70% ethanol solution, then pinned through the abdomen and dried. Exuviae were collected from the terrariums and measured as soon as possible.

In the behavioural choice experiments, adult *Platymeris biguttatus* and larvae of the third instar were used. The bugs were kept in two plastic terrariums and were fed alternately on meal-worm beetle larvae, cockroaches and house crickets. Animals were kept at room temperature. The terrariums' bottoms were covered with a layer of bark mulch that covered a layer of quartz-sand. Pieces of bark, wood or cardboard were supplied to provide hiding places for the animals. Light was provided for 12 hours every day, using a time-switch. Water was supplied daily via a spray bottle to keep humidity high. Eggs and young larvae in general were not removed from the terrariums and kept with the adults.

Adult bugs were individually marked, using Edding touch-up pencils of various colours. Markings were drawn onto the animal´s pronotum.

Larvae that were destined for the behavioural tests were removed from the adult terrariums and kept individually in small, numbered, transparent boxes. The larvae boxes were essentially the same as the terrariums, but smaller and with the identical arrangement of bark mulch over quartz-sand. The feeding and watering scheme was the same that was used with the adults, although smaller prey was used to feed the larvae. Larvae were held under the same 12:12 light/dark schedule as the adults.

Adult bugs are easily recognizable, since they possess fully developed wings. In contrast, larvae cannot be easily assigned to one particular instar. Therefore, photographs of the live

larvae were taken using a binocular stereoscope, and the pronotum width and tibia length were measured and used to identify the larval stage (Figure 1).

Larvae were usually put into the individual boxes while still in their second instar, which proved useful for two reasons. (i) The exuviae could be assigned to individual bugs after ecdysis into third instar. This was important because the exuvial tibia could be used to conclusively determine the larval stage of the animal. (ii) As soon as the animal was observed to have concluded the ecdysis, tests could begin the following day, which maximised the time in which trials could be performed before the next ecdysis.

If a larva died or began its ecdysis while tested, the trial series was discarded, unless at least eight out of ten tests were already concluded.

Fifteen adult *Platymeris biguttatus* were used in the optokinetic response experiment. The animals were kept and fed in the same way as in the choice experiment. Eggs and young larvae were not removed from the terrariums but kept with the adults.

To identify each bug individually a small piece of cardboard with a number on it was glued to the pronotum using beeswax and an electric soldering iron. The cardboard additionally served to fixate the animals in the arena with a small clip linked to a piece of wire.

2.2 Morphology

Morphological measurements were done using a Nikon 5m2-U binocular, a Nikon MicrophotoT FXA microscope, a micrometer and the image-processing program ImageJ.

To make estimates about *Platymeris biguttatus´* eye development and acuity, several parameters were chosen and examined for each instar. The parameters chosen were: pronotum width at its widest point, fore tibia length, number of facets, facet diameter and interommatidial angle. Pronotum width and tibia length have been used previously to estimate body size in the milkweed bug *Lygaeus kalmia* (Fox and Caldwell 1994).

Pronotum width was measured by taking photographs of the animals and comparing distances on the photographs with a photograph of a standardized scale using ImageJ (Figure 1)

Tibia length measurements were done in essentially the same way as pronotum width measurements (Figure 2) the only difference being that for tibia measurements either the left or right fore-tibia of either a dead bug or an exuviae was used. The tibiae had to be cut off in order to be photographed, whereas for pronotum width measurements the animals were left intact.
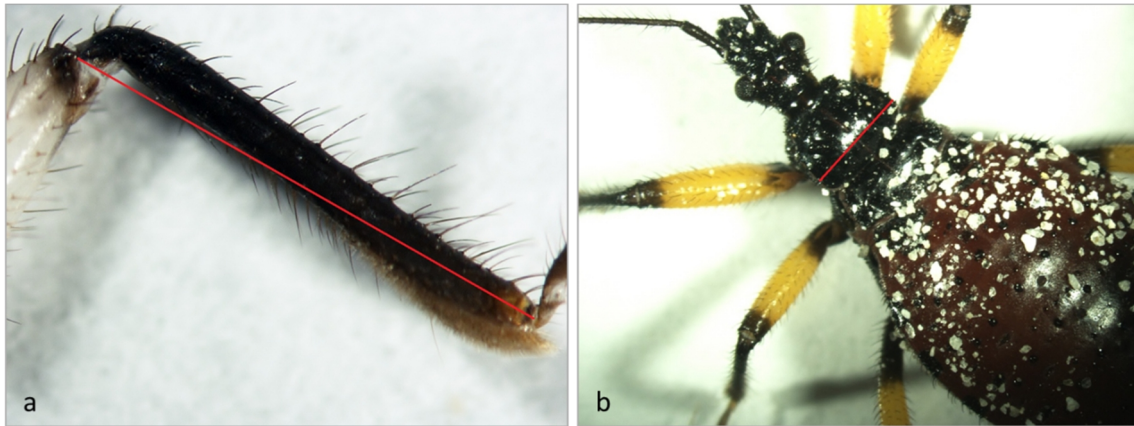
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Figure 1 Photographs used for measurements of tibia length (a) and pronotum width (b). The tibia (a) was taken from an exuviae shortly after ecdysis, the photograph used for the pronotum width was taken of a live larva of the 3rd instar. The red lines indicate where the measures were taken using the program ImageJ.

To count the number of facets or ommatidia in one eye of the animals, it was necessary to produce a picture in which all facets could be seen clearly. Due to the curved surface of the eyes of *Platymeris biguttatus*, this could not be achieved by simply photographing the eye. Therefore an imprint of one eye of each specimen was made using commercially available, clear nail polish. The nail polish was spread over the eye, and pulled off as soon as it was dry enough. The resulting imprint was then incised, flattened, transferred onto an object slide and photographed using a camera mounted onto a Nikon MicrophotoT FXA microscope. If the imprint was too large to fit on to a single photograph, several pictures were taken and stitched together using Adobe Photoshop (Figure 2).
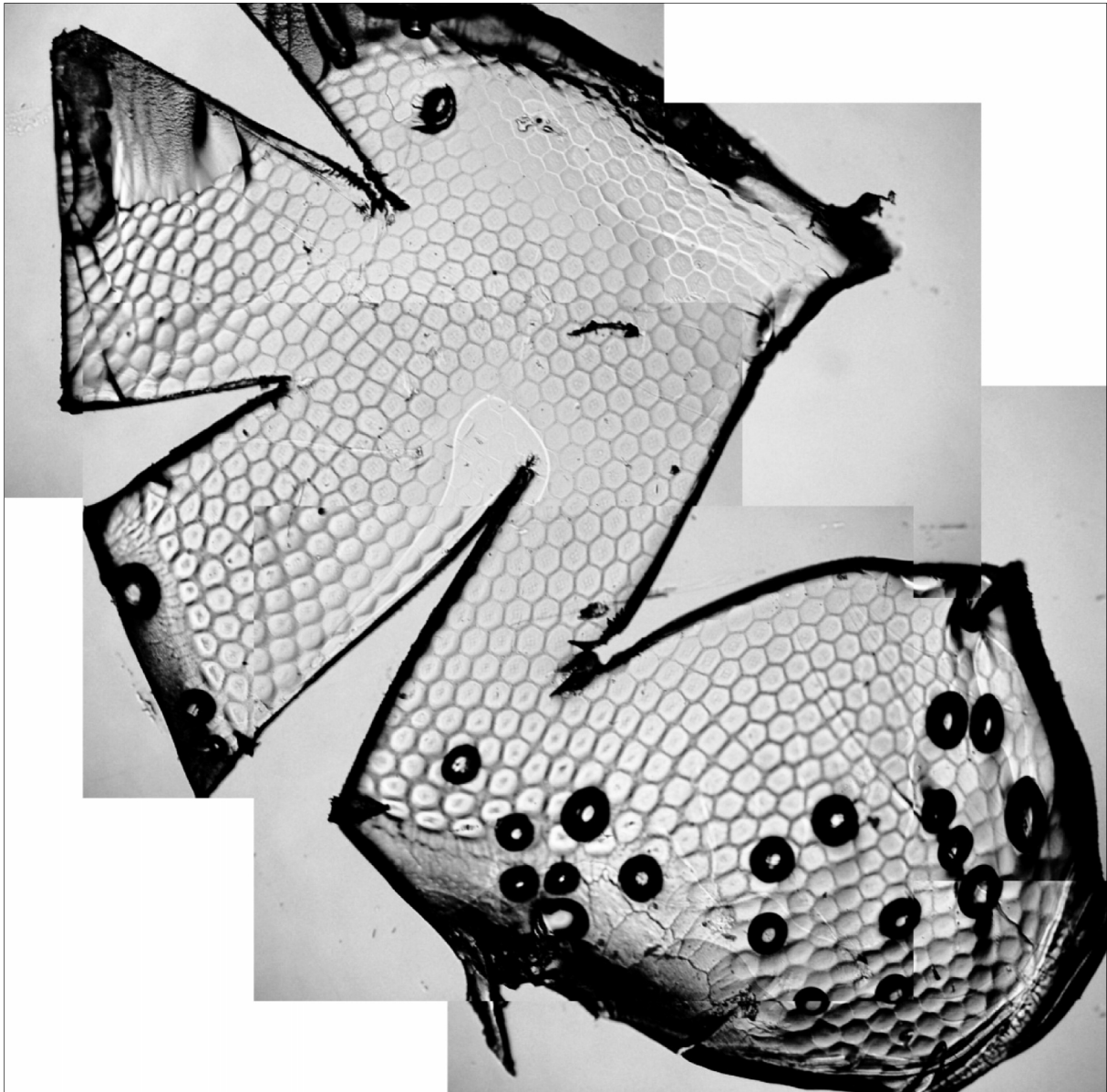
Figure 2 Picture used for counting the number of facets in the eye of an adult specimen of *Platymeris biguttatus*. The picture is a composite of five photographs of a nail polish imprint of the compound eye. Photographs were taken using a digital camera mounted onto a microscope.

This approach worked quite well for adult animals and the fifth, fourth and third instar. Using nail polish on the first and second larval stages, however, turned out to be impractical. Since the exoskeleton of these animals was so small and weak, it was not possible to remove the dried nail polish from the eye without excessive tearing.

Therefore, exuviae were used to count facets. The corneal lenses of larvae are, along with the rest of the exuviae, shed off by the animal during ecdysis. These corneal lenses were extracted carefully from the exuviae, put onto an object slide, embedded in nail polish and photographed. In the course of the embedding, the array of corneal lenses was deliberately

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crushed using the covering glass, so that it would lie flatly on the slide, allowing for a more focused picture (Figure 3).
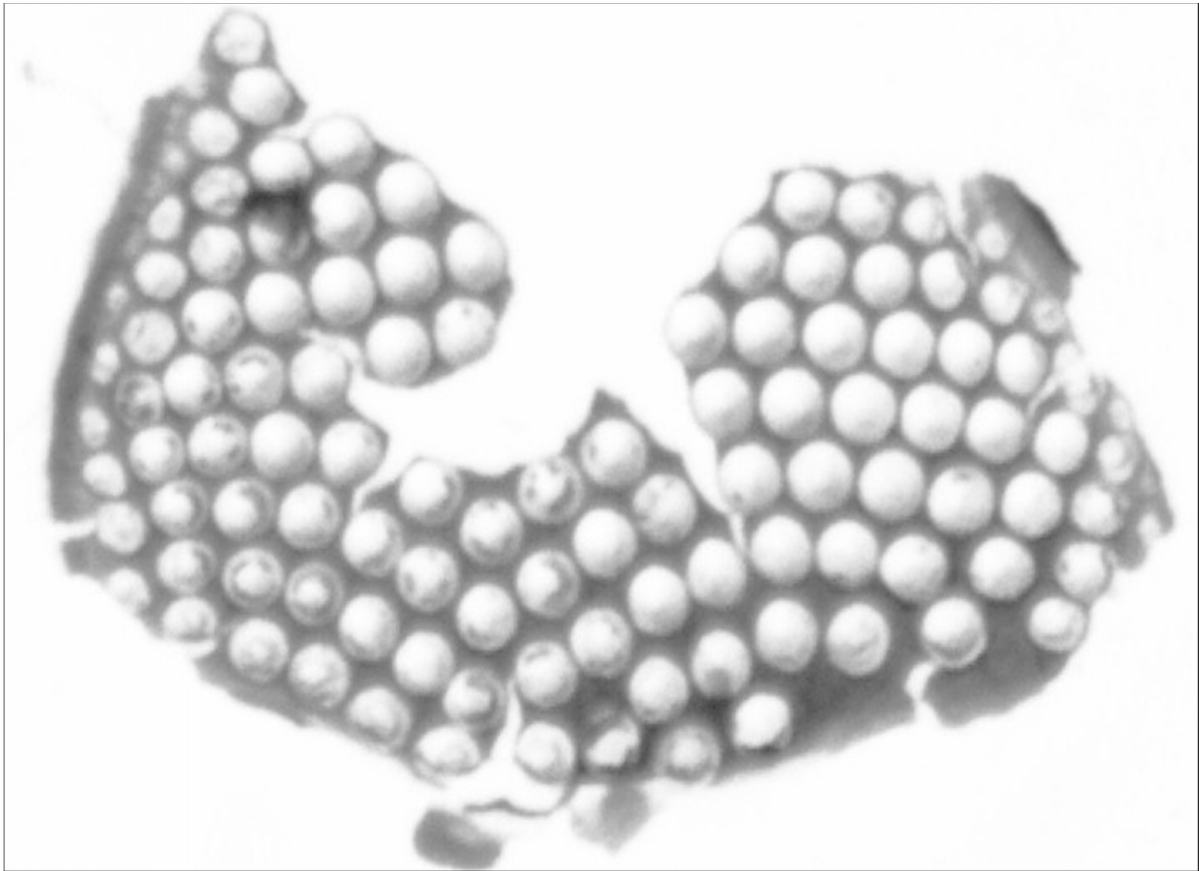

Figure 3 Photograph of facets taken from the exuviae of a *Platymeris biguttatus* larva after its first ecdysis. The cornea was deliberately broken so that the corneal lenses could lie flat on the object slide, thus allowing for a more focused picture. The photograph was taken using a binocular and a digital Nikon camera.

The facet diameter was determined by taking pictures of the eye and measuring the width of four facets in a row, using ImageJ, and dividing the measured value by four. For this purpose pictures of corneal lenses from exuviae were used, as well as pictures taken from pinned animals.

The interommatidial angle was estimated by taking pictures of the bug's eye from the dorsal view (Figure 4). This was done for the anterior and dorsal part of the eye. The next step was to fit a circle to cling to the row of ommatidia using ImageJ plug in "Circle Fit", which gives the central point of the described circle. Using that central point and two points lying in the middle of two ommatidia on the circle, the angle enclosed by those two ommatidia was calculated. That angle was then divided by the number of ommatidia enclosed in the angle to

obtain an estimation of the interommatidial angle ($\Delta\Phi$). Ommatidia used for these calculations had to be at least 4 ommatidia apart.
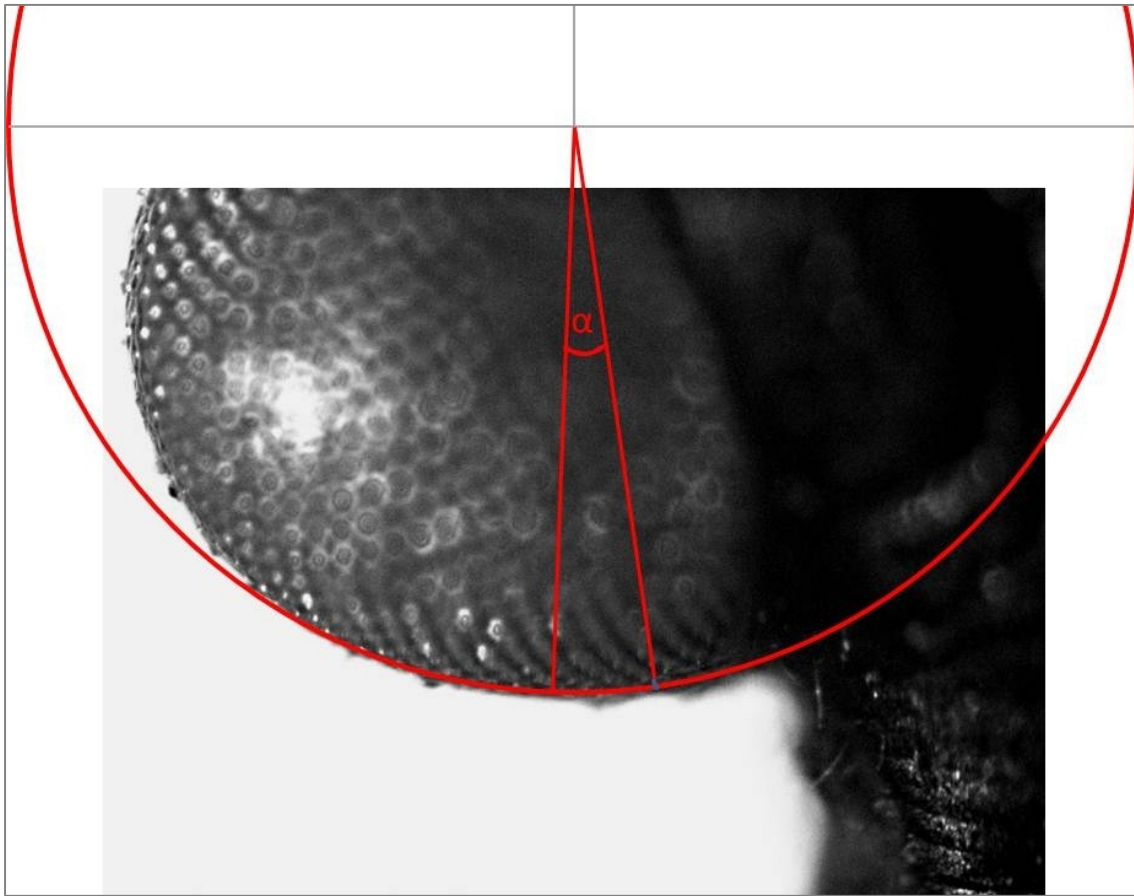

Figure 4 Picture used to assess the interommatidial angle in the frontal region of the eye. The central point of the osculant circle touching the facets on that part of the eye was established using the ImageJ plug-in "Circle Fit". The angle between two facets, at least 4 facets apart (α), was calculated and divided by the number of facets included in that angle to obtain the interommatidial angle. The red line depicts a section of the circle used to calculate the interommatidial angle and the angle α enclosing four ommatidia touching that circle. The red lines in the picture were not produced by the ImageJ plug in "Circle Fit" but were added to the photograph for illustration purposes only.

2.3 Behavioural Experiment

The behavioural experiments were conducted in a simple V-shaped arena. The V consisted of two aisles, 30cm squared, meeting at a 90 degree angle (Figure 5). The walls of the arena were 30cm high. The whole arena was lined with white photocopy paper. The backplane of one of the two aisles contained the stimulus: a black bar; the other back wall was either kept white or was covered with grey paper, matching the percentage of darkened surface of the other backplane.
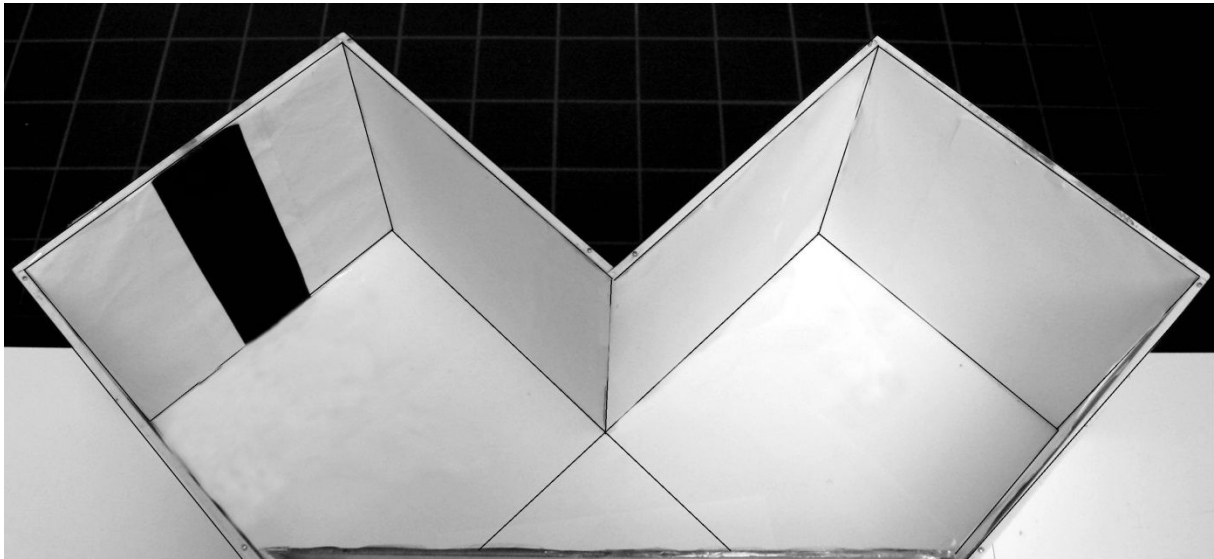
Figure 5 Photograph of the V-shaped arena used in the behavioural experiment. Stimulus attached to the backplane of the left aisle of the arena.

Adults were tested on six different stripe sizes. When seen from the entrance to the aisle the bars enclosed a visual angle of 30°, 10°, 5°, 3°, 1.5° and 0.5° respectively in the horizontal plane. Larvae were tested against the same set of stripes, except that instead of 0.5° a 15° degree stripe was used since after evaluating the results from the tests with adult animals, it was determined that the larvae could not resolve visual angles smaller than 3°.

The assumption behind the experiment was that the bugs would seek to flee or take shelter when being exposed to the very bright surroundings of the arena, since reduviids are known to show photonegative-reactions (Reisenman and Lazzari 2006). If that were the case, the animals should run into the aisle holding the dark stripe more often than into the other aisle, as long as they can perceive the stripe. To rule out the possibility that the bugs reacted to one aisle being darker than the other due to the black bar on its back panel, a second series of tests was performed in which a grey sheet of paper was placed on the back wall of the non-stimulus aisle that matched the percentage of blackness in the stimulus aisle.

Each individual was randomly tested 10 times for each stripe width during the experiment, resulting in a total of 60 trials for each individual. Whether the stripe was to be positioned in the right or left aisle was decided by a coin toss.

The bugs were placed into the arena using a non-transparent cup, and manoeuvred into the centre point between the two arms of the arena. At the beginning of each test the cup was removed. If the bug ran into one of the aisles before a minute had passed, the trial was stopped and considered successful. Which aisle the bug entered was recorded. The animal was

then returned back to the starting position if another test was to be made, or put back into the terrarium if not. Each individual was tested no more than five times per day.

Quite often the animals would not move on their own account, but remained at the same place. In that case after 30 seconds, an attempt was made to startle them by either blowing on them or nudging them with a ruler. To ensure that they were not pushed in any direction when nudging them, they were nudged squarely on the prothorax from directly above.

2.4 Optokinetic Response Experiment

The testing arena consisted of a cylindrical drum measuring 30cm in diameter and 30cm in height. The drum was set to spin in motion by means of a small electric motor and gears connected by hard rubber bands. The turning speed of the drum was controlled via an adjustable power supply unit and the setting of the gears (Figure 6). The drum's inside wall was clad with interchangeable gratings of varying fineness, made either of white cardboard with black stripes attached to the cardboard, or printed black and white stripes, producing a regular pattern with alternating black and white stripes of the same dimension.
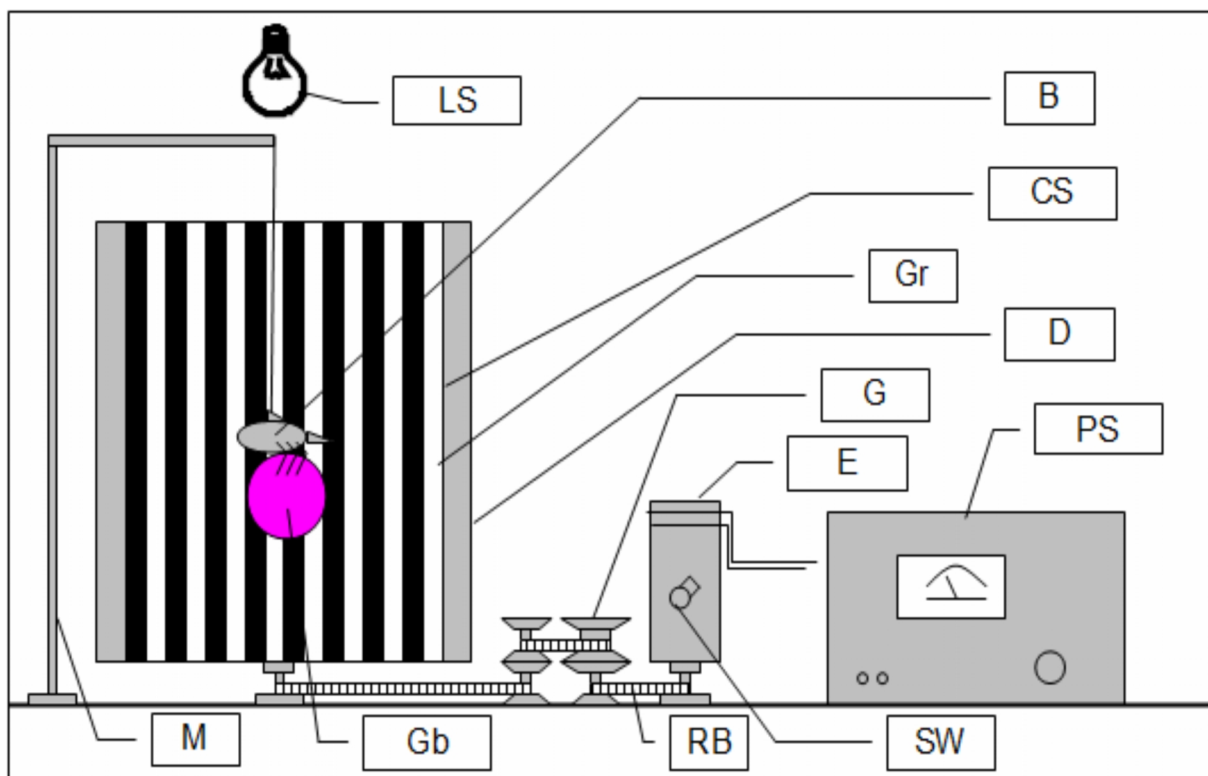

Figure 6 Setup of the optokinetic reaction experiment: LS, Light source; B Bug fixated to mounting via piece of cardboard glued to its pronotum, clamp and wire; CS , Cut section - depiction of view into the drum to show grating and bug; Gr, Grating; D, Drum; G, Gears; E, Electric engine; PS, Power supply; SW, Switch allowing to arrange drum to turn clockwise or counter clockwise without shifting cables; RB, Rubber bands connecting engine, gears and drum; Gb, Globe held by tested bug; M, Mounting

The bugs were led to grab onto a table tennis ball, draped in crepe paper for better grip. They were then tethered in the centre of the arena via a small clamp which held the cardboard on the back of the animal and which was bound to the mounting by wire. The table tennis ball was used instead of a Y-maze globe since preliminary studies showed that the bugs would not run along the Y-maze.

After arranging the bug to hang in the arena with the ball at its feet, as depicted in Figure 6, the drum was set in motion and the grating started to spin.

If the tethered bug tried to move in the direction of the spinning grating, it would move the ball in the opposite direction of the drum. This behaviour was interpreted to be an optokinetic reaction. Usually before the bug moved its feet and the globe with them, movement of the antenna and head could be observed.

In preliminary tests the bugs were exposed to gratings of varying fineness moving at two different frequencies, 7Hz and 48Hz. The animals performed equally well at both frequencies, which is why a frequency of 20Hz was chosen for the test set-up. This value was chosen to avoid either getting too close to the cut-off frequency off the animals' eyes, at which the black and white bars would be flickering past the animals too fast to be resolved by the eye, or going too slowly to incite reaction from the animals.

Four different gratings and a grey background were tested. The single bars of the gratings amounted to a visual angle of 15°, 5.5°, 3°, and 1.5° when seen from the centre of the arena. Consequently the periods of the gratings were 30°, 11°, 6° and 3°, respectively.

The grey background used as a control was designed to match the brightness of the gratings, 50% white and 50% black, and moved at the same speed as the widest grating (15°).

Each animal was tested once a day only and was exposed to three consecutive runs on that occasion. Preliminary tests indicated that longer or more frequent stimulation in the drum lead to diminishing reactions. After setting the drum in motion, the bug and the globe were observed for a maximum of thirty seconds if a distinct spinning, i.e. rotation of the globe more than 180 degrees in either direction, occurred.

The direction of the turn, or the absence of a distinct spin, were recorded.

2.5 Statistics

To find out whether the results of the behavioural choice experiment differed significantly from chance, a one-sample t-test was conducted using SPSS. The expected value used in this test was 0.5 (50%) – based on the assumption that without any stimulus the bug would choose randomly between the two aisles. The level of confidence was set to be 0.05 (5%).

To evaluate the results of the optokinetic response experiment a two-sided sign-test was conducted. The level of confidence was set to be 0.01 (1%). Morphological data was collected and analysed using Microsoft Excel.

3 Results

3.1 Results of Morphological Study

By plotting tibia length against pronotum width of the examined animals, six distinct groups could be identified (Figure 7). The six clusters seen in Figure 7 represent the five larval stages of *Platymeris biguttatus* (L1-L5) and the adult stage (A).
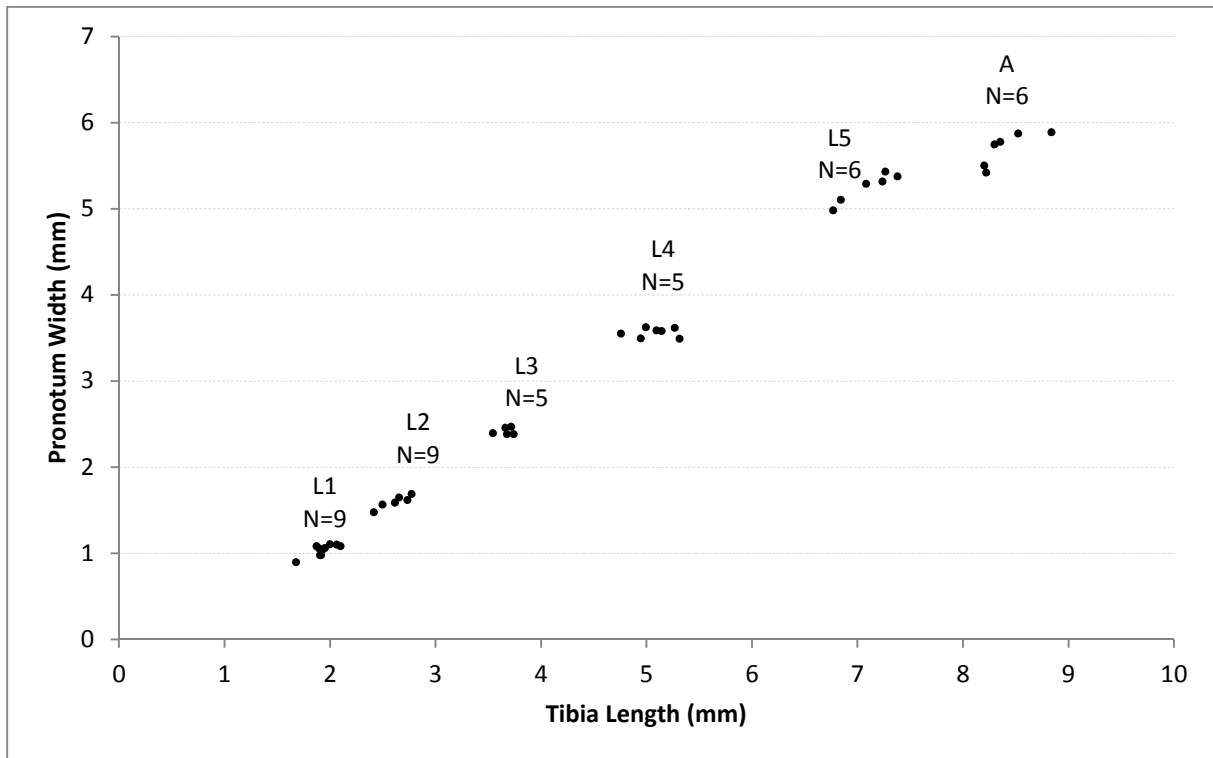
Figure 7 Graphical depiction of tibia length compared with pronotum width of each developmental stage of *Platymeris biguttatus*. The pronotum width is applied on the X-axis; Tibia length is applied on the Y-axis. L1 to L5, first to fifth larval stage; A, Adult; N= Number of animals measured per developmental stage. Each dot in the diagram represents the tibia length and pronotum width of a single animal at the respective developmental stage.

The same data was used to create Figure 8. In Figure 8 mean values of tibia length and pronotum width were used, and the standard deviation is given by the error bars.
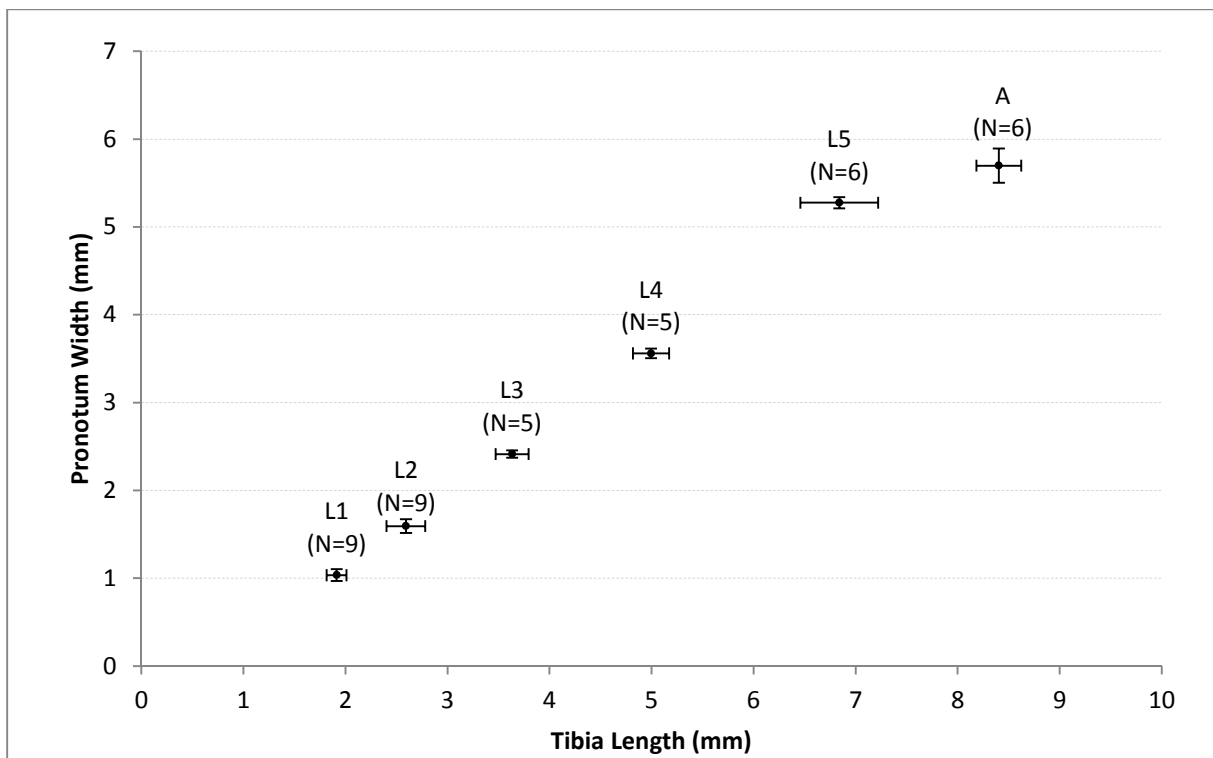
Figure 8 Graph of mean tibia length plotted against the mean width of the pronotum for each developmental stage. Pronotum width is applied on the Y-axis, tibia length on the X-axis. L1-L5, first to fifth larval stage; A, Adult. Each dot in the diagram represents the arithmetic mean of the tibia length and pronotum width for the respective developmental stage; the horizontal error bars for each dot depict the standard deviation of the arithmetic mean. N= Number of animals measured for each developmental stage, all measures in millimetre.

The correlation between tibia length, pronotum width and stage of development in *Platymeris biguttatus* was used to determine the exact larval stage which a larva was currently going through. This was crucial to the behavioural experiment since only 3rd instar larvae and adult animals were used (see below). Adult animals can be easily identified by the presence of fully mature wings; however young larvae of the 2nd, 3rd and 4th instar are difficult to distinguish. To ascertain to which instar a larvae belongs, the larvae were kept individually. Photos of their pronotum were taken before and after ecdysis to determine its width. After ecdysis the exuviae were collected, and the front tibia measured. These measurements were compared with the data in Figure 8 and used to confirm an animal's current developmental stage.

The number of facets found in a single eye of *Platymeris biguttatus* increases throughout its larval development (Table 1). The mean number of facets composing one eye of a first instar larvae amounts to a rounded 106, the number of ommatidia in a 3rd instar larvae is 325, and the eye of an adult animal is composed of about 880 ommatidia. As is shown in Figure 9 the number of facets seems to increase linearly with the tibia length.
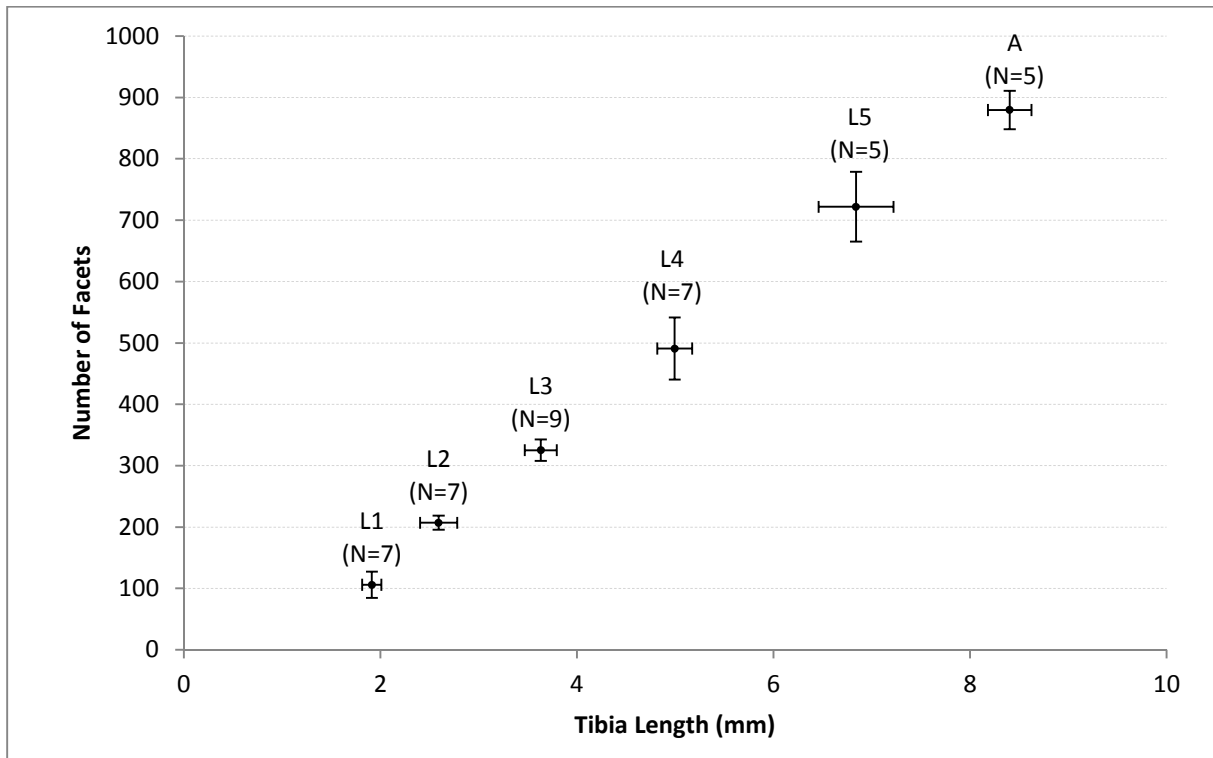
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Figure 9 Graphical depiction of mean tibia length compared with the mean number of facets in one eye of each stage of development. The horizontal error bars for each dot depict the standard deviation of the arithmetic mean. L1 - L5, first to fifth larval stage; A, Adult; N= Number of animals measured per developmental stage.

Measurements of the facet diameter show that the diameter of facets increases during development. The mean value doubles from 34 micrometres in the first instar to 69 micrometres in the adult bugs (Table 1, Figure 10). The increase in facet diameter does not appear to be as linear as is the case with the increase of facet numbers throughout the animal´s development.
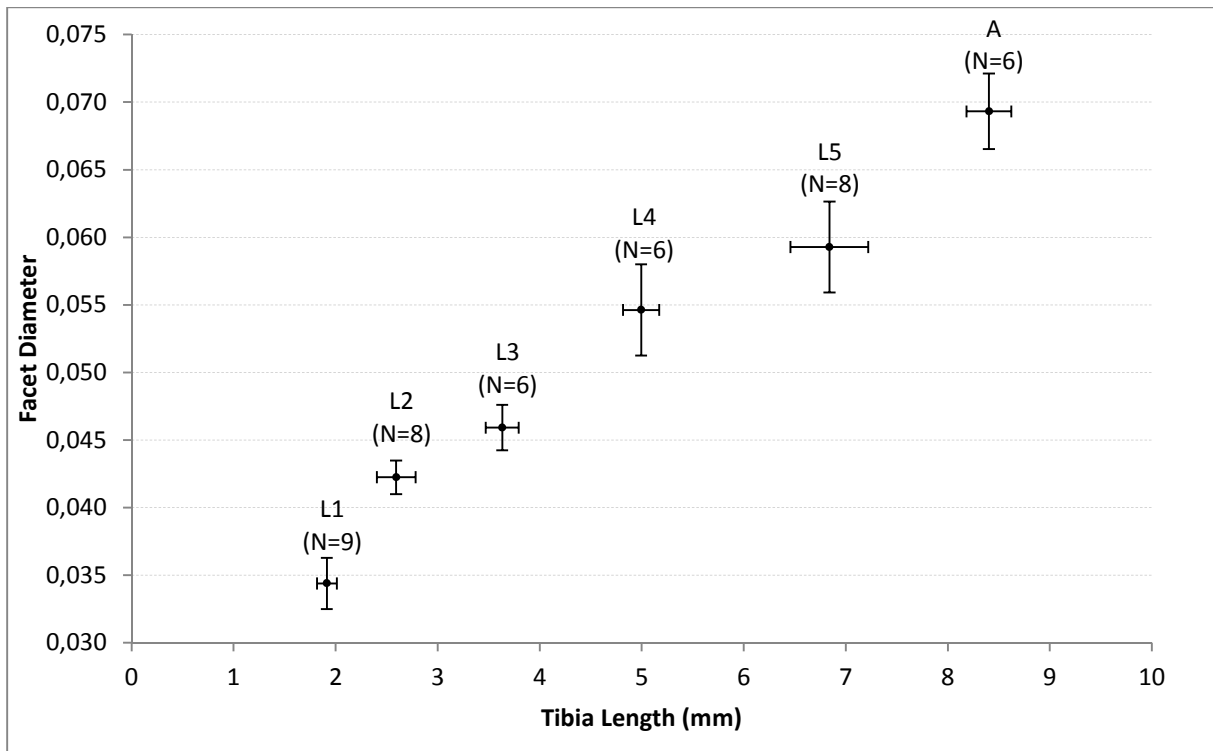
Figure 10 Graphical depiction of mean tibia length compared with the mean facet diameter in the eye of each developmental stage. The facet diameter is applied on the X-axis; tibia length on the Y-axis. L1 - L5, first to fifth larval stage; A, Adult; N= Number of animals measured per developmental stage.

Figure 11 shows the mean interommatidial angle of each developmental stage of *Platymeris biguttatus*, measured for two regions of the eye (anterior, posterior) and compared with tibia length. The interommatidial angle in both the anterior and posterior part of the eye declines from one instar to the next.

The interommatidial angle of the caudal part of the eye is larger than that of the rostral part, except for the first instar, where the interommatidial angle of the rostral part is larger. The interommatidial angles range from 12.5° in the first instar to 3.7° in the adults (Table 1).
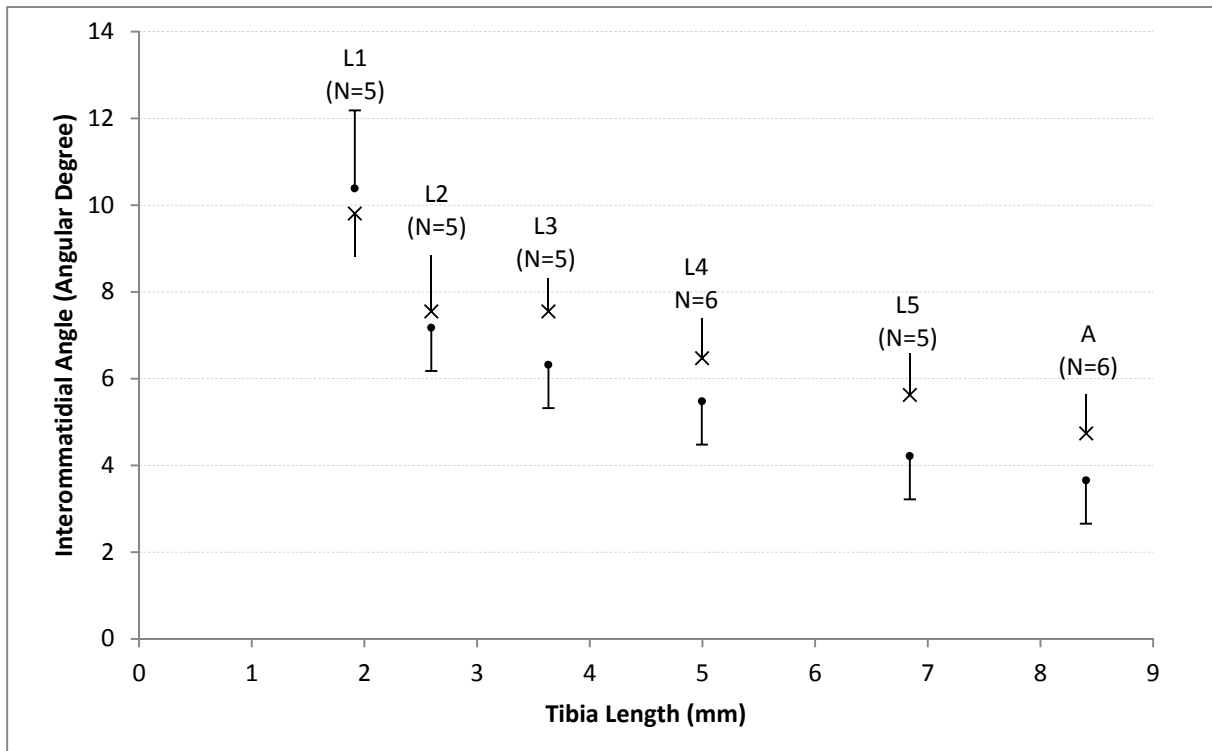
Figure 11 Graphical depiction of mean tibia length compared with the interommatidial angle measured at the rostral and caudal part of the eye of each developmental stage. Dots represent the interommatidial angles of the anterior, cross-marks (X) that of the posterior part of the eye. The interommatidial angle is applied on the Y-axis; tibia length is applied on the X-axis. L1 to L5, first to fifth larval stage; A, Adult. Each dot in the diagram represents the arithmetic mean of the tibia length and interommatidial angle of a particular developmental stage, the horizontal error bars for each dot depict the standard deviation of the arithmetic mean. Standard deviations of the interommatidial angle are given as vertical bars. Measures of tibia length are given in millimetre, interommatidial angle in angular degree. N= Number of animals measured per developmental stage.

When measurements taken on the eye are compared with each other and not with tibia length, it is apparent that the number of facets, and therefore the number of ommatidia and the diameter of the facets increase more or less continuously, while the decrease of the interommatidial angle diminishes toward the adult state.

This trend is particularly apparent when the graphs in Figure 12 and Figure 13 are viewed. Obviously, the interommatidial angle nearly reaches its minimum at the 5th instar, while the number of facets and the size of the lenses increase to the last ecdysis.
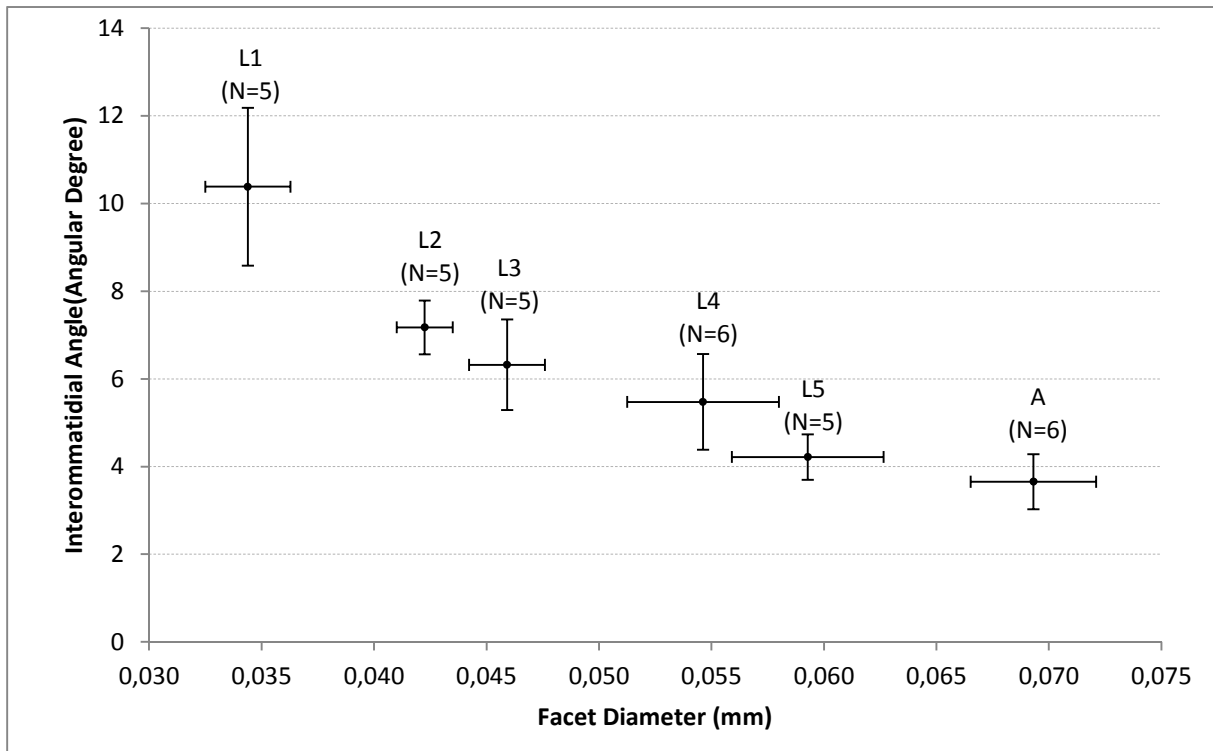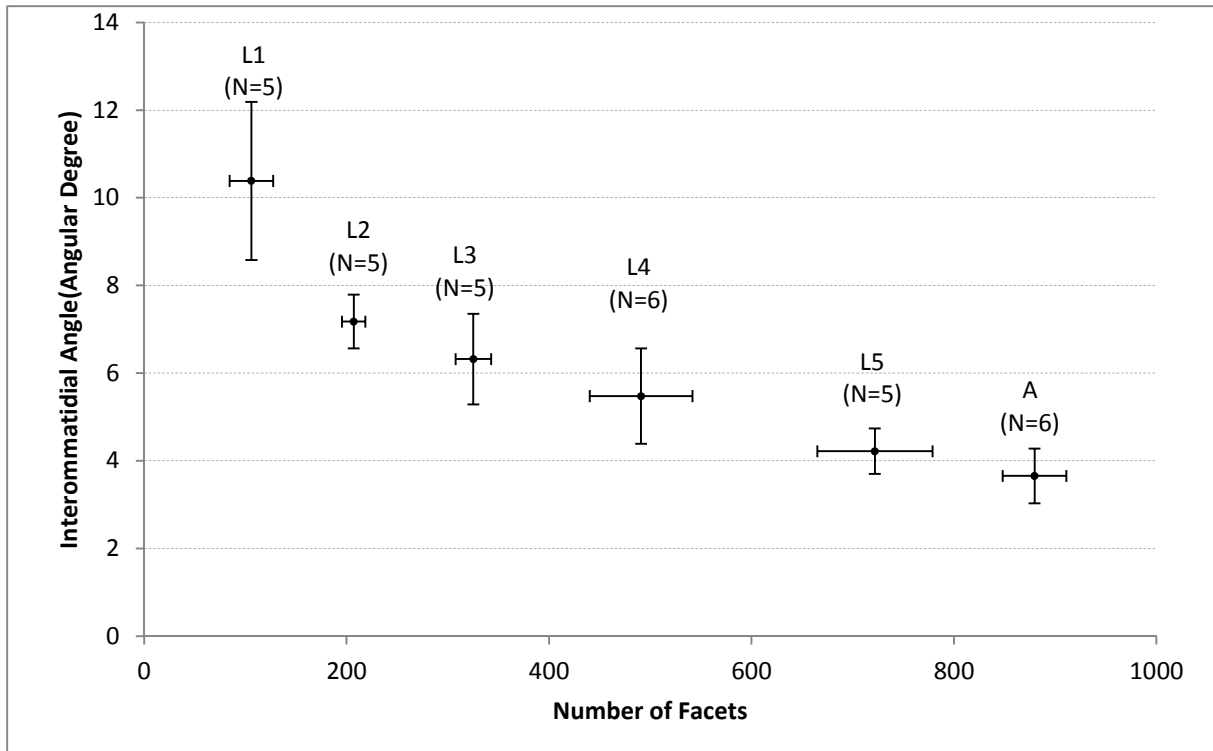
Figure 12 Graphical depiction of the mean interommatidial angle compared with the mean facet diameter in the eye of each developmental stage. Each dot in the diagram represents the arithmetic mean of the interommatidial angle and facet diameter of a larval stage or the imagines, the error bars on each dot depict the standard deviation of the arithmetic mean. Standard deviations of facet diameter are given as horizontal bars , standard deviations of the interommatidial angle as vertical bars . N= Number of animals measured per developmental stage.

Figure 13 Graphical depiction of the mean interommatidial angle compared with the mean number of facets in the eye of each developmental stage. Each dot in the diagram represents the arithmetic mean of the interommatidial angle and the number of facets for each developmental stage, the error bars on each dot depict the standard deviation of the arithmetic mean. Standard deviations of facet number are given as horizontal bars, standard deviations of the interommatidial angle as vertical bars. N= Number of animals measured per developmental stage.

Table 1 Mean-values (MV) and standard-deviations (+/-) of the parameters tibia length (Tib L), pronotum width (Pro W), facet number (Fac No), facet diameter (Fac D), anterior and posterior inter-ommatidial – angle (IOA and IOP) in mm in all stages of development. L1-L5 Larval stages 1-5, A – Adult.

	L1		L2		L3		L4		L5		A	
	MV	**+/-**	**MV**	**+/-**	**MV**	**+/-**	**MV**	**+/-**	**MV**	**+/-**	**MV**	**+/-**
Tib L	1.91	0.10	2.59	0.19	3.63	0.16	4.99	0.18	6.84	0.38	8.40	0.22
Pro W	1.04	0.07	1.59	0.08	2.41	0.04	3.56	0.06	5.28	0.06	5.70	0.20
Fac No	105.9	21.4	207.0	11.5	325.2	17.6	490.9	50.8	721.8	56.9	879.6	31.4
Fac D	0.034	0.002	0.042	0.001	0.046	0.002	0.055	0.003	0.059	0.003	0.069	0.003
IOA	10.38	1.80	7.18	0.61	6.32	1.03	5.48	1.09	4.22	0.52	3.66	0.63
IOP	9.81	1.70	7.55	1.29	7.06	0.76	6.47	0.92	5.62	0.96	4.74	0.90

To provide a general view of the development of all eye-parameters Table 1 gives the mean-values of all eye-parameters for all developmental stages.

3.2 Results of Behavioural Experiment

To evaluate the data gathered from the behavioural experiment, the performance of each tested bug was calculated for every bar-size. The performance is the percentage of test runs for each given stripe size, to which the bug chose to run into the aisle where the bar was fixed. These performance values were put together into groups according to bar size; the mean

values and standard deviation were calculated (Figure 14, Table 2). To determine whether the mean values differed significantly from chance results, a one-sample t-test was conducted using SPSS. The expected value used in this test was 0.5 (50%) – based on the assumption that without any stimulus the bug would randomly choose between the two aisles. The level of confidence was set to be 0.05 (5%).

The best performance was observed in adult animals, when tested against a white background. The one-sample t-test showed that the bugs, when confronted with a stimulus as narrow as 1.5° chose the aisle containing the stimulus significantly more often than the aisle lacking the stimulus. The performance was above 60% for all tested stripe sizes, except for the smallest size, which from the entrance to the aisle could be seen at an angle of 0.5°.

For example, in 71.9% of the trials, the animal ran into the aisle with the black strip when tested with the broadest stripe, which amounted to 30° of visual angle (Table 2, Figure 14). In 69.1% of the trials, the assassin bug ran into the aisle containing the stripe when the stripe amounted to 10° of visual angle.

The percentage of trials in which the bug ran into the aisle containing the stripe was always lower when the aisle lacking the stripe was grey instead of white. The t-test showed that only when confronted with the broadest stimulus did the bugs chose significantly more often the aisle with the black stimulus than the aisle with the grey backplane.

Larval bugs of the third instar were tested in the same way as the adults, but performed poorer in every aspect. As evident in Table 2 and Figure 14, the percentage of larval bugs choosing to run into the aisle holding the black stripe is lower than that of the adult animals throughout the entirety of the test-setup. The broadest stripe attracted the larvae in 60.5 % of all runs. However the percentage of larval bugs choosing the aisle with the stripe when the stripe was 15° wide was 64.3 – higher than when the 30° stripe was used. The test runs using the black 15° stimulus in one aisle and the white backplane in the other aisle was also the only series of runs in which the results differed significantly from randomness. The results of all other groups do not differ significantly from random.

When larvae were used, no significant results could be derived from any of the test conditions in which a grey backplane was fixed in the aisle lacking the stimulus.
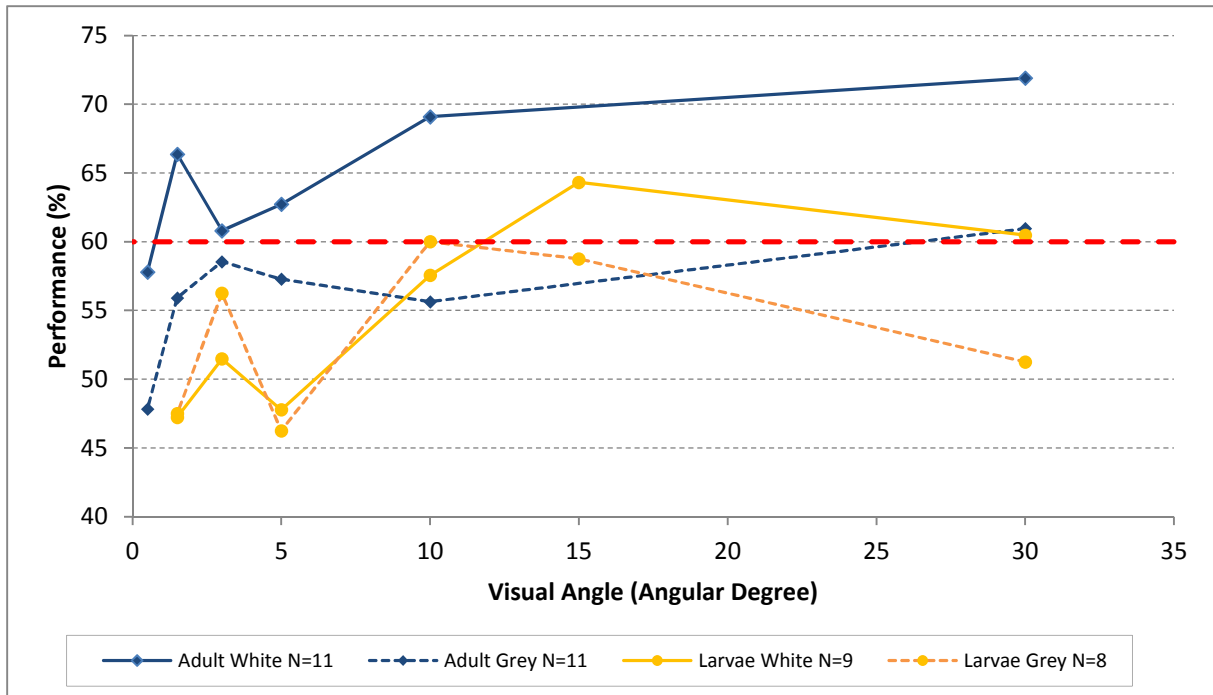
Figure 14 Graphical depiction of the mean performance of adult and larval *Platymeris biguttatus* in various behavioural test conditions. Blue lines show the performance of adult individuals, orange lines those of third instar larvae. Solid lines represent the performance attained in those tests in which the empty aisle had a white back wall, broken lines represent tests where the back wall lacking the stripe was covered with grey paper that matched the amount of darkness of the striped aisle. Red dashed line indicates a performance 0f 60%.

Table 2 Mean values of the performance of adult and 3rd instar *Platymeris biguttatus* in the behavioural experiment. Numbers in the top row indicate the angular degree at which the tested stripe was visible from the entrance of the aisle. N indicates the number of animals tested; A Adult; L Larvae of 3rd instar. W White, G Grey indicate colours of the back wall of aisle lacking the black stripe. Cross-marks (X) indicate no tests performed.

	30.00		15.00		10.00		5.00		3.00		1.50		0.50		N
Ad W	71.90	14.62	X	X	69.10	17.00	62.70	12.72	60.80	14.24	66.40	22.92	57.80	17.63	11,00
Ad G	61.00	13.05	X	X	55.60	15.62	57.30	11.04	58.50	14.65	55.90	16.34	47.80	15.76	11,00
L W	60.50	14.67	64.30	15.17	57.60	18.71	47.80	17.87	51.50	18.74	47.20	19.18	X	X	9,00
L G	51.30	15.53	58.80	17.27	60.00	11.95	46.30	15.06	56.30	16.85	47.50	18.32	X	X	8,00

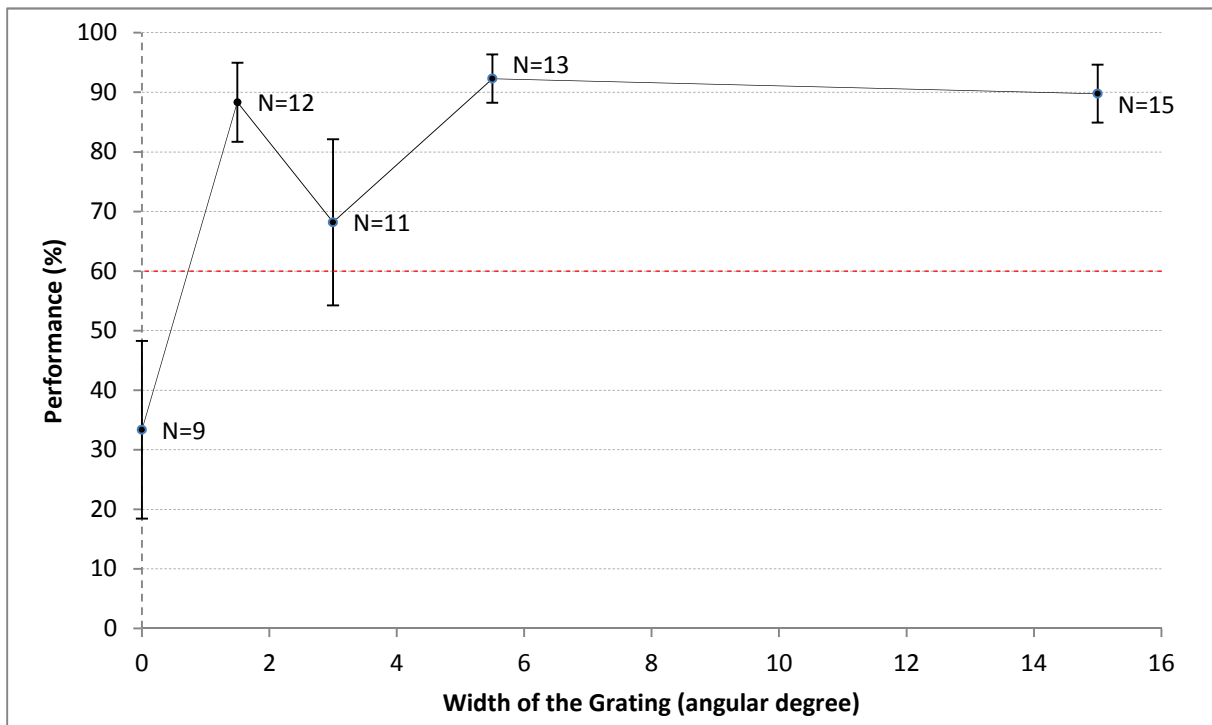## 3.3 Results of Optokinetic Response Experiment

Figure 15 Graphical depiction of the mean performance of adult *Platymeris biguttatus* in the optokinetic experiment showing the mean percentage of turning-movements that matched the direction in which the drum was turned (with rotation). Values on the X-axis represent the broadness of the stripes that made up the grating of the drum, in angular degree as seen from the centre of the drum. The gratings from left to right: 1,5°, 3°,5,5°,15°. The grey background that the animals were exposed to is depicted as 0°, since an infinitely fine grating of black and white bars would be perceived as grey. Standard deviations of the mean values are depicted as vertical error-bars at the corresponding point in the graph, N= Number of animals with at least one evaluable run at the given grating.

When exposed to the finest grating used, in which every bar enclosed a visual angle of 1.5°, a mean value of 88% of all countable turning-movements occurred in the direction that would have enabled the animal to follow the motion of the drum had it not been fixed in space by the clamp and mounting. The movements of the gratings, which consisted of bars 15° and 5.5° broad, were met with movements going "with the drum" in over of 80% of cases. The only grating, in which less than 80% (68.8%) of the movements went with the drum, contained bars 3° broad.

In contrast, the bugs did go against the drum more often than with it when the drum was lined with the grey background instead of a grating, as evident in Figure 15.

It is also notable that the animals' disposition to rotate the ball at all coincided with the broadness of the bars that make up the grating. The number of bugs that moved the ball (N) was highest when the coarsest grating (15°) was used. With this stripe width, all 15 bugs

moved the ball at least once in the course of the 10 runs (see Table 4, Supplements). The number of bugs that moved the ball in at least one of the 10 runs for every grating diminishes as the grating becomes finer. Using the 5.5°, 3° and 1.5° gratings, the number of bugs that moved is 13, 11 and 12, respectively. When a grey background was used only 9 out of 15 bugs spun the ball.

4 Discussion

The aim of this study was to test the visual performance of the compound eyes of *Platymeris biguttatus* over the course of the animals' development.

The morphological data suggest a continuous increase of spatial resolution and light sensitivity from the first instar to the adult animals. The number of ommatidia increases from about 100 ommatidia to almost 900. The facet diameter doubles from 34µm to 69µm and the interommatidial angle in the anterior part of the eye decreases from 10.4° in the first instar to 3.7° in adult animals.

The combined development of these parameters should enable those animals that have just completed an ecdysis to resolve finer details under poorer light conditions than in the preceding ecdysis.

For comparison, the eyes of *Triatoma infestans*, which is a closely related bug, start with an average of only 34 ommatidia in the first instar and adults possess an average of 312 ommatidia per eye (Settembrini 1984). Likewise, the mean diameter of the facets increases from 34µm to a maximum of just above 100µm in the course of larval development.

Triatoma infestans, however, is a nocturnal hematophagous bug and may not be as dependant on visual acuity as other reduviids. *T. infestans* sneaks up on its involuntary donors by using quite an array of senses; thermal orientation and olfaction being the main guides of host location (Reisenman et. al. 2000). It is therefore not surprising that *T. infestans* has larger facets and a smaller number of ommatidia per eye than *P. biguttatus*. The developmental trends, however, are quite similar, an increase of ommatidia per eye by a factor of roughly nine, and at least a doubling of facet-diameter.

Similar trends have been found in the development of the shore-bug *Saldula saltatoria*. Like *S. saldtatoria, P. biguttatus*, is an active hunter that pounces on its prey. *T. infestans* in contrast is a nocturnal blood-sucking parasite. Therefore *T. infestans* might be less dependent on its visual acuity than the more active predators. As Griesinger and Bauer (1990) showed the number of ommatidia in the eye of *S. saltatoria* increases from 139 (first instar) to 1081 (imago), and the diameter of the corneal lenses in the frontal part of the eye increases from 12µm to 22µm. The similarity in the development of the number of ommatidia in *P. biguttatus* and *S. saltatoria* is striking, as well as the difference in the diameter of their facets. The reason for the difference in facet diameter might be that *P. biguttatus´* eyes are adapted to a more nocturnal or crepuscular life style than those of *S. saltatoria*. Another possible explanation stems from the fact that adult *S. saltatoria* only reach a body length slightly more than 4mm, whereas *P. biguttatus* reaches sizes up to ten times that measure, and therefore has

more space available for larger facets. Interestingly, the first larval stage of *P. biguttatus* is roughly the same size as adult *S. saltatoria* and possesses facets 1.5 times the size of *S. saltatoria* but less than a tenth of its ommatidia. This fact suggests that sensitivity may be more important than visual acuity for the early larval stages of *Platymeris*.

Morphological data suggest that the anterior part of the eye is most important for *Platymeris biguttatus*, since the anterior interommatidial angle reaches its smallest value during the 5th instar and is narrower than the posterior interommatidial angle. The posterior angle in the smaller larval stages is almost as narrow or even slightly more narrow (L1) than the anterior angle. This condition changes with the second ecdysis, after which the anterior angle is considerably smaller than the posterior angle (Table 1, Figure 11). A possible explanation for this might be that the smaller larvae, such as the L1, have only about 100 ommatidia and therefore cannot rely entirely on vision as the primary method of prey location. Instead the main function of the bug's eyes at that stage might be predator location. Predators would, of course, be considerably larger than the animals' desired prey-size and therefore more easy to locate visually even with cruder resolution. Although predators may approach their prey from any given direction, the prey can only be attacked frontally with the bugs' beak. Thus if the main objective is predator location, it is not efficient to concentrate their limited ommatidial capacity to one area of the eye. In the behavioural experiments, it was observed that before a stationary bug moved in any direction it pointed its antennae in that direction. This suggests that olfaction is an important source of orientation in *P. biguttatus*.

It is possible that in the development of *Platymeris biguttatus* a shift from a mainly olfactory guided mode of hunting to a more visual guided mode occurs. This hypothesis stems from the observation that the antenna of the early larval stages appear to be larger than those of the adults relative to body size.

Furthermore on the one hand, Freund and Olmsted (2000) showed that vision is more important in predator avoidance than olfaction in two predatory bugs, *Sinea diadema* (Reduviidae) and *Nabicula subcoleoptrata* (Nabidae). The same study postulated that olfaction in predatory hetereopterans might be more important than vision in foraging for prey. The animals used in that study were considerably smaller than adult *P. biguttatus* (*S. diadema* 5th instar ≈ 13.5mm, *N. subcoleoptrata* ≈ 8.1mm) but comparable in size to the younger larvae of *P. biguttatus* and might therefore be exposed to similar predatory pressure as the first few instars of *P. biguttatus*.

On the other hand, *Haematorrhphus niggroviolaceus*, a reduviid bug that preys on millipeds, when starved and confronted with artificial bait, has been shown to react appreciably more often to unscented moving bait than to stationary scented bait (Haridass and Ananthakrishnan 1980a). The impact of antennectomy, eye blinding and tibial comb coating on the predatory behaviour of the reduviid *Rhynocoris kumarii* was studied by Claver and Ambrose (2001). That study showed that impairment of any of the senses results in a delayed arousal response. The act of approaching prey, however, was only significantly affected in antennectomised animals, not in blinded ones. Capturing response was significantly affected in those bugs whose eyes had been blinded, as well as, in those whose antenna had been cut off and those whose tibial combs had been rendered useless (Claver and Ambrose 2001). These studies illustrate the complex collaboration of predatory bugs' senses during prey location, approach and capture.

Platymeris biguttatus possesses a cave organ along with a number of differentiated trichobottria, which was first discovered in the bloodsucking *Triatoma infestans* (Barth 1952, Lazzari and Wicklein 1994). However, the larvae of Reduviidae do usually not possess more than a single trichobottrium (Weirauch 2003). Cave-organs are thought to register heat-radiation (Barth 1952 cited in Weirauch 2003, Lazzari and Wicklein 1994) or chemical cues (Catala 1994). Weirauch (2003) demonstrated that this organ is present on the antennae of *P. biguttatus*, but it is as yet unknown how *P. biguttatus* benefits from the organ, since heat-stimuli are probably not as useful in locating arthropod prey as in finding mammals or birds for obvious reasons. Moreover, trichobottria may function to register air movement (Weirauch 2003) and may readily benefit prey-tracking and predator avoidance.

With such an array of sensory systems including vision, air movement registration, olfaction, and the cave-organ, which possibly detects heat-stimuli or chemical cues, *Platymeris* bugs are superbly equipped in locating food and not getting devoured in the process, as well as finding other bugs of their species to mate with.

However, to clarify which of these senses is used for what task and how well it performs more research will have to be conducted.

The observations made during the behavioural experiment suggest that it is possible to use spontaneous preferences to determine the visual acuity in hemipterans. Although the animals may often remain inactive instead of running through the V-shaped arena, there is a preference for running toward dark objects. A method to coax the bugs to show more active

behaviour, such as flight reaction, without influencing the direction in which they flee might greatly improve the experiment.

The behavioural experiment clearly suggests that resolution improves during larval development, since the adults show a positive reaction toward objects which could not be detected by the 3rd instars. The behavioural experiment, however, was hindered by the animals' reluctance to move spontaneously - a disposition that most likely stems from their "wait and grab" (Haridass et al. 1987) strategy of feeding. Additionally, the animals' photo-negative reaction does not seem to be as profound as that of their reduviid relatives, such as *Triatoma infestans*, whose strong photonegative reaction has been used to study its spectral sensitivity by Reisenman and Lazzari (2005).

In the behavioural choice experiment a grey back wall, placed in the aisle which lacked the stimulus, severely diminished the likelihood of the bug choosing the aisle with the stimulus. An explanation for that is that the bugs, when no grey back wall was fixed in the arena, reacted to the aisle containing the stimulus which was darker. However, the results of the optokinetic experiment and the behavioural choice experiment when conducted without a grey back wall matched very closely, which led me to assume that the animals can perceive single objects as narrow as 1.5°, but are almost equally attracted to the grey back wall and to the black stimulus.

Both the behavioural and the optokinetic test show that the animals react to stripes at a visual angle considerably more narrow than the interommatidial angle in the frontal part of their eyes. In both experiments the animals still react to bars of 1.5° width.

Given the equation $vs=1/(2\Delta\Phi)$, which postulates that there must be two receptors for every circle of a grating in order for the grating to be resolved properly (Land and Nilsson 2002), the interommatidial angle in the eye of *Platymeris biguttatus* would be assumed to be 1.5° or smaller.

A possible explanation for the discrepancy between values for the interommatidial angle gathered from the morphological examination and the optokinetic response experiment lies in the arrangement of the ommatidia within the eyes. In a hexagonal arrangement of ommatidia the question arises which interommatidial angle should be used. In case of the hexagons "standing on their tips" some argue that ommatidial axes are separated vertically by less than the interommatidial angle and that in some circumstances the proper basis for measuring acuity should not be $\Delta\Phi$ but $(3)^{\frac{1}{2}}\Delta\Phi/2$ (Land 1997).

The mean value of the interommatidial angle in the frontal part of adult specimens of *Platymeris biguttatus* is 3.7. However, using the formula mentioned above, this value is

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reduced to roughly 3.0. The value suggests that *P. biguttatus*` visual abilities are worse than they evidently are.

Another explanation for the actual acuity being better than the morphological data suggested would be the presence of an acute zone somewhere in the eye of the animals, although nothing of that kind became apparent during the morphological studies.

The spatial acuity of the adult bugs determined by using the optokinetic response and the behavioural choice experiment gives a value of 1.5° or lower for the interommatidial angle $\Delta\Phi$. This is a fairly average value for insects (for comparison, see Land 1997).

Table 1 and Figure 11 show that the interommatidial angle in the anterior part of the eye diminishes rapidly from the first instar to the fifth, the smallest decrease of the interommatidial angle happens during the last moulting. Parallel to this development the facet diameter increases from one ecdysis to the other, but the greatest increase occurs in the last ecdysis – from the fifth larval stage to the adult animals. This suggests that early in the larvae's development acuity is a key issue, and that the quality of vision of the fifth instar is almost as acute as that of the adults, albeit much dimmer. During the last ecdysis the facet diameter increases from 59μm to 69μm, which is an increase of one sixth. This is interpreted to mean that, when compared to the largest larvae, adult *P. biguttatus* do not gain much more acuity, which would enable them for example to detect prey from further away or to pounce at prey with more precision, but instead increase sensitivity by a large margin, which should enable them to hunt further into dawn or even at night.

It was shown that the parameters contributing to the visual capabilities of *Platymeris biguttatus* increase drastically during larval development. This increase was shown to be apparent in the eye parameters: facet diameter, interommatidial angle and number of ommatidia.

This increase in acuity was successfully demonstrated to affect the animals' ability to resolve objects visually, by showing that adult animals are able to react to much more narrow objects than larvae of the third instar.

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6 Register of Illustrations

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8 German Summary

Ziel dieser Diplomarbeit ist es die Entwicklung des optischen Auflösungsvermögens der räuberischen Raubwanze *Platymeris biguttatus* (Reduviidae) zu erforschen. Zu diesem Zweck werden morphologische Untersuchungen der wichtigsten Augenparameter sowie Verhaltensversuche in Form eines Wahlversuches und eines optokinetischen Versuches durchgeführt. Augenparameter wie Facettendurchmesser, Interommatidialwinkel und die Anzahl der Facetten pro Auge werden für alle fünf Larvalstadien sowie die adulten Tiere untersucht. Wanzen im dritten Larvalstadium sowie adulte Tiere werden im Wahlversuch verwendet. In dem optokinetischen Versuch werden ausschließlich adulte Tiere verwendet. Der Wahlversuch basiert auf der Annahme, dass Wanzen der Spezies *Platymeris biguttatus*, photonegatives Verhalten zeigen und von dunklen Stimuli angezogen werden wenn sie einer hellen Umwelt ausgesetzt werden. Die Wanzen werden in einer V-förmigen Arena ausgesetzt und können entweder in einen Gang laufen, an dessen Rückwand ein dunkler Stimulus angebracht ist, oder in einen anderen Gang ohne einen solchen Stimulus. Der optokinetische Versuch basiert auf dem Experiment von Bernd Hassestein (1949). Die morphologischen Daten zeigen, dass der Facettendurchmesser und die Anzahl der Ommatidien während der Larvalentwicklung mehr oder weniger kontinuierlich anwachsen. Der Interommatidialwinkel hingegen scheint sich nicht derart kontinuierlich zu entwickeln. Die Anzahl der Facetten nimmt von 105 im ersten Larvalstadium auf durchschnittlich 880 bei den Adulten zu. Während der Larvalentwicklung vergrößert sich der Facettendurchmesser von 34 μm auf 69 μm, und der Interommatidialwinkel im anterioren Anteil des Auges schrumpft von 10,4° auf 3,7°. Im Wahlversuch wählen die adulten Wanzen signifikant häufiger jenen Gang, in dem der dunkle Stimulus fixiert ist, bis hinunter zu Stimulusgrößen die einen Sehwinkel von 1,5° einschließen. Ist eine graue Rückwand in jenem Gang angebracht, der keinen Stimulus enthält, verringert das die Wahrscheinlichkeit, dass eine Wanze den Gang mit dem Stimulus wählt, deutlich. Tiere, die sich im dritten Larvalstadium befinden, wählen nur bei einem Stimulus der 15° breit ist signifikant häufiger den Gang mit Stimulus. Im optokinetischen Versuch zeigen adulte Versuchstiere signifikante Reaktionen auf Streifenmuster, die aus schwarzen und weißen Streifen von je 1,5° Breite zusammengesetzt sind. Besonderheiten der Entwicklung der Augenparameter und die Aussagekraft der Verhaltensversuche werden besprochen.

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10 Supplements

Table 3 Morphological measurements of individual *Platymeris biguttatus* of all developmental stages. Mean-values (MV) and standard-deviations (ST D) of the parameters tibia length (Tib L); Pronotum width (Pro W); Facet number (Fac No); Facet diameter (Facet D), Anterior and posterior inter-ommatidial – angle (IOA and IOP) in mm in all stages of the bugs development. Developmental stage (Dev. St.) L1-L5 Larval stage 1-5, A – Adult.

Specimen	Dev. St	Facet D	Tibia L	Pro W	Fac No	IOA	IOC
3	L1		1.95	1.06	60.00		
4	L1	0.037	1.87	1.08	103.00		
5	L1	0.035	1.90	1.05			
6	L1		2.00	1.10		12.50	9.42
17	L1	0.030	1.68	0.89			
Exh1	L1		1.84		119.00		
Exh2	L1		1.81		109.00		
Exh3	L1		1.96		120.00		
Exh4	L1		1.87		108.00		
Exh5	L1	0.035	1.90		122.00		
28	L1	0.033	1.92	0.98		11.60	9.70
29	L1	0.036	2.10	1.08		8.90	12.50
30	L1	0.035	1.91	0.98			
50	L1	0.035	1.93	1.04		10.00	7.60
52	L1	0.035	2.06	1.10		7.80	7.20
	MV	0.034	1.91	1.04	105.86	10.38	9.28
	ST D	0.002	0.10	0.07	21.44	1.80	1.88
1	L2	0.043	2.62	1.59			
2	L2	0.044	2.42	1.47		6.70	9.30
7	L2	0.042	2.73	1.61			
8	L2	0.043	2.78	1.69			
36	L2	0.042	2.80			7.80	6.40
38	L2	0.040	2.52			7.60	6.80
ExhT	L2		2.70		205.00		
Exh6	L2		2.47		195.00		
Exh7	L2		2.63		219.00		
Exh8	L2		2.98		222.00		
Exh9	L2		2.45		206.00		
Exh10	L2		2.21		191.00		
Exh13	L2		2.49		211.00		
31	L2	0.043	2.50	1.56		6.60	7.70
47	L2	0.043	2.66	1.65		8.20	8.70
48	L2		2.56				
	MV	0.042	2.59	1.59	207.00	7.18	7.55
	ST D	0.001	0.19	0.08	11.47	0.61	1.29
9	L3	0.045	3.68	2.38	297.00		
26	L3	0.049	3.66	2.45		5.80	6.30
27	L3	0.047	3.72	2.47		5.00	6.70
41	L3	0.044	3.69			7.80	7.20
42	L3	0.046	3.66			6.50	6.80
live1	L3		3.55	2.39	318.00		
live3	L3		3.74	2.38	346.00		
L4 Exh3	L3		3.63		325.00		
L9	L3		3.63		334.00		
L10	L3		3.32		310.00		

Specimen	Dev.St	Facet D	Tibia L	Pro W	Fac No	IOA	IOP
L11	L3		3.54		338.00		
L14	L3		3.91		348.00		
L15	L3	0.045	3.33		311.00		
T32	L3		3.80			6.50	8.30
	MV	0.046	3.63	2.41	325.22	6.32	7.06
	ST D	0.002	0.16	0.04	17.58	1.03	0.76
10	L4		5.00	3.62		4.45	6.91
11	L4		5.27	3.62	464.00	5.61	6.52
12	L4	0.057	5.31	3.49		6.82	6.87
20	L4	0.053	4.76	3.55	405.00	4.16	7.63
21	L4	0.055	5.09	3.59	481.00	5.21	4.99
39	L4	0.054	4.89			6.60	5.90
L4 Exh4	L4		4.89		516.00		
L8	L4		4.97		558.00		
L18	L4		4.79		477.00		
L11	L4		4.88		535.00		
51	L4	0.057	5.14	3.58			
53	L4	0.054	4.95	3.49			
	MV	0.055	4.99	3.56	490.86	5.48	6.47
	ST D	0.002	0.18	0.06	50.78	1.09	0.92
14	L5	0.058	7.26	5.43		3.82	6.18
15	L6	0.059	7.08	5.29	684.00	3.42	4.88
16	L7	0.057	7.38	5.37	646.00	4.45	6.76
19	L8	0.053	7.24	5.31			
Exh12	L9		6.49		729.00		
Exh14	L10	0.062	6.36		780.00		
Exh15	L11	0.063	6.57		770.00		
Exh16	L12		6.40				
45	L13	0.063	6.77	4.98		4.40	5.30
46	L14	0.060	6.84	5.10		5.00	5.00
	MV	0.059	6.84	5.28	721.80	4.22	5.62
	ST D	0.003	0.38	0.06	56.87	0.52	0.96
13	A	0.066	8.52	5.87	912.00	4.24	
22	A	0.072	8.84	5.88	855.00	4.57	4.67
23	A	0.068	8.22	5.42	851.00	3.37	4.05
24	A	0.000	8.30	5.74	865.00		
25	A	0.072	8.35	5.78	915.00	3.55	6.26
43	A	0.063	8.20	5.50		2.90	4.10
44	A	0.064	8.38			3.30	4.60
	MV	0.069	8.40	5.70	879.60	3.66	4.74
	ST D	0.003	0.22	0.20	31.38	0.63	0.90

Table 4 Performance of individual adult *Platymeris biguttatus* in the optokinetic experiment. Numbers above the far left column give the angular degree at which a single stripe of the grating was visible from the centre of the drum. Double lined boxes contain the results of one kind of grating each. N gives the number of animals showing at least one valid response to the given grating during the course of the experiment. T1-TX are the markings of the animals, columns under these labels give the data for respective bug; WR indicates the with rotation-number of valid responses that matched the direction in which the drum was turning; AR indicates the against rotation-number of responses that went against the direction in which the drum was turning; WR% and AR% Percentage of valid movements with or against rotation respectively; MV mean value for % WR and % AR for all animals that showed at least one valid response.

15°	T1	T2	T3	T4	T5	T6	T11	T12	T14	T15	T16	T17	T18	T19	TX	MV	N=15
WR	2	8	5	5	1	2	1	1	1	2	1	2	2	1	2		
AR	0	0	1	0	0	0	0	0	0	0	1	0	2	0	1		
% WR	100	100	80	100	100	100	100	100	100	100	50	100	50	100	66.6	89.77	
% AR	0	0	20	0	0	0	0	0	0	0	50	0	50	0	33.3	10.22	

5.5°	T2	T3	T4	T5	T6	T11		T14	T15	T16	T17	T18	T19	TX	MV	N=13
WR	4	3	1	3	4	1		1	2	2	3	2	2	1		
AR	0	0	0	0	0	0		0	0	1	0	1	1	0		
%WR	100	100	100	100	100	100		100	100	66.66	100	66.7	66.6	100	92.3	
%AR	0	0	0	0	0	0		0	0	33.3	0	33.3	33.3	0	7.68	

3°	T2	T3	T4		T6		T14	T15	T16	T17	T18	T19	TX	MV	N=11
WR	2	1	2		1		1	0	1	0	3	0	1		
AR	0	0	0		0		0	1	0	1	0	1	1		
% WR	100	100	100		100		100	0	100	0	100	0	50	68.18	
%AR	0	0	0		0		0	100	0	100	0	100	50	31.82	

1.5°	T2	T3	T4	T5		T11		T14	T15	T16	T17	T18	T19	TX	MV	N=12
WR	1	1	1	1		3		1	1	2	1	3	1	2		
AR	0	0	0	0		0		0	1	0	0	2	1	0		
%WR	100	100	100	100		100		100	50	100	100	60	50	100	88.33	
%AR	0	0	0	0		0		0	50	0	0	40	50	0	11.67	

Grey	T1	T2	T3	T4	T5		T12		T15		T18		TX	MV	N=9
WR	0	1	1	0	0		1		0		0		0		
AR		0	0	1	1		0		3		1		1		
%WR	0	100	100	0	0		100		0		0		0	33.33	
%AR	100	0	0	100	100		0		100		100		100	66.67	