Visual vertical subtended angle of *Myrmica ruginodis* and *Myrmica rubra* (Hymenoptera, Formicidae)

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Abstract

The visual subtended angle of two species of *Myrmica* (*M. ruginodis* and *M. rubra*) were calculated on the basis of the maximum distance at which the ant workers perceived black squares of different dimensions. This distance equaled 18 and 15 times the edge of the presented squares for *M. ruginodis* and *M. rubra*, respectively. The subtended angle of these species thus equals 3°10' and 3°50', respectively. The subtended angle of *M. sabuleti* workers was previously determined to equal 5°12'. These three angular values agree with the morphometric characteristics of the eyes of the studied species and are significant to the species' visual perception.

Keywords: conditioning, ants' eyes, visual perception

Introduction

The subtended angle of vision of an animal is the smallest angle under which this animal can visually perceive an object. When an object is perceived under the smallest angle of vision, it is located at the maximum distance of vision from the individual. If the object is at a greater distance than the maximum, the angle under which it should be perceived is smaller than the subtended one, and the individual can no longer see it.

Although ants mainly respond to pheromonal odours, they can also use their visual perception to find their way through their environment and to perform several tasks (PASSERA & ARON, 2005). We previously studied the visual perception of *M. sabuleti* workers (CAMMAERTS, 2004a, b, 2007) assessing, among other aspects, the visual subtended angle of these ants, which equals 5°12'. To negotiate their way, these workers primarily use olfactory cues and secondarily visual cues (CAMMAERTS & LAMBERT, 2009; CAMMAERTS & RACHIDI, 2009). Their compound eyes are small compared to those of *M. ruginodis* and *M. rubra* (RACHIDI *et al.*, 2008). On the contrary, the eyes of *M. ruginodis* are larger and have a well-developed postero-dorsal region (RACHIDI *et al.*, 2008). For orientation, *M. ruginodis* workers exclusively use visual cues located above them and use olfactory cues only when visual ones are no longer available (CAMMAERTS *et al.*, 2011). It can be presumed that visual perception in workers of *M. ruginodis* is superior to that in *M. sabuleti* workers (while the reverse may be true regarding olfactory perception).

The present work is the first step of our study of the visual perception in workers of *M. ruginodis* and *M. rubra*. We here seek to determine the visual subtended angle of these ant species and to compare the values with that obtained for *M. sabuleti* workers.

To assess the subtended angle of vision of *M. ruginodis* and *M. rubra* workers, we measured their maximum distance of vision, then using trigonometry we calculated the subtended angle. To measure the ants' maximum distance of vision, we conditioned them to visual cues and subsequently presented the cues to the ants, first from a large distance, then with successively closer distances, until the ants clearly reacted, thus indicating visual perception of the cues. When this occurred, the cues were located at the maximum distance of vision from the ants.

Material and Methods

Collection and maintenance of ants

The experiments were conducted on six and four experimental colonies of *M. ruginodis* and *M. rubra*, respectively. The colonies of *M.*

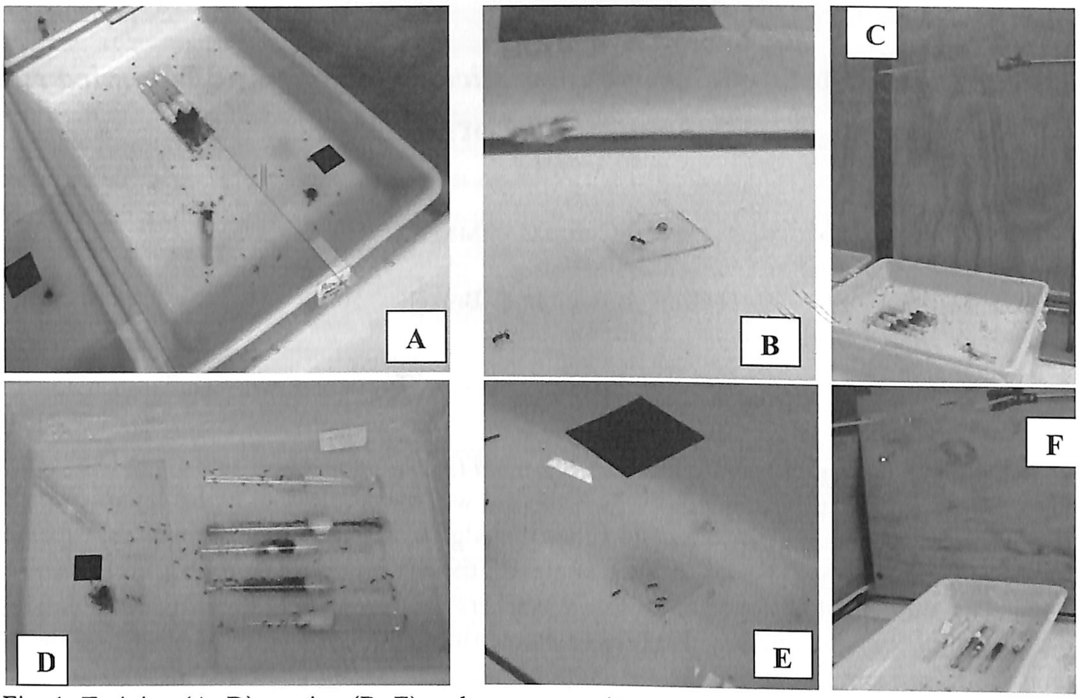


Fig. 1. Training (A, D), testing (B, E) and assessment (C, F) experiments performed on *Myrmica ruginodis* (A, B, C) and *Myrmica rubra* (D, E, F) to measure their subtended angle of vision. The ants were conditioned to a black square of a given dimension which was presented above them on a glass-slide (A: on the right; B: on the left) and extent of conditioning was checked (B, E). The cue was then brought closer to the ants starting from a great distance (C, F) until the ants show obvious reaction. At that point, the cue was located at the ants' maximum distance of vision.

ruginodis came from four large colonies collected in the Aise valley along a forest edge and round a forest clearing in Petigny (Ardenne, Belgium). The colonies of *M. rubra* came from colonies collected on grasslands in the Aise valley and in Marchin (Belgium). The largest colonies were split so that the experimental ones were demographically similar, each containing a queen, brood and about 500 workers. They were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water. A cotton-plug separated the ants from the water. The glass tubes were deposited in trays with borders covered with talc to prevent ant escape. For *M. ruginodis*, the tray size was 37 x 52 x 8 cm, since this species requires a larger foraging area than *M. rubra* for which the tray size was 27 x 42 x 7 cm. The trays served as foraging areas, as well as for training and testing (Fig. 1 A, D).

Temperature was maintained at 20° ± 2°C. Humidity was about 80% and remained constant over the course of an experiment. A lighting intensity of about 600 lux was maintained when caring, conditioning or testing the ants. Sugared water was permanently offered in a small glass tube plugged with cotton, and pieces of dead cockroaches were supplied twice a week on a glass-slide. No meat food was offered during experiments since it served as a reward during training (Fig. 1 A, D).

Experimental apparatus

Conditioning and testing of the ants

Since the first task was to assess the ants' maximum distance of vision, the experimental setup must allow the experimenter to vary the distance between the ants and the presented cues. In other words, the ants should not be able to approach the cues. The cues were thus presented to the ants on glass slides located above the foraging area, lying on the borders of the ants' tray (Fig. 1 A, D). The glass sides for *M. ruginodis* were 29 x 20 cm, and for *M. rubra* 24 x 18 cm, proportional to the different dimensions of the artificial foraging area of the experimental colonies.

The cues were squares made of strong black paper (Canson®), the width (the edge) of these squares equaling 1, 2, 3, 4, 5 or 6 cm for each of the six *M. ruginodis* colonies and 2, 4, 5 or 6 cm for each of the four *M. rubra* colonies.

Maximum distance of vision assessment

The cues were moved toward the ants, step by step, starting from a large distance (larger than the maximum distance of vision). They were placed on a transparent polyacetate sheet that was horizontally tied to a vertical gantry thanks to pairs of forceps that could be adjusted to different heights. The equipment was placed near the ants' nest such that the ants could see the cue but neither the gantry nor the forceps (Fig. 1 C, F). For each assessment, the forceps holding the polyacetate sheet with the cue was initially located the farthest from the ants and was thereafter, step by step, lowered closer to the ants' tray.

Experimental protocol

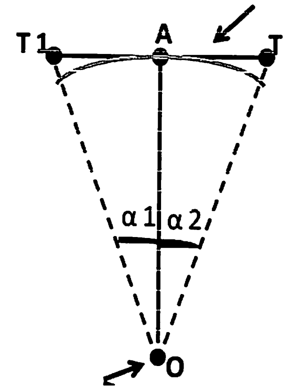
A distinct cue - a black square of a different dimension - was used for each of the six *M. ruginodis*, as well as for each of the four *M. rubra* colonies. The ants were conditioned to find their food under the presented cue during 6 training days, and a first test was performed on the seventh day. The ants were again trained for 6 days after which a second test was performed. Since each test is an experiment of extinction, 3 supplementary training days were conducted before final assessment of the ants' maximum distance of vision.

Training (= conditioning) (Fig. 1 A, D)

A black square with specific dimensions (diam = 1, 2, 3, 4, 5 or 6 cm according to the colony) was horizontally presented above the ants' tray and a piece of dead cockroach was placed on the ants' tray directly beneath the black square. During the 6-day and the 3-day training periods, the squares were relocated 6 and 3 times, respectively, and the meat was renewed when necessary but never after a time lapse of 24h to avoid the establishment of a trail (CAMMAERTS-TRICOT, 1974; CAMMAERTS & CAMMAERTS, 1980) and to prevent the possibility of spatial and temporal learning (CAMMAERTS, 2004b).

Testing (Fig. 1 B, E)

A control experiment was performed prior to any training, in which a black square was presented to each colony, as detailed in the above section 'Conditioning and testing of the ants'. The number of ants present on the tray directly beneath the square was counted 15 times, that is once every 15 sec, and the mean values of these counts were calculated. For the



→ T1 A T2 = cue → O = individual looking at the cue

If OA is the individual's maximum visual distance of the cue,

then, its subtended angle = α1 + α2 and can be calculated

since tgte α1 = AT1 / OA = |AT2| / OA = |tgteα2|

Fig. 2. Assessment of the subtended angle of an individual based of its maximum distance of vision and trigonometry.

actual tests on each colony, performed after the ants' training, the presented black square and the delivered meat were removed and, one hour later, the black square was again presented but without the food. The number of ants present on their tray beneath the square was counted 15 times (once every 15 sec), and the mean values calculated. The values obtained for the six or four colonies were compared to the corresponding control using the non-parametric test of Wilcoxon (SIEGEL & CASTELLAN, 1989) (Tab. 1).

Assessment of maximum distance of vision and calculation of subtended angle (Fig. 1 C, F)

The glass slide bearing the black square and the meat were removed from the colonies. The black square was deposited on the polyacetate sheet and tied to the gantry at a distance from the bottom which was greater than twenty times the square edge. The equipment was set near the colony tray, and the ants beneath the square were counted 15 times (once every 15 sec). Then, the forceps was clamped a few cm lower to the gantry. Again, the number of ants beneath

Table 1. Results of the experiments testing the ants' acquisition of operant conditioning to a black squared cue. Control: assessment prior to conditioning; Test 1 and test 2: assessment after 6 and 9 training days, respectively. Statistics: results of Wilcoxon non-parametric tests.

Species Experiment	N^{os} of reacting ants in each colony						Mean n^{os} of reacting ants	Statistics N	T	P
M. ruginodis										
Control	0.87	0.00	2.27	0.00	0.13	0.13	0.57			
Test 1	1.40	0.93	8.06	2.67	0.80	0.33	2.37	6	21	0.016
Test 2	1.00	0.87	6.07	0.66	1.00	0.54	1.69	6	21	0.016
M. rubra										
Control	0.20	0.40	0.33	1.40			0.58			
Test 1	0.80	1.93	1.27	2.47			1.62	4	10	0.06
Test 2	2.40	2.47	2.40	5.73			3.25	4	10	0.06

the square was counted 15 times (once every 15 sec). The same operation was repeated until the number of ants beneath the square became first similar to those obtained during the tests and thereafter less numerous. The mean value of the 15 counts was established for each experiment. The distance (between the bottom and the forceps) at which the ants suddenly became more numerous than in the previous distances represents the maximum distance of vision of the presented square for the studied species. This was statistically checked by performing Wilcoxon non-parametric tests between the control and the test values (Tabs 2 - 3). With the maximum distance of vision at which the two ant species perceived cues of different dimensions, it was possible to calculate the subtended angle of the two ant species using trigonometry, as explained in Fig. 2.

Results

Ants' response to the learned cues

Prior to conditioning, almost no workers of *M. ruginodis* or *M. rubra* moved to or stayed under the presented black squares (Tab. 1, control).

After six days of training (test 1) and three additional (test 2) training days, statistically more ants were present on the area located directly beneath the black square (Tab. 1, test 1, test 2; Fig. 1 B, E).

Ants' maximum distance and subtended angle of vision

Myrmica ruginodis (Fig. 1 C; Tab. 2)

The workers, which were trained with a 1 cm^2 black square, responded to that cue (i.e. became more numerous under it) when it was located at a distance of 18 cm above them. When the cue was presented closer, the ants progressively ceased to respond since the cues were presented in extinction experiments.

Likewise, workers trained with black squares with edges of 2, 3, 4, 5 and 6 cm responded to these cues (i.e. became more numerous under them, thus perceiving them) when the cues were at distances of 36, 55, 70, 90 and 110 cm, respectively, the ants becoming less numerous at smaller distances.

Using trigonometry, the tangent of half of the subtended angle of *M. ruginodis* workers equals: 0.5/18 ≈ 1/36 ≈ 1.5/55 ≈ 2/70 ≈ 2.5/90 ≈ 3/110 ≈ 0.0275. Half of the subtended angle equals 1°35' and the subtended angle 3°10'.

Myrmica rubra (Fig. 1 F; Tab. 3)

Workers of this species, which were trained to a 4 cm^2 (2 x 2 cm) black square, became more numerous beneath the square when it was located at a distance of about 30 cm. After that, when the square was presented at shorter distances, the ants became less numerous since each presentation was an experiment of extinction.

Similarly, *M. rubra* workers, which were trained to black squares with edges of 3, 4 and 5 cm, responded to such squares when they were at distances of about 45, 60 and 75 cm, respectively. When the squares were moved closer, the ants no longer responded since they had been previously submitted to experiments of extinction.

The tangent of half of the subtended angle of *M. rubra* workers equals: 1/30 ≈ 1.5/45 ≈ 2/60 ≈ 2.5/75 ≈ 0.033. Half of the subtended angle thus equals 1°55' and the subtended angle 3°50'.

Table 2. Assessment of maximum distance of vision in *Myrmica ruginodis*. Ants from 6 colonies (first column) were previously trained to a squared cue of a given dimension(second column). The cue was then presented to the ants to determine their largest distance (that still induces a reaction). The cue was gradually moved towards the ants (third column), this being repeated (1rst time, 2nd time) and the reacting ants were counted each time (fourth column). The largest distance inducing a reaction is the visual maximum distance for the species (fifth column = OA); it allows calculation of that ant's subtended angle of vision (Fig. 2 where A, T1 and T2 can be seen).

Colony	Dimension of the cues (T1-T2; cm)	Distances of presentation (OA; cm)	Mean n^{os} of reacting ants		Conclusion: maximum distance of vision (OA) [for AT1 = AT2]
			1rst time	2nd time	
1	1	24	-	0.27	
		20	0.47	0.13	
		18	1.00	1.00	→ ≈ 18 cm [0.5 cm]
		16	1.00	0.47	
		14	0.60	0.13	
		12	0.33	0.13	
2	2	40	0.60	0.07	
		36	2.00	0.80	→ ≈ 36 cm [1 cm]
		32	0.73	0.00	
		28	0.20	0.00	
		24	0.33	0.00	
		20	0.40	0.00	
3	3	60	1.73	2.13	
		55	5.00	4.00	→ ≈ 55 cm [1.5 cm]
		50	3.33	2.47	
		45	2.93	1.80	
		40	2.47	0.53	
		36	1.80	0.40	
4	4	80	0.00	0.07	
		75	1.00	0.40	
		70	2.20	1.53	→ ≈ 70 cm [2 cm]
		65	0.53	0.20	
		60	0.33	0.27	
		55	0.00	0.13	
5	5	95	0.00	0.60	
		90	1.40	2.46	→ ≈ 90 cm [2.5 cm]
		85	0.30	1.07	
		80	0.07	0.80	
		75	0.27	0.00	
6	6	120	0.00	0.13	
		110	2.27	2.40	→ ≈ 110 cm [3 cm]
		100	1.80	0.67	
		95	0.20	0.40	
		90	0.00	0.27	

Discussion

The subtended angle of *M. ruginodis* workers equals 3°10' (present work). The number of ommatidia in each of the worker's eyes is relatively high, about 149, and there is a large posterior-dorsal region (RACHIDI *et al.*, 2008). The nests of this species are located under branches along forest edges and forest clearings which permit a partial view of the sky above (CAMMAERTS *et al.*, 2011). The workers orient themselves when foraging primarily using visual cues located above them, and they neglect odorous elements except when foraging under very low light intensity (CAMMAERTS *et al.*, 2011). The visual perception abilities of this ant species are high since it is able to distinguish

Table 3. Same legend as for table 2, except that 4 (instead of 6) colonies of *Myrmica rubra* was tested..

Colony	Dimension of the cues (T1-T2; cm)	Distances of presentation (OA; cm)	Mean n^{os} of reacting ants 1^{rst} time	2nd time	Conclusion: maximum distance of vision (OA) [for AT1 = AT2]
1	2	40	0.47	0.33	
		36	0.93	0.33	
		32	2.33	1.27	→ ≈ 32 - 28 cm [1 cm]
		28	2.13	1.73	
		24	0.60	0.53	
		20	0.13	0.60	
		16	0.33	0.60	
2	3	60	0.33	0.13	
		55	0.20	0.07	
		50	0.53	0.13	
		45	1.73	1.93	→ ≈ 45 cm [1.5 cm]
		40	0.60	0.60	
		36	0.33	0.67	
		32	0.00	0.33	
3	4	80	0.00	0.53	
		75	0.40	0.73	
		70	0.13	0.53	
		65	0.33	0.47	
		60	2.33	2.67	→ ≈ 60 cm [2 cm]
		55	0.80	0.73	
		50	0.33	0.13	
		45	0.47	0.47	
4	5	95	0.33	0.33	
		90	0.80	0.13	
		85	0.87	0.13	
		80	0.60	0.47	
		75	2.73	1.33	→ ≈ 75 cm [2.5 cm]
		70	1.67	0.53	
		65	0.67	0.27	
		60	0.87	0.40	

very small luminous points in a dark ceiling (CAMMAERTS, submitted). In all likelihood, *M. ruginodis* uses cues from the canopy and sky, and its small subtended angle of vision allows it to distinguish small visual cues.

The subtended angle of vision of *M. sabuleti* equals 5°12' (CAMMAERTS, 2004a). Its eyes are small, each having about 109 ommatidia, and there is no large posterior-dorsal region as in *M. ruginodis* (RACHIDI *et al.*, 2008). These ants primarily use odorous elements to negotiate their way, although they are able to use visual cues in the absence of odours, using their frontally oriented visual system (CAMMAERTS & LAMBERT, 2009; CAMMAERTS & RACHIDI, 2009). The visual perception of this species is not high; the workers are not able to distinguish convex shapes or lines (squares and circles, rectangles and ellipses, squares and triangles, etc.); they can only discriminate concave forms and lines (CAMMAERTS, 2008). However, they are capable of distinguishing cues that contain different numbers of elements, cues that are differently oriented (CAMMAERTS, 2008) and cues that have different dimensions or that are located at different heights above the ground (CAMMAERTS, 2004a). This species nests in open biotopes containing several odorous plants (RACHIDI *et al.*, 2008; CAMMAERTS & RACHIDI, 2009). We experimentally demonstrated that these workers orient themselves primarily by olfaction and secondarily by vision (CAMMAERTS & RACHIDI, 2009).

The subtended angle of vision of *M. rubra* equals 3°50' (present work). Its eyes are rather large each containing about 129 ommatidia, and the posterior-dorsal region is not enlarged, as in *M. ruginodis* (RACHIDI *et al.*, 2008). The orientation system of *M. rubra* is yet unknown. Presently, we conclude that the visual perception in this species is of medium quality: indeed, the workers are able to distinguish convex filled shapes but not convex hollow ones, being however able to discriminate lines of different dimensions (CAMMAERTS, unpublished data). The species nests in rather open grassy biotopes containing several small non-odorous plants (CAMMAERTS, personal observations). Studies on course elucidate *M.* rubra workers' system of navigation and correlate the results with the species' subtended angle of vision and visual perception.

Based on our results, we hypothesize that, with respect to insects, a relationship exists between the subtended angle of vision and the number of ommatidia. Indeed, in the studied species of *Myrmica*, the eyes have a mean of 149, 129 and 109 ommatidia, while the subtended angles for these species are 3°10, 3°50' and 5°12, respectively. This suggests that there is a decreasing logarithmic relation between the two variables.

The visual subtended angle of insects has scarcely been determined. WILSON (1975) reported that the subtended angle of vision in the western honeybee may equal one angular degree, but HORRIDGE (1996) and GIURFA & VOROBYEV (1998) obtained a larger value (about 3 angular degrees). Among ants, a large variability exists regarding their visual perception. Some species (*Leptothorax albipennis*, MC LEMAN *et al.*, 2002; PRATT *et al.*, 2001) have very small eyes; others (*Giganthiops destructor*, BEUGNON *et al.*, 2001) have very large eyes while numerous other species (*Cataglyphis spp, Formica spp.*, HÖLLDOBLER & WILSON, 1990) have middle-size eyes. The orientation systems of ants correlatively differ (PASSERA & ARON, 2005). Although orientation and visual perception have been rather well studied in ants, no assessments have been made for their subtended angle of vision. Even in AVARGUES-WEBER *et al.* (2011), there is no mention of specific values of the subtended angle of vision in the social insects cited in these author's otherwise well documented review of visual learning in insects. It would be beneficial to know a species' subtended angle of vision and its visual perception abilities before designing experiments to investigate its system of orientation. Even closely related species, such as those of the genus *Myrmica,* may differ in their visual subtended angle and their perception abilities, and thus in their orientation and foraging behaviour.

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The diversity of ladybirds (Coleoptera: Coccinellidae) and grasshoppers (Orthoptera) surveyed on coal tips in the Walloon Region (Belgium)

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Abstract

Coal tips are artificial hills characterized by very heterogeneous soil. Nowadays, the natural colonization is taking place and coal tips are more and more considered as highly valuable habitats for the biodiversity. We detail the results of standardized sampling of Ladybird beetles (*Coccinellidae*) and grasshoppers (Orthoptera) performed during 2009 and 2010 in 55 sites in the Province of Hainaut (Belgium). The species richness and the diversity are analysed and discussed at the level of sites and habitats within sites. Coal tips are likely to host rare or endangered species which find there substitute habitats of their natural sites. 21 species of ladybirds and 21 species of Orthoptera were identified and counted by mean of the method that we implemented. A special focus was dedicated to the invasive Multicoloured asian ladybird *Harmonia axyridis* (Pallas, 1773). Our method seems to be convenient to detect the species composition of ladybirds but it has some weaknesses in the detection of few grasshopper species. On the other hand, we emphasize that the list of Orthoptera can be easily supplemented with use of good knowledge of their songs. In addition, we evaluated the possibility of adding other insect group such as Lepidoptera Rhopalocera into the methodology.

Keywords: Coccinellidae, Orthoptera, coal tips, diversity index, Belgium, biological assessment

Introduction

The coal-mining extraction stopped in 1984 in the Walloon Region and left behind numerous coal tips along the coal-mining basin: ca. 340 sites in the Walloon Region and 250 in France (data Service Public de Wallonie; PETIT, 1980 *in* RASMONT & BARBIER, 2000). These hills, which are made of a mixture of soil, rock and coal, are characterized by very heterogeneous soil composition, soil grain size, exposition of the slopes to the sun, albedo (i.e. the reflecting