Morphometric study of the eye of three species of *Myrmica* (Formicidae)

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Abstract

We slightly modified a previously devised method to obtain detailed drawings of the external surface (i.e., corneal replicates) of the eyes of eleven workers of *Myrmica rubra, M. ruginodis* and *M. sabuleti.* On these drawings, we counted the ommatidia and measured the perimeter and the axes (length and width) of the eyes. Based on serial photos of the replicates, we assessed three vertical distances (the height) of the eyes. These measurements and assessments yielded four distances of the eye surface and two angles of their convex base. The mean values allowed us to reproduce the exact shape and size of the eye of the three species. The unsuspected interspecific differences have potential implications for the visual perception abilities of the ants. The technique for obtaining precise drawings as well as the quantification of the eye shapes and sizes have been fully tested and can now be applied to most microscopic and opaque structures.

Keywords: ants, eye, morphology, *Myrmica* species, replicate, visual perception.

Introduction

Ants successfully negotiate their way using landmarks (i.e. visual cues), which they can see and memorise. We studied several aspects of the visual perception of the ant *Myrmica sabuleti* MEINERT 1861 (CAMMAERTS, 2004, 2007a,b) and analysed their path negotiation (CAMMAERTS & LAMBERT, 2008; CAMMAERTS & RACHIDI, personal observations). The next step was to detail the eye morphology and to compare it with that of *M. rubra* (LINNAEUS 1758) and *M. ruginodis* NYLANDER 1846 workers. The three species were found in a same valley but each in a different environment, provided with different visual elements. This points to a potential relationship between eye morphology and the visual elements that ants can perceive in their environment.

Fig. 1. Biotope of the Aise valley (Ardennes, Belgium) inhabited by the ants *M. rubra* (A), *M. ruginodis* (B), *M. sabuleti* (C).

The eye of a *Myrmica* worker is small (about 200 μm) and opaque. It is only poorly visible under a stereomicroscope or a microscope. An appropriate technique was devised, to satisfactorily observe the eyes of *Myrmica* ants (CAMMAERTS *et al.*, 2008). In the present work, this technique was slightly modified in order to conduct a morphometric study of the eyes of *M. rubra, M. ruginodis* and *M. sabuleti*, being as free as possible of specialised software. This new technique can be applied to most microscopic and opaque structures.

Material and methods

Collection and maintenance of ants

Colonies of *M. rubra, M. ruginodis* and *M. sabuleti* were collected from the Aise valley (Belgium) in autumn 2005. *M. rubra* nested near the river, in grassy land (Fig. 1A); *M. ruginodis* was located higher, in partly wooded areas (Fig. 1B); *M. sabuleti* colonised old slate quarries invaded by several plants such as *Leontodon hispidus, Trifolium repens, Galium mollugo, Daucus carota, Lotus corniculatus, Leucanthemum vulgare, Centaurea sp., Fragaria vesca, Cytisus scoparius, Euphorbia cyparissias, Hieracium sp., Prunella vulgaris* (Fig. 1C).

These colonies were maintained in a laboratory (20 ± 2°C; 80% humidity; 600 lux). The ants nested in glass tubes half-filled with water, a cotton-plug

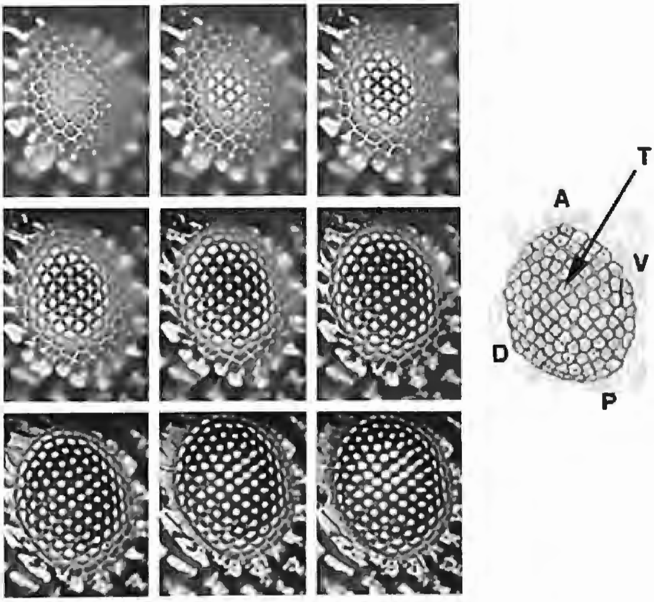

Fig. 2. Left: part of the serial photos of the corneal replicate of an eye of a *Myrmica sabuleti* worker, printed in a contact sheet mode. Right: the drawing of this eye, made by superimposing successive tracing off the photos. A, D, P, V, T as in Fig. 3. The ommatidia can be counted on the drawing; this eye contains 111 ommatidia. Perimetric as well as axial parameters can be measured on the drawing, using a curvometer (explanation in the text and in Fig. 3; results in the text and in Fig. 5).

separating the ants from the water. The glass tubes were deposited in trays (7 x 23 x 47 cm) whose borders were covered with talc and in which food (pieces of dead cockroaches, sugared water) was delivered twice a week.

The manipulation and analysis detailed below were conducted on 11 workers of each species, collected while moving on their foraging area.

Material

Corneal replicates were obtained as previously (CAMMAERTS *et al.*, 2008), using a stereomicroscope, pairs of Brucelle forceps, transparent varnish and acetone. Microscopic preparations of the replicates were made using glass slides, paper frames, glass slides cover slips and lute in paraffin. The eye replicates were observed under a microscope (Zeiss Axioskop, objective magnitude: 40 X) to which a camera (Sharpvision Co., Ltd., Guangzhou, China, chipset Omnivision 3.1 Mpixel, USB2.0) was adapted. The camera was coupled to a PC, to view each replicate. The subsequent manipulation and analysis only required a printer, transparent paper and a curvometer.

Serial photos and drawings of the eyes

For each studied replicate, a digital photo was taken and saved in the JPEG image format (width: 2048 pixels, height: 1536 pixels), focussing firstly on the highest zone of the replicate, then on each of its successive lower zones, ending on the lowest one. The step between two successive photos was 2 μm. The serial photos were printed in a contact sheet mode (Fig. 2, left). The printed serial photos of each file were then traced off. The successive drawings were superimposed, yielding a precise, whole drawing of each studied eye (Fig. 2, right).

Measured and calculated parameters

The four perimetric distances AD, DP, PV, VA as well as the four axial distances At, tP, Dt, tV were measured on the drawings of the eyes using a curvometer (scale: 1/800,000) (Fig. 3, the two first sketches).

The total height of the eye (Tt), the difference of height between the ventral-most and the dorsal-most point (D'D) as well as the difference of height between the anterior-most and the posterior-most point (P'P) (Fig. 3, third sketches) were assessed by evaluating, in μm, the gap between focussing on the upper point (T, V, A respectively), then on the lower point (respectively t, D, P) of the respective distances. For these purpose, the corneal replicates could be observed under the microscope or on the printed serial photos.

Four distances of the external eye surface were calculated using the measured axial distances At, tP, Dt, tV and the assessed total eye height Tt (Fig. 3, fourth sketches). The distances AT, PT, DT, VT nearly equal the hypotenuse of the respective right-angled triangles AtT, PtT, DtT, VtT.

The base of the eye is not plane but curved, convex, i.e. the angle AtP and VtD > 180°, because the posterior-most point is not at the same height as the anterior-most (but further below) and because the dorsal-most point is not at the same height as the ventral-most (but also further below). The exact values of these two angles are 180° plus an angle (respectively x and y, Fig. 3, fifth sketches) whose sinus equals PP'/ Pt and DD'/Dt, respectively. The centre of the circumference is P and D, respectively; t is on the circumference and P' (and D') are on the sinus axis. PP'/Pt and DD'/Dt are the sinus of the angles x' and y', respectively, equal to the angles x and y (see details in Fig. 3).

Results

Number of ommatidia

The distance between the two eyes of each studied ant was measured using a stereomicroscope provided with a camera lucida (magnification = 28 X), before making the corneal replicate (i.e. before placing the ant's head into an acetonic solution of varnish). The ommatidia of each studied eye were counted on the drawing obtained as explained above. The two parameters (distance between the two eyes and number of ommatidia) were plotted (Fig. 4). The results show that the head of all three species are similar in size. Moreover, the eye of *M. sabuleti* is smaller than that of the two other species. Indeed, the eye

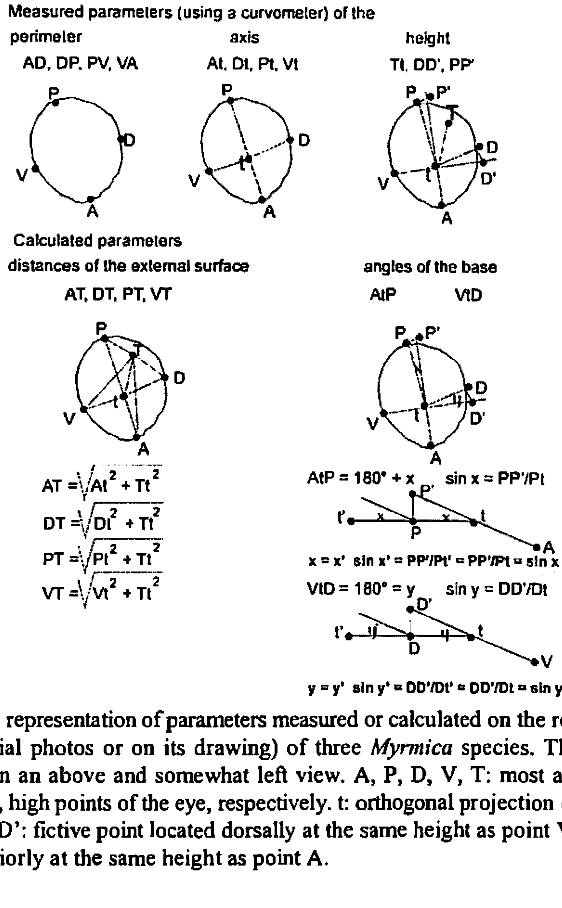

Fig. 3. Schematic representation of parameters measured or calculated on the replicate of the eye (or on its serial photos or on its drawing) of three *Myrmica* species. The parameters are schematised in an above and somewhat left view. A, P, D, V, T: most anterior, posterior, dorsal, ventral, high points of the eye, respectively. t: orthogonal projection of T on the fictive plane ADPV. D': fictive point located dorsally at the same height as point V. P': fictive point located posteriorly at the same height as point A.

of *M. sabuleti* contains 103 ± 8.2 ommatidia (mean number ± standard deviation), *M. rubra* 129 ± 19.5, and *M. ruginodis* 140 ± 11.8 ommatidia.

Parameters of the base of the eye: perimeter and axis

A micro-calibrated scale was photographed, then measured exactly like were the perimetric and the axial distances of the corneal replicates. This allowed the curvometer measurements of the base of the eyes to be converted into μm (Fig. 5). These values also showed that *M. sabuleti* has smaller eyes than the two other species. Its mean eye perimeter was 670 ± 52 μm, versus 746 ± 96 μm for *M. rubra* and 741 ± 56 μm for *M. ruginodis*. Its eye axes were also smaller: AP = 225 ± 27 μm and DV = 178 ± 14 μm versus 233 ± 23 μm and 198 ± 16 μm for *M. rubra* as well as 247 ± 8 μm and 196 ± 9.5 μm for *M. ruginodis*, respectively. On the other hand, the posterior part of the eye of *M. ruginodis* is larger than in the two other species: tP = 147 μm for *M. ruginodis*, but only 135 μm for *M. rubra* and 134 μm for *M. sabuleti*.

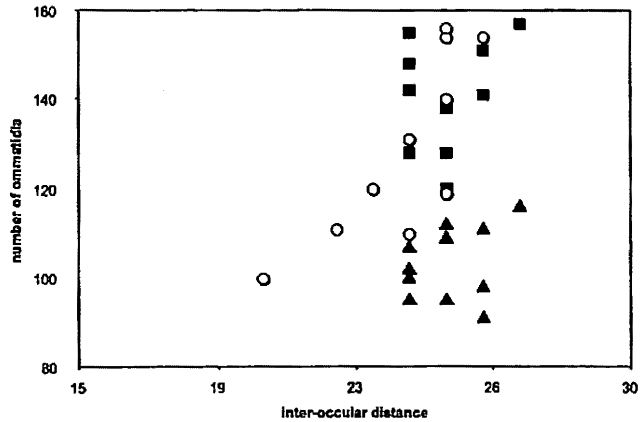

Fig. 4. Distance between the two eyes and number of ommatidia of eleven workers of*Myrmica rubra* O, *M. ruginodis* ■ and *M. sabuleti* ▲. The distances between the two eyes were measured on each ant using a stereomicroscope provided with a camera lucida (Mag.: 28 X) before making the corneal replicate. The ommatidia were counted on the drawings of the eyes (Fig. 2 and explanation in text).

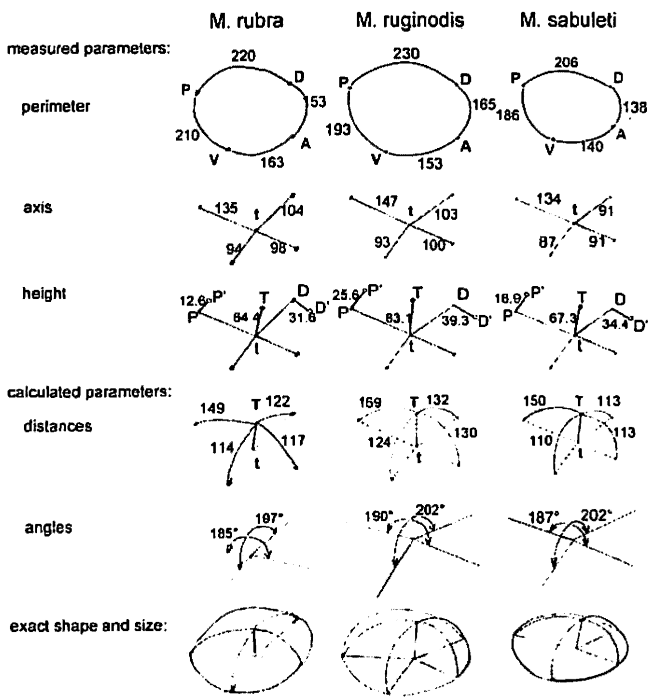

Fig. 5. Morphometric representation, step by step, of the eye of three species of *Myrmica*. The procedure is detailed in the text and illustrated in Fig. 3. The labelling is that of Fig. 3. Each parameter was assessed for 11 individuals of each species. Their mean values are given on the sketches. Standard deviations are given in the text.

Eye height parameters

Mean values of the three assessed parameters relative to the height of the eyes (Fig. 5, third line) show that *M. ruginodis* has the highest eyes: mean total height (Tt) = 83.1 μm (σ = 12.9 μm) versus 64.4 ± 15.4 μm for *M. rubra* and 67.3 ± 6.5 μm for *M. sabuleti. M. ruginodis* also has the largest height differences between the ventral-most and the dorsal-most points (39.27 μm) as well as between the anterior-most and the posterior-most points (25.64 μm). The values are 31.64 μm and 12.55 μm for *M. rubra*, and 34.36 μm and 16.91 μm for *M. sabuleti*, respectively.

Calculated distances of the external surface of the eyes

The calculations of the four distances of the external eye surface show that, although at a quick glance the eyes have a similar shape and size, the eye mean values of the three studied species differ (Fig. 5, fourth line). In all three species, the distances AT and VT are smaller than PT and DT. The top of the eye is thus located not in the centre of its surface but more frontwards and more ventrally. The eye of *M. ruginodis* has the largest external surface since all four calculated distances are larger. The eye length is nearly the same for *M. rubra* and *M. sabuleti*, the anterior part being somewhat larger for *M. rubra*. Eye length is clearly larger in *M. ruginodis*, especially the posterior part. As for the distance between the ventral-most and the dorsal-most zone (eye width), it is larger in *M. rubra* (236 μm) than in *M. sabuleti* (223 μm), which is mostly reflecting a difference in the dorsal part. Total eye width is clearly the largest for *M. ruginodis* (256 μm), mostly due to the dorsal part. Thus, the eye of *M. ruginodis* is more developed dorsally and posteriorly than that of the two other species. The eyes of these two latter species extend similarly posteriorly, but the anterior and dorsal parts are larger in *M. rubra* than in *M. sabuleti*.

Calculated angles of the convex base of the eye

The calculations of the angles AtP and VtD of the base of the eye (Fig. 5, fifth line) are in agreement with the previously calculated distances of the eye surface and help to determinate the exact shape of the eyes. The angle AtP equals 190° in *M. ruginodis*, 187° in *M. sabuleti* and 185° in *M. rubra*. The angle VtD is 202° in *M. ruginodis* and *M. sabuleti*, but 197° in *M. rubra*. The eyes of *M. ruginodis* and *M. sabuleti* are thus the more curved ones, those of *M. rubra* less curved.

Three-dimensional (shape and size) aspect of the eyes

The above measurements and calculations were combined to obtain an exact representation of the eyes (Fig. 5, last line). The eye of *M. rubra* has the least convex base. It is nearly as wide as that of *M. ruginodis* but shorter, making it the most spherical. Its dorsal and its posterior zones are more extended than the ventral and anterior zones, but to a lesser extent than in the two other species. The eye of *M. ruginodis* has the most convex base. It is longer than wide (AP/DV = 1.2) and therefore resembles an ellipsoid more than a sphere.

Its posterior zone is a little more extended than its anterior one. Its dorsal zone is larger than its ventral one, as in *M. rubra*, but has a more curved base. The eye of *M. sabuleti* is the smallest and the least high; its base is nearly as convex as that of the eye of *M. ruginodis*. It is somewhat longer than wide (AP/DV = 1.2). Its posterior zone is a little larger than its anterior one. The dorsal part is only slightly more extended than the ventral one, but has a more curved base.

Discussion

The present work firstly reports on an accessible technique to morphologically and morphometrically study microscopic and opaque structures of insects. It is based on a previously described technique (CAMMAERTS *et al.*, 2008) that has already been successfully applied to conduct morphological studies using standard material such as a microscope, a camera and a PC, together with appropriate software. This technique has been modified here in order to be more software independent. The replicate of the structure is obtained using the earlier technique (CAMMAERTS *et al.*, 2008), but all the subsequent analyses now merely require a printer, transparent paper and a curvometer.

The present paper then applies this approach to precisely describe the eye of three species of *Myrmica*. It yields the number of ommatidia, the dimensions of the eye perimeter and axis, and distinguishes the dorsal and the ventral as well as the anterior and the posterior zones of these parameters. It evaluates four distances of the external surface of the eyes, from their top to their perimeter. It quantifies the eye height, at their top, as well as between the ventral-most and dorsal-most points and between the anterior-most and posterior-most points. It describes the convexity of the base, therefore providing information on the total visual field from the anterior-most part to the posterior-most one, as well as from the ventral-most part to the dorsal-most one.

Obtaining an entire view (or a 3-D model) of a structure based on serial photos using appropriate software (CAMMAERTS *et al.*, 2008) takes as long as obtaining a whole drawing of the structure by tracing off and superimposing the photos (present study). Counting the elements of a structure directly on its whole drawing (present study) is faster than doing so using computer software (CAMMAERTS *et al.*, 2008). Assessing the surface of the external area of a structure and of its elements using software (CAMMAERTS *et al.*, 2008) is somewhat faster than measuring and calculating several parameters of the structure (present study), but the former assessment is approximate while the latter is exact. The previous and the present methods are equivalent in their efficiency - the choice of one or the other depends on the users' particular skills.

It would be possible to calculate, for each eye, the distances and the angles not only for the points A, P, D, V, but also for every point of the perimeter, using exactly the same material and the same method. This would enable a very precise 3D representation of each eye. But, a mean representation of the eye of each studied species would then have been difficult. The presently measurements and calculations do yield such 'mean sketches'of the three kinds of eyes (last paragraph of the 'Results' section and Fig. 5), revealing valuable insights into differences between the three species.

In the course of the present study, it was observed that the ommatidia of one eye were not all identical. They did not all have an identical external surface. For instance, some of them, apparently specifically located, had a triangular external surface. This point warrants future study at a morphological and histological level.

The minimum angle of vision (i.e. minimum angle of subtense) of *M. sabuleti* workers was assessed via ethological experiments (CAMMAERTS, 2004). Its value (5° 12') is less than that of the approximate visual field of one ommatidia (15°) deductible from the present morphometric study. Each ommatidia is thus fully efficient *per se*.

In the Aise valley, each species nested in a different environment provided with different perceptible visual elements. The nests of *M. rubra* were surrounded by rather high Graminaceae and a few dicotyledonous plants (*Epilobium sp., Silene dioica…*) (Fig. 1A). The eye of *M. rubra* has a shape which could allow the perception of such relatively tall plants. *M. ruginodis* were always found near wooded area; their nests were surrounded by only few, not very tall plants, and were over-hanged by several branches (Fig. 1B). Thanks to their long, dorsally and posteriorly developed eyes, *M. ruginodis* is very probably able to see the elements located above their nests and their foraging areas. *M. sabuleti* always inhabited small tree-less zones provided with many small plants (see 'Introduction') some being odorous (Fig. 1C). The small eyes of that species may be appropriate for perceiving such a low vegetation. We presume that, in the course of their successive nest moving, *Myrmica sp.* foragers choose places of which they can easily perceive the different environmental elements, that is places which potential cues are in agreement with, among other, their eyes morphology.

Ethological studies on *M. sabuleti* show that this ant uses primarily odours and secondarily visual cues to negotiate its paths (CAMMAERTS & LAMBERT, 2008; CAMMAERTS & RACHIDI, personal observations). In the case of *M. sabuleti*, the morphological, ecological and ethological observations are thus in agreement. It would therefore be interesting to conduct similar ethological studies on *M. rubra* and *M. ruginodis* to determine the relative importance of visual and odorous cues for these two species that have morphometrically different eyes and live in different environments.

The site "www.myrmecofourmis.fr/spip.php?article" provides the most complete collection of photos of ant eyes: three species of *Lasius*, *Camponotus truncatus*, *Formica sanguinea* and *Formica rufibarbis*. All these eyes are larger than those studied here. However, these photos provide a less precise indication of the exact shape and size than the here given sketches. Other works dealing, at least partly, with ant eye morphology include MENZEL & WEHNER (1970), BERNSTEIN & FINN (1971), KLOTZ *et al.* (1992), MOSER *et* *al.* (2004). Books and reviews on insect eye morphology are availlable (e.g. HORRIDGE, 1975; LAND, 1989) but are less numerous than those on insect vision. Studies on the morphology of the eye of insects other than ants abound (e.g. RIBI, 1978; BAUER & KREDLER, 1993; COLLINS, 1997; RUTOWSKI, 2000; ZHANG *et al.*, 2007). The latter authors, among others, measured the angles of the visual field of *Chrysopa pollens*, i.e. the angle from the anterior-most to the posterior-most part of the eye (180°) and the angle from the dorsal-most to the ventral-most part (200°). All these investigations focus on eyes much larger than those studied here and utilise electron microscopes (transmitting and scanning) and/or histology.

Conclusion

The uncomplicated and efficient method we propose here may thus be useful to all those who aims to detail the morphology of a microscopic structure yet have only a traditional microscope, a PC and a camera at their disposal. This approach enabled us, for the first time, to precisely describe (shape and size) the eye of three common species of ants. It also revealed agreement between morphological, ecological and ethological characteristics of an animal.

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