Journal of Evolutionary Biochemistry and Physiology, Vol. 37, No. 4, 2001, pp. 398—404. Translated from Zhurnal Evolyutsionnoi Biokhimii i Fiziologii, Vol. 37, No. 4, 2001, pp. 301—305.
Original Russian Text Copyright © 2001 by Frantsevich, Zolotov.

COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Response to Contrast Objects of the Seven-Spot Ladybird *Coccinella septempunctata* (Coleoptera, Coccinellidae)

L. I. Frantsevich and V. V. Zolotov

Shmalgauzen Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, Ukraine

Received July 30, 2000

Abstract—The goal of the work was to establish whether the seven-spot ladybird *Coccinella septempunctata* has a spatial constancy towards the shape of images. The beetles were presented with black figures on the white wall of the cylindrical arena. The beetles were walking with different orientation of the body relative to the force of gravity: in the horizontal plane, on a three-ray labyrinth or on the flat ring in the bottom of the arena; with an inclination of 90° on the cylindrical ring wall; upwards on the vertical labyrinth or upside down, under a thin wire labyrinth stretched over the arena. The beetles in the horizontal position did not discriminate from each other differently orientated images, except for two types of the figures: vertical or horizontal bands (the choice was 80 : 20%) and meanders orientated downwards and upwards (the choice was 56 : 44%). The bands oriented vertically towards the Earth were preferred by the beetles when observed from the inclination position, but not in the case of a vertical ascension. The meanders were not discriminated when observed from the upside down position. The ascending oblique bands were preferred over the descending ones. No ability to discriminate the shape of objects was found in the ladybirds, and, correspondingly, they have no spatial constancy. The discrimination of figures and ornaments has the simplest mechanism: detection of the movement (directional) of a contrast margin.

INTRODUCTION

The spatial constancy is an ability to perceive the stimulus direction in some system of coordinates regardless of position of the sense organ in this system. In arthropods, known are spatially constant visual orientational responses (see the review [1]); for example, in the walking stick, the ability to discriminate a figure from its overturned copy has been revealed [2].

The existence of the spatial constancy with respect to recognition of figures would be of a peculiar interest for understanding primary processes of the image processing. Search for the insect species that have this ability should meet several criteria: the insect is to naturally move with an arbitrary inclination in the gravity field, to use vision for detecting objects important for behavior, and to be sufficiently active for massive recordings of behavior. A good candidate might be the seven-spot ladybird. This voracious predator examines intensively plants, searching for aphids [3, 4]. The distant orientation is provided by vision. The ladybirds are "trichromates," they perceive light polarization [5], notice large and contrast objects [6—8] and small models of victims [9, 10] and are attracted from a long distance to colored traps [11], plant stems, and places of copulation, estivation, and wintering [3, 12]. They are known to have an optomotor response [13, 14], defensive shadow reflex [7], and chorotaxis, i.e., an intention to move along the right or rounded lines [15]. Collett [7] believed the ladybirds to discriminate the object shape. When running on plants, the ladybirds occupy any posi-

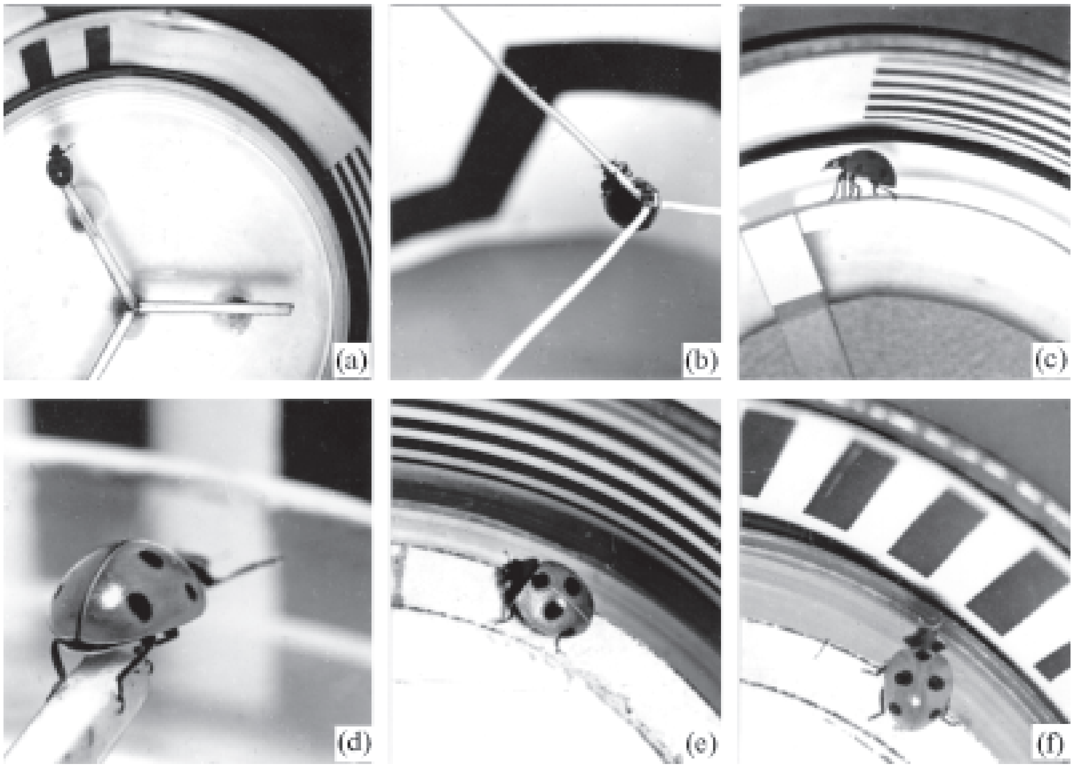

Fig. 1. Behavior of the seven-spot ladybirds on the arena. (a) On horizontal labyrinth, (b) on the labyrinth above the arena, (c) on the side on the cylindrical track (view from above), (d) attempt to catch figure, (e) passage on the flat circular track, (f) catching from the flat track.

tion relative to the vertical line. Since they are touching, at every step, the substrate with their maxillary tentacles, their head preserves a constant position relative to the substrate [16].

After numerous experiments, we have found that the ladybirds are hardly able to discriminate figures with different orientation, apart from vertical and horizontal bands. The bands vertical towards the Earth surface are more attractive when observed from the horizontal body position and from inclination of 90°, but not from the vertical body position. These preferences can be explained by effect of the simplest mechanism of perception of flashings or directional shifts produced by movement of the "retinal" image.

MATERIALS AND METHODS

The beetles were collected in the field. Selected for the experiments were seven-spot ladybirds with the body length of 6–7 mm. The experiments were carried out in a cylindrical arena of 49 mm in radius and 45 mm in height above the substrate. The background of the wall and large figures were made of white, black, or gray paper (Plagan Colour System), the figures with small details were printed with a printer. The background reflection, etc., were measured in green rays (530 nm) with a FEU-68 photomultiplier, the reflection of white paper taken as 100%. The arena was illuminated with a fluorescent lamp, the wall illumination was 70–100 lux.

So as not to take the beetles with hands, a wire hook was put under them and, from it, the beetles were placed either on a labyrinth or on a circular track. In most experiments the labyrinth made of three matches and mounted above the arena floor was used (Fig. 1a). The matches-rays did not reach the wall by 15 mm, so the beetle could not pass from the ray to the wall (Fig. 1d).

The figures of two types (Figs. 2a, 2b) were

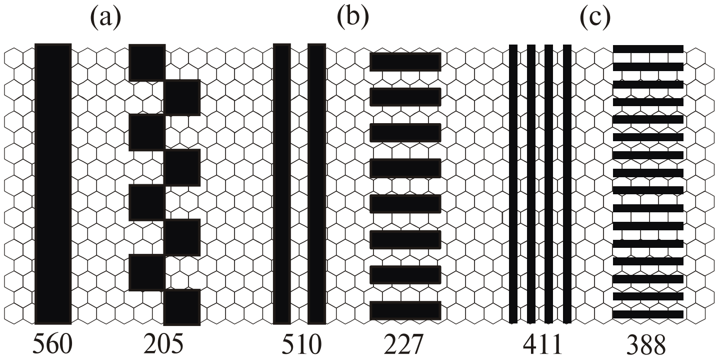

Fig. 2. Choice from the pair of ornaments (figures—the number of choices). Ornaments are shown on the background of a hexagonal net with a periodicity equal to the interommatidium angle (3.3°). The ornament period is 15° in (a), 7.5° in (b), and 3.8° in (c).

presented as a combined triplet (Fig. 2, (aab) or (abb)) in front of the rays. The beetle was walking along the ray until the center and, in the point of bifurcation, chose one of two directions towards identical or different figures (Fig. 2, (ab) or (ba)) depending on the way of approaching. At the ray end, the beetle either turned to continue the search or descended.

To balance the effect of secondary reference points, the wall, from time to time, was turned round relative to the labyrinth by steps of 120° and the whole arena together with the labyrinth, by steps of 60°. The triplet of figures was usually shown in the experiment with 10–20 beetles and 500–600 choices. About a third of choices concerned identical figures; using them, the asymmetry of turns was evaluated. In some experiments, only two figures were demonstrated (Fig. 2, (ab) or (ba)). The beetle was let out to the start ray and the choice was recorded in the bifurcation.

To observe the overturned beetles, they were let out on the labyrinth of three wires in a Teflon insulator (0.7 mm in diameter) which was installed above the arena (Fig. 1b).

Besides the three-ray labyrinth, a narrow circular track of 40 mm in radius (flat or cylindrical) was used. Along the former, the beetles were walking in a horizontal position (Figs. 1e, 1f), along the latter, with an inclination of 90° (Fig. 1c). Two pairs of figures (ab, ab) occupied, on the arena walls, the sectors of 45° width separated by the 45° white spaces. From the circular track, the beetle could pass to the wall. The positions of captures and crosses were recorded in 32 sectors of the ring. The width of such sector is approximately equal to the size of the beetle.

The beetles could walk along the labyrinth ray or the ring with an arbitrary body inclination: on the upper, side, or lower surfaces. Therefore, in three–ray labyrinth there were recorded only the passes on the upper edge of the ray, while on the flat track, on the upper, and of the cylindrical track, on the side surfaces. The experiment with the cylindrical track was recorded on the videotape for subsequent analysis: during the 90° inclination, a space between the track and the beetle silhouette was to be seen.

The feeding and statistical analysis of the data was performed according to original programs. The statistical significance of differences was evaluated by the χ^2 criterion.

The ommatidium sizes, curve radius, and interommatidium angle in the 90° sector of the eye were measured with an ocular-micrometer in cornea preparations.

Recorded were 23 500 behavioral responses of beetles.

RESULTS

Interommatidium angle. The measurements on seven-spot ladybird individuals of different sizes showed that in the beetles with the body length of 6.5 mm, the kidney-shaped eye contains 850–

Attraction of seven-spot ladybirds to white and black vertical bands of a width of 10° on the gray background at the paired choice in the labyrinth

Reflection, %			Choice, %		Number of choices	Probability, p_0, %
Background	black band	white band	black band	white band		
100.0	5.5	100	75.9	24.1	1537	< 0.1
52.8	1.2	100	79.4	20.6	613	< 0.1
41.8	1.2	100	72.5	27.1	608	< 0.1
25.9	1.2	100	47.1	52.9	240	> 5
12.5	1.2	100	28.5	71.5	639	< 0.1

900 ommatidia, and the angle between adjacent ommatidia is, on average, 3.3°; hence, the resolution amounts to 6.6°.

Congenital asymmetry of search. In a part of passes along the three-ray labyrinth, the beetles were before the choice from identical figures: interestingly, in this case, the beetles turned to the left more often than to the right (53.8 and 46.2%, respectively, from 7815 choices, $p_0 < 0.1\%$). The remarkable preference of a certain side of the turn was also noted in the ladybirds earlier [7]. In 2% of passes, the beetles turned back before reaching the bifurcation.

Attraction to the contrast band. When observing the wide black and wide white sectors (120°) before the labyrinth rays, the ladybirds chose predominantly the white one (63.5% from 455 choices, $p_0 < 0.1\%$). However, the white vertical band of a 10° width in the middle of the black sector was more attractive than the wide white sector (62.6% from 447 choices, $p_0 < 0.1\%$).

The response to the black and white vertical bands on the gray background is shown in the table. The choice depended on the contrast: on the black background, preferred was the white band, on the white one, the black band. On the background of a 26° reflection, the black and white bands were equally attractive.

Resolution of vision. Figure 2 shows the choices in the figure pairs depending on the space period of the ornament. The vertical bands were preferable than the horizontal ones or the chess ornament with a periodicity of 17.5–8.8° ($p_0 < 0.1\%$); different ornaments with a periodicity of 4.5° and lower did not differ from each other. In subsequent experiments, presented were the ornaments and figures with an angle size of 25–40° and angle distance between the details more than 10°, i.e., within the limits of the bud's vision resolution.

Discrimination of figures and their turned-over copies. We tried finding the figure pairs discriminated in the three-ray labyrinth on the bottom of the arena to subsequently present them to the turned-over beetles in the labyrinth above the arena. Most figure pairs shown in Fig. 3 produced a similar response. The figures from the pair no. 7

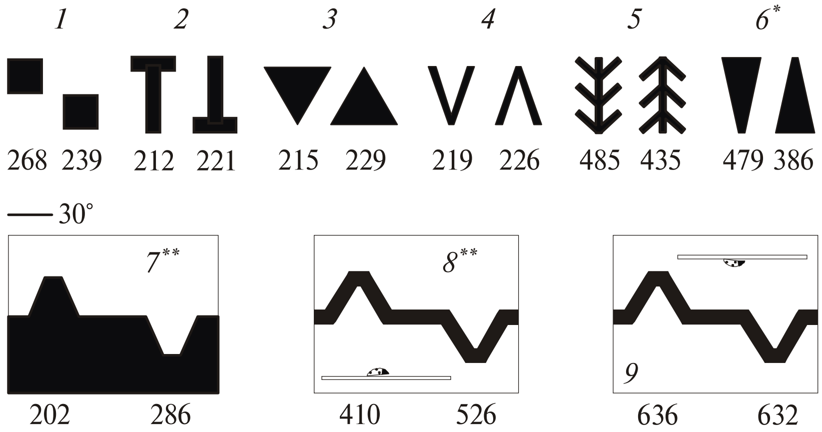

Fig. 3. Choice from the pair of differently orientated figures. (*1*)–(*8*) In the horizontal labyrinth on the arena bottom, (*9*) in the labyrinth above the arena from the upside down position. *Asterisks* mark the p_0 significance levels: *—5%, **—0.1%.

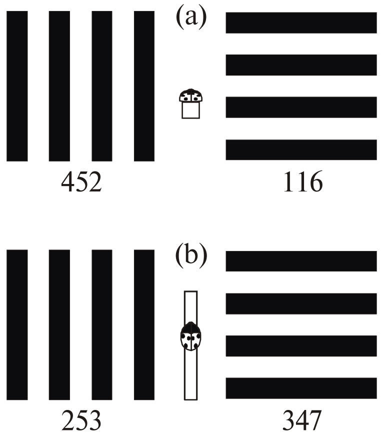

Fig. 4. Choice between meanders of the bands, vertical and horizontal relative to the Earth surface, in the ladybirds on the horizontal labyrinth (a) and the labyrinth installed vertically (b).

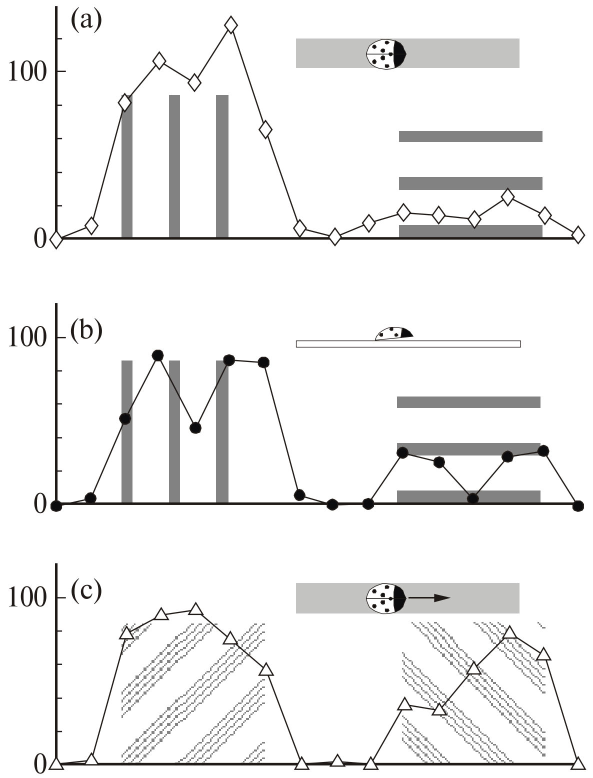

Fig. 5. Distribution of attempts to catch the ornament of black bands in the beetles on the circular tracks on the flat ring (a) with an inclination of 90°, on the cylindrical track (b) and on the flat ring with taking into account of the movement direction (c).

were not mirror images of each other, but were a prototype of the meander pair no. 8, with which a better discrimination was obtained: 56.2 and 43.8% ($p_0 < 0.1\%$). The same pair was presented in the labyrinth above the arena; however, a negative result was obtained: the choice of the figures turned out to be equal.

Choice of differently orientated parallel bands (Fig. 4). The band period amounted to 14°. In the horizontal labyrinth (Fig. 4a), the beetles showed the obvious preference for vertical bands as compared with horizontal ones (79.6% from 568 choices, $p_0 < 0.1\%$). A different result was obtained in the experiment with the arena and labyrinth inclined by 90° (Fig. 4b). In this experiment, two figures were presented, the start ray being orientated vertically upwards. In this case, the choice was in favor of the bands horizontal towards the Earth surface and perpendicular to the labyrinth plane. The discrimination was less pronounced than in the horizontal labyrinth (57.7 *versus* 42.2% in 600 choices, $p_0 < 0.1\%$).

Choice of figures on the circular track. On the flat track, the beetles were walking in the plane of the ring. We took into consideration both the positive responses, i.e., attempts to catch the band (Fig. 1f) and pass from the ring to the figure, and passes by the figure without attempts to catch it (Fig. 1e). In this case, the vertical bands also attracted the ladybirds much stronger than the horizontal ones (Fig. 5a): 477 attempts of catch *versus* 81, respectively, or 47.7 and 8.1 per the recorded sector. There were few attempts to pass to the white part of the ornament-free wall: 2.3 per the recorded sector. In this experiment, the clockwise and counterclockwise passes were summarized.

The responses to oblique parallel bands were analyzed taking into consideration the direction of approach (Fig. 5c). A marked preference was shown for the ascending bands over the descending ones (399 *versus* 275 attempts of catch from the total number of 683).

In experiments on the cylindrical track, only the passes with an inclination of 90° were counted. In this case, the bands vertical against the Earth were preferred over the horizontal ones (Fig. 5b): 366 and 125 attempts of catch. Correspondingly, during the experiment, the ladybirds passed only 12 times by the ornament of vertical bands without attempts to catch it, whereas disregarded the horizontal ornament 58 times.

DISCUSSION

The goal of the present work was to establish whether the ladybirds have the spatial constancy in perception of the object shape. After an elementary check of the visual acuity of the insects, it was necessary to find the figures or ornaments that attract the beetles and differ from their copies turned over by 90 or 180°. Two types of such figures were found: the ornaments of parallel bands (Fig. 5a) and the meanders directed upwards or downward (Fig. 3, nos. 8 and 9).

The beetles that were walking in the horizontal plane discriminated reliably the ornaments from vertical and horizontal bands (the vertical ones were definitely attractive). The same ornaments were presented to the beetles walking with a body inclination of 90° in the inclination plane or tangage. In the former case, preferred were the bands vertical relative to the Earth, in the latter, the horizontal ones. A common feature of the preferred ornaments was their orientation (perpendicular) relative to the movement direction, rather than to the Earth or retina. The spatial constancy with respect to the inclination by 90° turned out to be false, it can be easily explained by excitation of receptors with the moving image. The insects were attracted by the moving contrast edges, whereas in images of the bands parallel to the movement, by short ends of bands at the ornament edges.

The experiments with the band on the gray background showed that the ladybirds were attracted by images with a great contrast regardless of its polarity. These results agree well with the known fact that the ladybirds prefer contrast objects and nearby objects with a great movement parallax [7, 8]. The preference for stripped ornaments over the chess ones in our experiments and for vertical edges over the oblique ones in the experiments of the work [8] can also be explained by preference of a greater contrast, when taking into account a lesser blurring of the discontinuous vertical edge in the image after the optics with poor resolution.

It is not necessary to ascribe to ladybirds an ability to detect vertical objects (bands, stalks). It is sufficient for them to detect movement of a contrast image on the retina. This primitive ability is added by selectivity to a directed movement, which was revealed in the experiment with ascending oblique bands. The turn to the ascending edge is to result in orientation of the beetle along the ornament bands (chorotaxis), although this behavior might also be explained by frontal fixation of the contrast edge. The presence of a band in front of the beetle is known to decrease its sensitivity to the band presented from a side. [7].

The ability in ladybirds to discriminate figures is very poor or absent at all. The choice of the meander faced downwards might have been explained by phototropism, attraction to an open space. The ladybird in a turned-over position has no ability even to such discrimination. However, we observed the visual choice to be attenuated in the beetles walking upwards, when, apart from vision, their orientation was affected by geotropism. Probably, in turned-over beetles, the visual orientation is attenuated.

The indifference to the shape of objects in freely walking beetles does not rule out, in principle, their capability for visual discrimination in other circumstances, for example, in learning with reward and punishment [17, 18]. However, a known vision-controlled behavioral repertoire in ladybirds can be explained by the simplest mechanisms: phototropism, detection of a moving contrast, of a directed movement, frontal fixation. This simplicity and absence of specialization in visual analysis seems to provide a wide ecological plasticity of the seven-spot ladybirds.

ACKNOWLEDGMENTS

The work was supported by the International Research Foundation (project QSU 084211).

REFERENCES

1. Frantsevich, L.I., *Prostranstvennaya orientatsiya zhivotnykh* (Spatial Orientation of Animals), Kiev, 1986.
2. Frantsevich, L. and Frantsevich, L., Space Constancy in Form Perception by the Stick Insect, *Naturwissenschaften*, 1996, vol. 83, no. 7, pp. 323–324.
3. Savoiskaya, G.I., *Koktsinellidy (sistematika, primenenie v bor'be s vreditelyami sel'skogo khozyaistva)*, (Coccinellidae (Taxonomy, Use against Agricultural Pests)), Alma-Ata, 1983.

4. Turchin, P., *Quantitative Analysis of Movement*, Sunderland, Mass.: Sinauer Assoc., 1998.
5. Lin, J.T., Identification of Photoreceptor Locations in the Compound Eye of *Coccinella septempunctata* Linnaeus (Coleoptera, Coccinellidae), *J. Insect. Physiol.*, 1993, vol. 39, pp. 555–562.
6. Michieli, S., Analiza skototakticnih (perigramotakticnih) reakcij pri Artopodih, *Acad. Scient. Art Slovenica, Cl. IV, Hist., nat., med.*, 1959, vol. 5, pp. 237–286.
7. Collett, T.S., How Ladybirds Approach nearby Stalks: A Study of Visual Selectivity and Attention, *J. Comp. Physiol. A.*, 1988, vol. 163, pp. 355–363.
8. Khan, K.S., Ali Shah, M., and Khan, B.V., Optical Orientation in Predatory Coccinellids, *Pakistan J. Agric. Res.*, 1985, vol. 6, pp. 40–44.
9. Nakamuta, K. and Saito, T., Recognition of Aphid Prey by the Lady Beetle *Coccinella septempunctata bruckii* Mulsant (Coleoptera, Coccinellidae), *Appl. Entomol. Zool.*, 1985, vol. 20, pp. 479–483.
10. Stubbs, M., Another Look at Prey Detection by Coccinellids, *Ecol. Entomol.*, 1980, vol. 5, pp. 179–182.
11. Maredia, K.M., Gase, S.H., and Landis, D.A., Visual Response of *Coccinella septempunctata* (L.), *Hippodamia parenthesis* (Say) (Coleoptera, Coccinellidae), and *Chrysoperla carnea* (Stephens) (Neuroptera, Chrysopidae) to Colors, *Biol. Control.*, 1992, vol. 2, pp. 253–256.
12. Obata, S., Johki, Y., and Hidaka, T., Location of Hibernation Sites in the Ladybird Beetle, *Harmodia axyridis*, *Ecol. Aphidophaga, vol. 2, Proc. Symp. Zvikovske Podhradi, Sept. 2–8, 1984,* Prague, 1986, pp. 193–198.
13. Gaffron, M., Untersuchungen über das Bewegungssehen bei Libellenlavren, Fliegen und Fischen, *Z. Vergl. Physiol.*, 1934, vol. 20, pp. 299–337.
14. Zanker, J.M. and Collett, T.S., The Optomotor System on the Ground: on the Absence of Visual Control of Speed in Walking Ladybirds, *J. Comp. Physiol. A*, 1985, vol. 156, pp. 395–402.
15. Tischler, W., Ein Beitrad zur Formensehen der Insekten, *Zool. Jb. Allg. Zool.*, 1937, vol. 57, pp. 157–202.
16. Frantsevich, L.I., Mokrushov, P.A., and Shumakova, I.D., Kinematics of Insect Walking on Narrow Supports, *Vestn. Zool.*, 1995, no. 4, pp. 41–48.
17. Gong, Z.F., Xia, S.Z., and Liu, L., Operant Visual Learning and Menory in *Drosophila* Mutant Dunce, Amnesiae and Radish, *J. Insect. Physiol.*, 1998, vol. 44, no. 12, pp. 1149–1153.
18. Wolf, R. and Heisenberg, M., Basic Organization of Operant Behavior as Revealed in Drosophila Flight Operation, *J. Comp. Physiol. A.*, 1991, vol. 169, pp. 699–705.