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Light thresholds for colour vision in workers of the ant *Myrmica sabuleti* (Hymenoptera, Formicidae)

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ABSTRACT. Previous studies suggested that workers of the ant species *Myrmica sabuleti* have different light thresholds for distinguishing different colours. Here we assess these thresholds and find that the light thresholds required to distinguish colours from grey are lower than those necessary to discriminate between two colours. The two thresholds are somewhat lower for ants trained under low versus high light intensity. In every case, the ants' threshold decreases from red to violet. All these thresholds are lower than those required for perceiving shapes. The visual system of workers of *M. sabuleti* under very low light intensity may thus consist of discriminating only coloured spots from grey and under slightly higher light intensity, differently coloured elements where the eyes are used in superposition mode. Under high light intensity, these ants see (although not sharply) shapes and lines, using their eyes in apposition mode. Moreover, workers of this species demonstrated their best colour discrimination in seeing the colours yellow and blue under high light intensity, and green and violet under low light intensity. Therefore, these ants' visual system may be adapted to the quantitative and qualitative variations in natural light during the day.

KEY WORDS : colour vision, eyes, *Myrmica sabuleti*, perception threshold, vision

INTRODUCTION

Though ants primarily use chemical signals to perform most of their tasks (communicating, following a trail, perceiving marked areas), they also need some visual perception to accomplish several activities (foraging, returning to the nest after having discovered a new food source or new nest site) (Oliveira & Hölldobler, 1989; Chameron et al., 1998; Salo & Rosengren, 2001; Passera & Aron, 2005). Insect vision has previously been studied in detail, but most authors have worked on insects with large eyes and good vision (Odonata, Lepidoptera, Diptera, Hymenoptera such as wasps, bees etc…) (Wehner, 1981 and references therein). In ants, this field of study is in its infancy and tends to be physiological in nature, e.g., Jander (1957), Vowles (1965) and Voss (1967). Generally, the ants studied so far have large eyes (e.g. *Formica* sp*., Cataglyphis bicolor, Gyganthyops destructor*) (Wehner, 1981). The visual system in ants with (comparatively) small eyes has scarcely been studied. Given that ethological studies have already been conducted on *Myrmica sabuleti* Meinert 1861 (Cammaerts & Cammaerts, 1980), we decided to investigate this species' visual perception (Cammaerts, 2004 a; 2005; 2006; 2007 a; b). During the study of colour perception in workers of *M. sabuleti*, it was found that, under high light intensity, these ants were sensitive essentially to yellow and blue, while under low light intensity their highest sensitivities occurred for green and violet. Consequently it was presumed that these workers may have different light thresholds for different colours (i.e. different minimum light intensities necessary to perceive different colours). In the present paper, we investigate this issue, connect the results to previous ones, propose a visual system for workers of *M. sabuleti*, and compare our conclusions to those of other authors. This may be of interest because visual thresholds have not yet been precisely assessed in insects even if many studies have been conducted on their visual perception (for instance Chittka, 1996; Giurfa et al., 1996; 1997; Wehner, 1981; Briscoe & Chittka, 2001; Vorobyev et al., 2001; Kelber et al., 2003).

MATERIALS AND METHODS

Collection and maintenance of ants

Colonies of *M. sabuleti* were collected from Höghe Martelingen (Luxembourg; 49° 40' 30" N, 5° 45' 00" E) and from the Aise valley (Belgium; 49° 49' 39" N, 5° 15' 26" E). They were divided into a total of 30 smaller experimental colonies (5 series, labelled A to E), of six colonies (numbered 1 to 6), demographically similar, each containing about 250 workers, a queen and brood. These colonies were maintained in a laboratory, in a window-less room, at constant temperature (20°C±1°C) and humidity. Light was provided by OSRAM concentra lamps (60W) attached to the ceiling. The spectrum of this light was measured using a grating spectrograph (an Acton Spectrapro-500i) with CCD camera (Princetin Instrument TEA/CCD-1100-PF) detection and an optical fibre probe. The slit opening was 100µm and the grating was 600grooves/mm (500nm blaze). The spectrum was obtained by registering successive sections of 80nm, with an overlap of 45nm, the probe being maintained in front of an OSRAM concentra lamp, after these sections were assembled. The entire resulting broadband spectrum (shown in Fig. 2) revealed that the illumination contained all wavelengths of visible light and almost no UV light. The light intensity was assessed using a luxmeter (a Testoterm 0500 luxmeter built by Testoterm GmbH & Co (D-7825, Lenzkirch)). Two light intensities, 10,000 lux and 600 lux, were used in the course of our study. Light intensity was adjusted using a dimmer such that the intensity of illumination and not the shape of the wavelength spec-

trum was changed. These light intensities were previously used to reveal the capability of workers of *M. sabuleti* to discriminate colours from greys and from one another (CAMMAERTS, 2007 a).

The ants nested in one or two glass tubes half-filled with water, a cotton plug separating the ants from the water (Fig. 1). The glass tubes were deposited into a polyethylene tray (47cm x 22cm x 7cm) whose borders were covered with talcum and the tray served as a foraging area. Sugared water was permanently provided in a small glass tube closed by a cotton plug. Pieces of dead cockroach were provided twice a week on a small piece of glass when no experiments were planned or performed, since this meaty food was used as a reward during the training phases of each experiment (see experimental protocol).

Experimental apparatus

Experimental apparatuses were built of paper (Canson ®) of the following colours: grey, red, yellow, green, blue, and violet, these names being the ones given by Canson, and expressing only how these colours appear to the human eye. The spectra of the broadband emission of the light reflected by (or transmitted through) each coloured paper were measured using the above- described spectrograph (with CCD camera detection and an optical fibre probe) by holding the probe in front of a piece of paper of each colour with a lighted OSRAM concentra lamp being located either above or, more easily behind, the paper. These measurements were difficult because very little light reached the fibre probe. Nevertheless, the light spectra reflected by (or transmitted through) the grey paper had no maximum whereas those by (or through) the other coloured papers yielded maxima around 640nm (red), 550nm (yellow), 525nm (green), 425nm (blue), 445nm and 375nm (violet), the last extending up to about 360nm. To confirm these measurements, pieces (2cm x 2cm) of paper of each colour were boiled for 5 minutes in 10mL of water, tingeing the water in the respective colour. We checked that the colours of the tinged waters were exactly the same as those of the corresponding boiled papers. Then, the spectra of the light absorbed by the different coloured waters were obtained using a CARY 50-varian UV-visible spectrophotometer (Cary, USA; sensitivity range: 200-1,000nm; maximum scan rate of 24,000nm per minute; Xenon lamp) and the Cary Win UV software. Next, based on the spectrum of the delivered light (Fig. 2, upper graph) and of the spectra of the light absorbed by the waters tinged by the papers, the spectra of the light reflected by each of the coloured papers were calculated using the relation $Lr(=t)=Lo/10^{La}$ where Lr(=t) is the reflected (or transmitted) light, Lo is the delivered light and La is the absorbed light. These calculated spectra are shown in Fig. 2 (lower graphs, above the photo of the apparatus) and are in agreement with the spectra obtained using the grating spectrograph. Small differences are likely due to the differences between the xenon lamp in the spectrophotometer and the OSRAM lamp used with the grating spectrograph. The light intensity reflected by grey and each coloured paper was measured using the luxmeter as detailed above and was the same for all the papers under low light intensity, and nearly identical under high light intensity. Evidently, the light intensity reflected by the used papers varied with the intensity of the delivered light, which was therefore standardised for all the manipulations (600 or 10,000 lux as stipulated in the above paragraph).

Each experimental apparatus consisted of a disk (diameter=8cm) made of two half-disks of two differently coloured papers attached by means of a piece of glued paper (Fig. 2). To determine the ants' thresholds that allow discrimination between grey and colour, one half-disk was made of grey paper and the other of either red, yellow, green, blue, or violet paper. To determine thresholds that allow ants to distinguish between two colours, the two half-disks were made of two differently coloured papers. The combinations used were red and yellow, yellow and green, green and blue, blue and violet, and violet and red (Fig. 2).

Each experiment was performed simultaneously on six colonies; identical but other (new) apparatus were used on the one hand for training the ants and on the other for testing them (see experimental protocol). Thus, a total of 12 experimental apparatuses were built to perform a single experiment.

Experimental protocol (Appendix, upper part)

The protocol used the differential operant conditioning system to obtain the ants' conditioned response to a colour in the presence of grey or of another colour. This system, like the operant conditioning one (CAMMAERTS, 2004 c), generally consists of initially performing a control experiment before any training, then placing the animals in a situation where they are rewarded each time they give the correct response. Progressively, the animals associate the correct response to the presence of a reward. The system finishes by a test experiment to check the acquisition of the conditioning by the animals. After successful conditioning, the threshold (in our case the minimum light intensity necessary) to elicit the conditioned response (i.e. for responding to the correct colour) could be assessed by testing the animals under stepwise increases in stimulation. In the present work, the timing of the successive steps of the protocol is identical to that used to reveal the ants' light and dark adaptation (CAMMAERTS, 2005).

The experiments were conducted either under 10,000 lux or under 600 lux, each time simultaneously on the six colonies of a series maintained for four days under the respective experimental light intensity and having received no meat during these four days. The ants' visual thresholds for distinguishing colours from grey as well as colours from one another were assessed under the two light intensities, yielding a total of four series of experiments (each series involving five experiments: see tables). All the threshold assessments followed the same protocol. First, an experimental apparatus was deposited horizontally, on the tray of each of the six colonies of a series (an identical apparatus was used for each colony) and a control experiment was performed (see below: quantification of the ant response). Immediately thereafter, the apparatus was removed from the tray of each colony, and identical ones (those designed for the training phases) were deposited, also horizontally, on each tray. A

piece of dead cockroach was deposited, on a small piece of glass, on one of the two half-disks of the apparatus; the half-disk of the same colour for each colony; which is hereafter referred to as the "correct half-disk" (the one associated with the reward) (Fig. 1). This protocol enabled the ants to go through differential operant conditioning within six days. During that 6-day training period, the apparatuses were turned and relocated 6 to 12 times in order to avoid some efficient pheromone deposit by foragers as well as spatial learning by the ants (i.e. the learning of a localisation) (CAMMAERTS, 2004 b). Meat was replaced whenever necessary but not periodically to avoid temporal learning (i.e. learning of a given hour or periodicity) (CAMMAERTS, 2004 b). During this training phase, the ants progressively associated the "correct half-disk" to the presence of meat. After this training phase, the experimental apparatuses were removed from the foraging area, and those used during the control (those designed for the tests, free from any pheromonal deposits) were presented (at places differing from those where the apparatuses were located at the end of the training phase). A test was then conducted (see below: quantification of the ants' responses). Thereafter, the ants were conditioned again over three more days in the presence of the apparatus designed for training, the apparatus being once more randomly and not periodically turned and relocated three to six times during the three training days. After this second training phase, a second test was performed using the appropriate apparatus. Next, the ants were conditioned again for one day, and the following day was used to assess the visual threshold necessary for discriminating the "correct half-disk" from the other (grey or coloured) one. Here, the light intensity was lowered to 1 or 0.5 lux (this being nearly darkness) and a first test was performed. Then, the light intensity was progressively increased, step by step (Tables 1 to 4, column 1), and a test was conducted at each step. The experiment ended when at least as many ants responded correctly as had correctly responded during each of the two previously conducted tests (see above, conditioning protocol).

Quantification of the ant response (Appendix, lower part)

During the control, during the two tests (to assess the ants' conditioning) and during each test made with increasing light intensity (to assess the ants' threshold), the ants present on each half-disk presented to the six colonies were counted once for each colony, as quickly as possible (usually in 12 seconds) to avoid light adaptation, this process being then immediately repeated 14 more times, yielding a total of 180 counts (2 x 6 x 15, usually in three minutes). What we counted was the ants' responses, one ant being thus able to give several responses in the course of the counting time. The mean value of the fifteen counts was established separately for each half-disk, for each of the six colonies. Two mean values were thus obtained for each colony (see the table in the appendix). These 12 mean values allowed statistical analysis of ant response as follows: the difference between the mean value corresponding to the "correct half-disk" and that corresponding to the "wrong one" was calculated for each colony (for each test) and for the corresponding control. Then, the six differences obtained for a test were compared to the six corresponding differences obtained for the previously performed control by using the non-parametric Wilcoxon rank test (SIEGEL & CASTELLAN, 1988). The ants' responses were considered to be significant at $P<0.05$. This level of probability indicated that the ants can see the difference between the "correct and the wrong half-disk", enabling us to detect the lowest light intensity the ants required to see this difference (i.e. the ants' light threshold).

Additionally, the mean of the above-mentioned six mean values was calculated for each half-disk, for the control and for each of the tests. Tables 1 to 4 give these 'global means' as well as the calculated proportion of ants present on the "correct half-disk", for each control and each tests.

Common legend to Tables 1 to 4: Under either 10,000 lux or 600 lux, the ants (of series A to E, each consisting of six colonies) were trained to find food on a coloured half-disk (**in bold in the tables)** *versus* the other, differently coloured half-disk, as detailed in 'Materials and Methods' and summarised in the appendix. During the control (before the training), the test 1 (after 6 training days), the test 2 (after 3 more training days) and each of the tests conducted with increasing light intensity in order to assess thresholds, the ants were confronted with the two differently coloured half-disks free of food and of any ant secretions. Their responses were quantified via 15 counts for each half-disk (see 'Materials and Methods' and the appendix) by: - columns 2 and 3: their mean numbers present on each coloured half-disk; - column 4: the proportion of 'correct' responses; - column 5: the results of non-parametric Wilcoxon tests applied to the counted numbers of ants.
NS: non-significant result at P=0.05; → indicates the ants' threshold, with [when lying between two values.

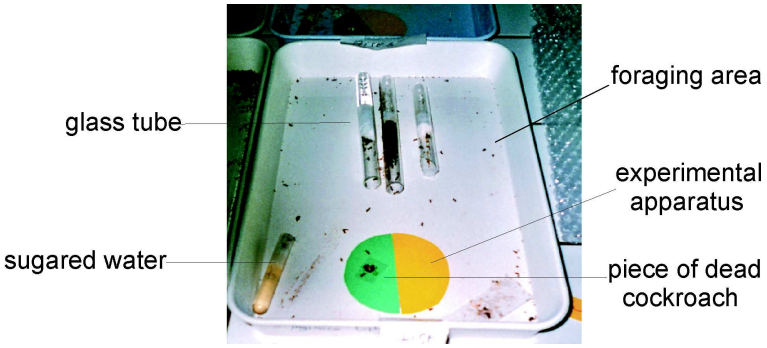

Fig. 1. – Experimental design. An experimental colony during a training phase.

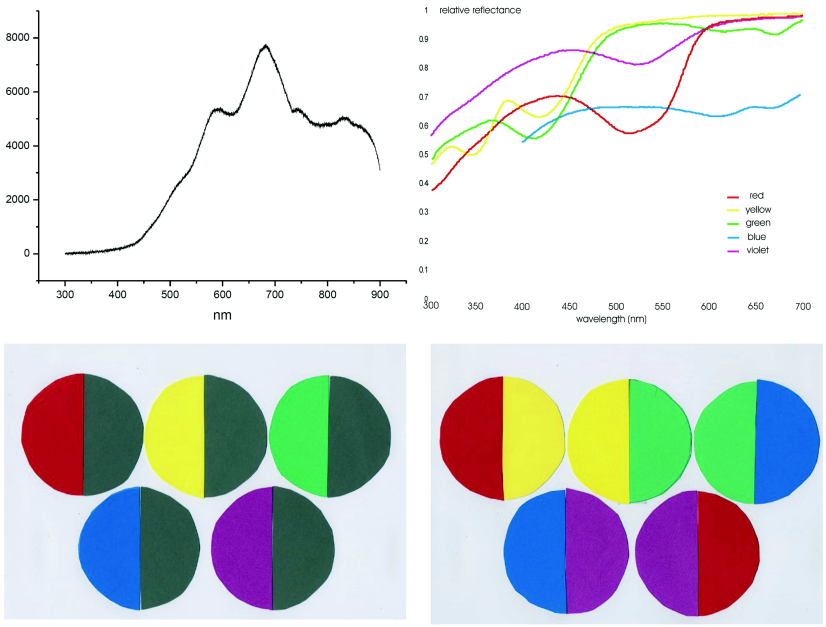

Fig. 2. – Spectra of the delivered light (upper left graph) and of the light reflected by the coloured papers (upper right coloured graphs). Experimental apparatus used to assess the ants' light thresholds for distinguishing colours from grey (lower left disks) and for distinguishing two colours (lower right disks).

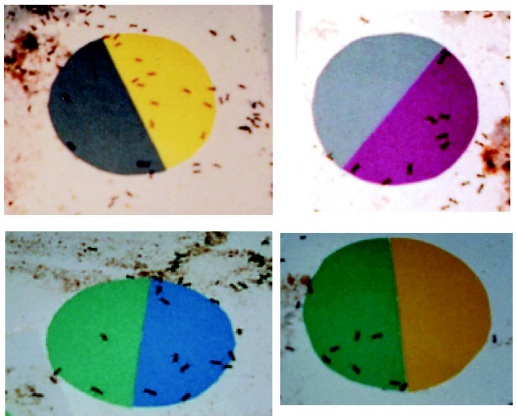

Fig. 3. – Examples of ant responses while assessing the lower light intensities they need to distinguish between colours and grey or between two colours.
Upper left: responses to yellow versus grey, after training under 10,000 lux
Upper right: responses to violet versus grey, after training under 600 lux
Lower left: responses to blue versus green, after training under 10,000 lux
Lower right: responses to green versus yellow, after training under 600 lux

TABLE 1

Ants' light thresholds for distinguishing colours from grey, after training under 10,000 lux

Series steps		Mean numbers on each colour		% correct responses	P
A		grey	**scarlet**		
Control		3.25	4.05	55	-
Test 1		4.51	8.09	64	0.031
Test 2		2.34	6.16	72	0.016
Lux	1	3.22	3.39	51	NS
	1.5	5.22	4.22	45	NS
	2	5.89	5.00	46	NS
	2.5	5.00	7.06	59	NS
	3	5.00	5.72	53	NS
	3.5	5.39	7.80	59	NS
	4	5.38	8.89	62	NS
	6	6.11	9.11	60	NS
	→8	6.17	9.83	61	0.031
	10	6.00	10.05	63	0.016
B		grey	**yellow**		
Control		1.96	2.16	52	-
Test 1		0.83	3.01	78	0.016
Test 2		0.44	1.99	82	0.016
Lux	1	3.28	3.34	50	NS
	1.5	2.78	2.78	50	NS
	2	2.33	2.00	46	NS
	2.5	1.67	2.72	62	NS
	3	1.78	2.39	57	NS
	3.5	1.56	2.45	61	NS
	→4	1.33	3.11	70	0.016
	6	1.83	3.50	66	0.016
C		grey	**green**		
Control		6.17	4.78	44	-
Test 1		1.22	3.32	60	0.031
Test 2		1.81	4.33	68	0.016
Lux	1	1.95	2.28	54	NS
	1.5	2.17	3.06	59	NS
	2	2.11	3.44	62	NS
	2.5	3.00	4.33	59	NS
	3	2.83	4.34	60	0.031
	→3.5	1.95	3.33	63	0.016
D		grey	**blue**		
Controle		2.34	1.98	46	-
Test 1		0.69	2.30	77	0.016
Test 2		1.26	2.93	70	0.016
Lux	1	3.33	3.89	54	NS
	1.5	1.94	2.56	57	NS
	2	2.39	2.56	52	NS
	→2.5	1.72	3.78	69	0.016
	3	1.89	3.61	66	0.016
	3.5	1.28	2.83	69	0.016
	4	1.72	3.06	64	0.016
	6	1.34	2.78	67	0.016
E		grey	**violet**		
Control		1.49	1.12	43	-
Test 1		1.33	2.76	67	0.031
Test 2		1.43	2.87	67	0.016
Lux	1	1.45	1.00	41	NS
	1.5	1.28	1.45	53	NS
	→2	0.78	2.28	75	0.016
	2.5	0.56	2.00	78	0.016
	3	0.50	1.78	78	0.016

TABLE 2

Ants' light thresholds for distinguishing colours from grey, after training under 600 lux.

Series Steps		Mean numbers on each colour		% correct responses	P
A		grey	**scarlet**		
Control		3.72	4.23	57	-
Test 1		2.82	7.24	72	0.016
Test 2		3.79	6.39	63	0.031
Lux	1	3.89	4.56	54	NS
	1.5	4.39	4.78	52	NS
	2	4.28	4.83	53	NS
	2.5	5.78	5.50	49	NS
	3	7.22	7.22	50	NS
	3.5	7.28	8.89	55	NS
	→4	5.95	11.28	65	0.016
	6	5.95	10.06	63	0.031
B		grey	**yellow**		
Control		3.37	3.25	49	-
Test 1		1.78	2.87	62	0.016
Test 2		2.91	4.86	63	0.016
Lux	1	2.94	2.33	44	NS
	1.5	2.83	2.89	50	NS
	2	2.00	2.94	60	NS
	→2.5	1.00	2.33	70	0.016
	3	1.33	2.83	68	0.016
C		grey	**green**		
Control		1.96	1.85	49	-
Test 1		1.49	4.34	74	0.016
Test 2		2.05	4.91	71	0.016
Lux	1	2.78	3.06	52	NS
	1.5	3.95	4.56	54	NS
	→2	2.83	4.72	63	0.016
	2.5	2.56	4.95	66	0.016
D		grey	**blue**		
Control		1.56	1.49	49	-
Test 1		0.97	2.11	69	0.016
Test 2		0.82	1.89	70	0.016
Lux	1	2.39	2.78	54	NS
	→1.5	2.06	3.72	64	0.016
	2	1.61	4.39	73	0.016
E		grey	**violet**		
Control		1.00	0.78	44	-
Test 1		0.56	2.26	80	0.016
Test 2		0.83	2.11	72	0.016
Lux	0.5	1.33	1.72	56	NS
	→1	0.67	1.22	65	0.031
	1.5	0.61	1.67	73	0.016

TABLE 3

Ants' light thresholds for distinguishing between two colours, after training under 10,000 lux.

Series	Steps	Mean numbers on each colour		% correct responses	P
A		scarlet	**yellow**		
Control		4.11	3.35	45	-
Test 1		3.53	6.68	65	0.016
Test 2		2.64	5.64	68	0.016
Lux	1	3.17	3.00	49	NS
	1.5	3.61	3.78	51	NS
	2	2.50	2.67	52	NS
	2.5	3.17	3.00	49	NS
	3	3.67	3.28	47	NS
	3.5	3.78	3.39	47	NS
	4	3.33	3.61	52	NS
	6	2.78	4.17	60	NS
	→8	2.61	8.72	77	0.016
	10	3.00	5.72	66	0.016
	12	3.50	5.33	60	0.016
B		yellow	**green**		
Control		2.26	1.58	41	-
Test 1		1.28	2.36	65	0.016
Test 2		1.82	3.13	63	0.016
Lux	1	2.22	2.95	57	NS
	1.5	3.45	3.56	51	NS
	2	2.89	3.56	55	NS
	2.5	3.39	4.33	56	NS
	3	3.22	3.06	49	NS
	3.5	3.56	3.44	49	NS
	4	2.67	3.17	54	NS
	→6	1.78	3.56	67	0.016
	8	1.17	3.67	76	0.016
C		green	**blue**		
Control		3.35	3.66	52	-
Test 1		0.94	2.44	72	0.016
Test 2		0.89	2.92	77	0.016
Lux	1	3.00	2.28	43	NS
	1.5	3.00	2.78	48	NS
	2	2.61	2.78	52	NS
	2.5	2.39	1.94	45	NS
	3	2.83	2.50	47	NS
	3.5	2.78	2.78	50	NS
	[4	2.72	3.56	57	NS
	→[6	1.89	4.42	70	0.016
	8	1.44	3.94	67	0.016
D		blue	**violet**		
Control		1.40	1.22	47	-
Test 1		0.53	1.41	72	0.016
Test 2		1.04	1.98	72	0.016
Lux	1	2.06	2.00	49	NS
	1.5	2.00	1.72	46	NS
	2	1.95	1.67	46	NS
	2.5	1.83	1.56	46	NS
	3	1.67	1.56	48	NS
	→3.5	1.00	2.06	67	0.031
	4	1.33	4.11	76	0.016
	6	0.50	1.89	79	0.016
	8	0.45	2.22	83	0.016
E		violet	**scarlet**		
Control		1.11	1.23	53	-
Test 1		0.47	1.86	80	0.016
Test 2		0.89	2.72	75	0.016
Lux	1	2.22	2.45	52	NS
	1.5	1.17	1.17	50	NS
	2	1.45	1.45	50	NS
	→2.5	0.83	2.28	73	0.016
	3	0.67	1.95	74	0.016
	3.5	0.72	2.17	75	0.016
	4	0.72	2.00	74	0.016

TABLE 4

Ants' light thresholds for distinguishing between two colours, after training under 600 lux.

Series	steps	Mean numbers on each colour		% correct responses	P
A		scarlet	**yellow**		
Control		6.20	5.53	47	-
Test 1		4.25	6.27	60	0.031
Test 2		2.25	3.99	64	0.016
Lux	1	2.83	3.11	52	NS
	1.5	4.45	5.45	55	NS
	2	3.50	3.28	48	NS
	2.5	3.22	4.11	56	NS
	3	3.17	4.33	57	NS
	3.5	3.06	4.95	62	NS
	4	2.94	4.39	60	NS
	→/ 6	3.11	5.22	63	0.016
B		yellow	**green**		
Control		2.51	2.54	50	-
Test 1		3.65	6.38	64	0.031
Test 2		2.07	5.44	72	0.016
Lux	1	4.06	4.99	55	NS
	1.5	2.34	2.39	50	NS
	2	2.50	2.67	52	NS
	2.5	2.00	2.72	58	NS
	3	2.61	3.72	59	NS
	→3.5	1.00	2.89	74	0.016
	4	1.28	3.39	73	0.016
	6	1.84	4.06	69	0.016
C		green	**blue**		
Control		4.25	3.60	46	-
Test 1		2.13	5.08	70	0.016
Test 2		2.33	4.90	68	0.016
Lux	1	1.22	2.05	63	NS
	1.5	2.28	2.89	56	NS
	2	1.72	2.95	63	NS
	→2.5	1.17	3.50	75	0.016
	3	1.72	3.33	66	0.031
	3.5	1.78	3.83	68	0.016
D		blue	**violet**		
Control		2.38	2.13	47	-
Test 1		0.62	1.85	75	0.016
Test 2		0.89	2.29	72	0.016
Lux	0.5	1.89	1.89	50	NS
	1	1.22	1.28	51	NS
	1.5	1.61	1.83	57	NS
	→2	0.78	2.22	74	0.016
	2.5	0.67	2.11	76	0.016
E		violet	**scarlet**		
Control		1.36	1.52	53	-
Test 1		0.61	1.39	70	0.016
Test 2		0.78	2.22	74	0.016
Lux	0.5	0.84	0.95	53	NS
	1	0.56	0.72	56	NS
	→1.5	0.67	1.17	64	0.031
	2	0.61	1.95	76	0.016
	2.5	0.61	1.89	76	0.016
	3	0.39	1.61	81	0.016

RESULTS

Thresholds for distinguishing colours from grey

After training under high light intensity, the ants could distinguish each presented colour from grey (Table 1). The lowest light intensities (=thresholds) required to perform this distinction were very low in general: about 8 lux for red, 4 lux for yellow (Fig. 3, upper left photo), 3.5 lux for green, 2.5 lux for blue and 2 lux for violet (Table 1). The threshold for discriminating a colour from grey thus decreased from red to violet.

After training under low light intensity, the proportion of ants having distinguished a colour from grey were generally lower than those having done so after training under high light intensity (Table 2). On the other hand, the lower light intensities required to see colours other than grey were generally smaller than those previously assessed (see above) but the same decrease from red to violet was observed. Indeed, the values were about 4 lux for red, 2.5 lux for yellow, 2 lux for green, 1.5 lux for blue and 1 lux for violet (Fig. 3, upper right photo) (Table 2).

Thresholds for distinguishing two colours from one another

After having been trained under high light intensity, workers of *M. sabuleti* were able to discriminate all the presented colours from one another with nearly the same efficiency (Table 3). The lower light intensities (=thresholds) required to correctly respond to two presented colours were somewhat higher than those needed to see a colour other than grey; again, these light thresholds decreased from red to violet. More precisely, the thresholds for colour discrimination were about 8 lux for yellow versus red, 6 lux for green versus yellow, 5 lux for blue versus green (Fig. 3, lower left photo), 3.5 lux for violet versus blue and 2.5 lux for red versus violet (Table 3).

After training under low light intensity, the ants also distinguished each colour from another one, but the mean proportions of ants doing so were lower than those observed after having trained the ants under high light intensity (Table 4). The lower light intensities required to respond correctly to the two presented colours were all smaller than those required for correct responses after training under high light intensity; again, the values decreased from red to violet. In fact, the corresponding thresholds were about 5 lux for yellow versus red, 3.5 lux for green versus yellow (Fig. 3, lower right photo), 2.5 lux for blue versus green, 2 lux for violet versus green and 1.5 lux for red versus violet (Table 4).

DISCUSSION

The present work attempts to measure the lowest light intensities (the light thresholds) that workers of *M. sabuleti* require to be able to see colours other than grey and to be able to discriminate between two colours. These thresholds are very low, and those allowing discrimination between colours and grey are lower than those required to distinguish between two colours. After training under high light intensity, the ants' thresholds are higher than those acquired after training under low light intensity. The lower thresholds observed after maintenance and training under low light intensity are in agreement with the light and dark adaptation previously revealed for the species (CAMMAERTS, 2005). In every case (colour versus grey or colour versus another colour; training under high or under low light intensity), the ants' thresholds decreased from red to violet.

Even if our experimental protocol and method of quantification may have their limitations, the conclusions remain valid since potential limitations are identical for all the experiments (use of same papers, lamps, instruments etc…) and the conclusions are qualitative and comparative in nature. Although one might criticise our experiments because of a potential bias due to ant pheromones, we believe this to be unlikely as the experimental apparatuses were often relocated in the ants' foraging areas. Consequently, olfactory cues were likely not used by foragers when making their choices between two colours.

In a keystone paper on ant vision (especially colour vision), KRETZ (1979) showed that, for a given light intensity, the visual response varies with the wavelength used, i.e. increases from longer wavelengths (red) to shorter ones (violet). To obtain a similar response for red and violet, the author would need to decrease the light intensity (from red to violet); accordingly, the ant's visual thresholds would be higher for red and lower for violet, as is in agreement with our results.

On the other hand, it was previously shown (CAMMAERTS, 2007 a) that the ability of workers of *M. sabuleti* to discriminate a colour from grey or one colour from another one was highest for blue and yellow under 10,000 lux and for violet and green under 600 lux. This observation is confirmed by the proportions of ants correctly responding during the two tests made before assessing the ants' thresholds obtained in the present work, and after having trained these ants under either 10,000 lux (Table 1) or 600 lux (Table 2). In these two mentioned tests as well as in the previous work on the subject (CAMMAERTS, 2007 a), the assessment concerned the ants' ability to discriminate between two broadband spectra. This is similar to the determination by VON HELVERSEN (1972, second part of his work) of the bee's abilities to discriminate between wavelengths. By detecting light thresholds for colour perception in ants, we estimate the spectral sensitivity of ants, similar to the measurements made by VON HELVERSEN (1972, first part of his work, Figs 6; 9; 11). Our results and those of VON HELVERSEN are in agreement: ants and bees present the best abilities for two wavelengths of the visible light and their sensitivity to wavelength increases from red to violet: i.e. with the light frequency.

Our results can also be compared to those of HORI et al. (2006) and of NEUMEYER (1981). HORI et al. (2006) trained bees with monochromatic lights associated with a reward of sucrose solution delivered to the antennae and proboscis for eliciting the proboscis extension reflex. The authors found that bees conditioned with a 540nm light stimulus also responded to a 618nm but not to a 439nm light stimulus, reacting, however, when this last stimulus

was switched off. According to the authors, this shows that the tested insects were not conditioned to increases in light intensity or temperature but effectively to colours (a fact we judge also true for our experiments) and that their results are in agreement with those of NEUMEYER (1981). This last author investigated successive colour contrast as well as colour constancy in bees by training freely flying insects to land on one of nine differently-coloured test fields. The author tested bees under various yellow and blue illuminations. In doing so, NEUMEYER (1981) revealed the bee's colour constancy and pointed out the chromatic adaptation of these insects, the most probable mechanism allowing colour constancy. In the present work, we show light adaptation for colour vision in workers of *M. sabuleti* and we obtained qualitatively identical colour discrimination under two light intensities, in favour of a colour constancy in this species.

CAMMAERTS (2005) measured light thresholds needed by workers of *M. sabuleti* to see an object. These "form thresholds" depend on the light intensity under which the ants are maintained. If maintained under 10,000 lux, the ants present a light threshold of 165 lux (an experimentally-assessed value); if maintained under 600 lux, they acquire a light threshold of 22.44 lux (a calculated value using the set up by CAMMAERTS (2005) function: $thr=11.6xe^{0.027i)}$. The present study goes beyond the perception of form and tests the ants' perception of colours other than grey as well as their distinction between two colours. The "form thresholds" (CAMMAERTS, 2005) are higher than the "grey/colour" and the "two different colours" thresholds. This suggests that, for precisely seeing form, the ants may use their eyes in apposition mode (WEHNER & GEHRING, 1999 p. 424), requiring higher light intensity. In contrast, superposition mode (WEHNER & GEHRING, 1999 p. 424) may be used to discriminate colours from grey or different colours, consequently requiring lower light intensity. In other words, superposition mode allows grey/colour distinction under very low light intensities, and colour/colour distinction under slightly higher light intensities. Under high light intensities, apposition mode allows ants to see lines and shapes (never very sharply because they fail in discriminating some lines and shapes from one another, CAMMAERTS, 2006). This succession of capabilities depending on light intensity differs from that of mammals, which perceive shapes and lines under low light intensities and distinguished colours only under rather high light intensities (WEHNER & GEHRING, 1999 p. 420). Under high light intensity, the ants' best abilities occur for yellow and blue; under low intensity, their best abilities are for green and violet (CAMMAERTS, 2007 a). Their colour perception ability shifts thus towards shorter wavelengths in response to decreasing light intensity. This allows for their colour vision to be adapted to shifting natural light conditions during the day. In other words, colour vision in ants may be qualitatively identical and quantitatively similar throughout the day.

Another, even lower light threshold might exist for ants: one in which they might see that something differs from darkness. Ants may be confronted with such a very low light threshold inside their nest, enabling them to orient themselves either towards the outside or the inside of the nest.

After having been maintained under low light intensity, the ants acquire a lower light threshold (CAMMAERTS, 2005; present paper). They thus became more sensitive to light after maintenance under low light intensity and even more so in darkness. Accordingly, the ants likely perceive being shifted from a bright environment to being placed under a red filter as being in near-darkness. On the other hand, a shift from darkness to red light would not be perceived as complete darkness. This resolves the polemic between authors about ants' sensitivity to red light and explains the results of DEPICKERE et al. (2004).

Light and dark adaptation (as conducted in the laboratory on *M. sabuleti:* CAMMAERTS, 2005 and present work) has been studied under natural conditions on *Formica polyctena* by MENZEL & KNAUT (1973). Those authors revealed two adaptations. A first adaptation occurs at sunrise while light intensity is still low and involves a major modification of pigment arrangement. A second light adaptation occurs thereafter, when light intensity increases. This cytological observation leads to the speculation that, during sunrise, the ants' eyes initially function in superposition mode and undergo some adaptation; they then change and function as apposition ones, once again undergoing some light adaptation. This interpretation agrees with our work (CAMMAERTS, 2005 and present study).

MENZEL & KNAUT (1973) studied the chromatic adaptation for several wavelengths in response to increasing light intensity, cytologically, in *F. polyctena*. This adaptation occurs in different cells and differs according to wavelength. This would explain one of our present results: thresholds for colour perception of workers of *M. sabuleti* depend on the colour and therefore on wavelength.

In one of his numerous works on bee visual perception, MENZEL (1981) demonstrated two thresholds in the detection of spectral stimuli: a lower one for the absolute detection of the stimulus and a higher one for the perception of colour hue. These observations echo those reported here for *M. sabuleti.* MENZEL (1981) termed the range lying between the two thresholds (the achromatic lower one and the chromatic higher one) the achromatic interval. The author concluded that, at light intensities near visual threshold, bees use neurally-derived achromatic signals: under such low light intensities, the output of all receptors from a single ommatidium is pooled in a neural strategy that produces achromatic signals. This deduction is also supported by the present results: workers of *M. sabuleti* use their eyes as superposition ones under light intensities nearly equalling the visual threshold values.

The capability of workers of *M. sabuleti* to distinguish colours versus grey on one hand, and different colours on the other, can be compared to the bee's ability to detect and discriminate coloured patterns (HEMPEL DE IBARRA et al., 2001; 2002). The summation of photoreceptor cell signals in bees under light intensity approaching thresholds values is corroborated by the study of VOROBYEV et al. (2001). At such low light intensities, the authors found that some experimentally-measured thresholds were lower than the theoretically-calculated ones. This is because, under very low light intensities, several photore-

ceptors 'work together' (i.e. their neural signals are pooled), yielding good visual perception, as also illustrated by the work of WARRANT et al. (1996).

Nonetheless, ants are not bees, and even if similarities with the bee visual system exist, ants present specific visual characteristics. As formulated in the review by MENZEL & BACKHAUS (1991), insects have specific adaptations in their colour vision systems, and research should concentrate on these species-specific adaptations. The present study, together with previous ones (CAMMAERTS, 2005; 2007 a), provides detailed information on the subject, supporting and expanding upon earlier knowledge.

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Appendix: experimental protocol and quantification of ant responses

Protocol of an experiment

Performed on six colonies successively:

a 4-day period of starvation and light intensity adaptation

a control (with the apparatus designed for tests)

a 6-day period of training (with the apparatus designed for training)

a first test (with the apparatus designed for tests)

a 3-day period of training (with the apparatus designed for training)

a second test (with the apparatus designed for tests)

a 1-day period of training (with the apparatus designed for training)

the assessment of the ants' threshold for the "correct" colour versus either grey or another colour by conducting successive tests (with the apparatus designed for tests) with stepwise increases in light intensities.

Quantification of ant responses

An example: threshold for **green (gr)** (the "correct" colour) *versus* yellow (ye) after adaptation and training under 600 lux.

Columns 2 to 7: mean numbers of ants present on each half-disk

Column 8: mean (=global mean) of the previous means

Column 9: proportion of "correct" responses

Column 10: results of non-parametric Wilcoxon tests applied to the mean numbers (specifically to the differences between the "correct" and the "wrong" mean numbers). N, T, P according to the nomenclature of SIEGEL & CASTELLAN (1988).

Colonies	**1**		**2**		**3**		**4**		**5**		**6**		**Mean**		**%**	**N**	**T**	**P**
Colours	Ye	**gr**	Ye	**gr**	Ye	**gr**	Ye	**gr**	Ye	**gr**	Ye	**gr**	Ye	**gr**				
Control	1.80	1.47	0.67	0.67	2.47	2.07	6.80	6.80	1.13	1.13	2.20	3.07	2.51	2.54	50			
Test 1	4.60	7.13	0.00	2.00	2.67	5.47	11.6	16.5	0.33	2.00	2.67	5.13	3.65	6.38	64	6	21	0.016
Test 2	1.40	4.67	0.33	2.67	2.00	4.93	6.40	13.1	1.00	3.33	1.26	3.93	2.07	5.44	72	6	21	0.016
Threshold assessment																		
Lux 1	2.67	5.33	2.67	5.33	6.67	8.33	5.67	4.33	3.00	2.33	3.67	4.33	4.06	4.99	55	6	15	NS
1.5	1.67	1.33	2.00	2.33	2.67	2.33	3.67	2.67	0.67	2.00	3.33	3.67	2.34	2.39	50	5	8	NS
2	2.33	6.00	1.00	1.67	5.00	3.00	3.67	2.67	1.00	1.00	2.00	1.67	2.50	2.67	52	5	- 9	NS
2.5	3.00	4.33	1.33	2.67	1.00	2.33	4.00	4.33	1.00	1.00	1.67	1.67	2.00	2.72	58	5	13	0.094
3	2.67	5.00	1.67	2.00	4.33	3.67	2.33	4.67	1.00	1.33	3.67	5.67	2.61	3.72	59	6	20	0.031
3.5	1.33	3.66	0.33	1.33	1.67	5.00	1.67	5.00	1.00	1.00	0.00	1.33	1.00	2.89	74	5	15	0.031
4	0.00	2.67	0.00	1.00	2.67	5.00	2.00	4.33	2.33	5.00	0.67	2.33	1.28	3.39	73	6	21	0.016
6	1.00	4.33	0.00	1.00	2.00	4.67	1.67	4.33	2.67	4.67	3.67	5.33	1.84	4.06	69	6	21	0.016

The ants' threshold lies between 3 and 3.5 lux.

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