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Colour vision in the ant *Myrmica sabuleti* MEINERT, 1861 (Hymenoptera: Formicidae)

Marie-Claire CAMMAERTS

Abstract

The differential operant conditioning method reveals that workers of *Myrmica sabuleti* MEINERT, 1861 distinguish colours from one another, as well as colours from greys. In high light intensity, these ants are slightly sensitive to red, sensitive to green, and highly sensitive to yellow and blue. In low light intensity, they are no longer sensitive to red, but sensitive to yellow and blue, and highly sensitive to green and violet. Moreover, these ants clearly perceive black objects under UV light: They are thus sensitive to wavelengths shorter than those corresponding to violet.

Key words: Colours, eyes, *Myrmica sabuleti*, operant conditioning, vision.

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Dr. Marie-Claire Cammaerts, Université Libre de Bruxelles, Faculté des Sciences, CP 160/11, 50 Av. F. Roosevelt, 1050, Brussels, Belgium. E-mail: mtricot@ulb.ac.be

Introduction

Behaviour depends, among other factors, on what animals perceive from their environment and how they perceive it. Ethology must take into account the parameters of this perception. In ants, the study of chemical perception has a long history (HÖLLDOBLER & WILSON 1990), whereas the visual perception has only recently been investigated (in ants with a good vision). This prompted me to undertake an ethological study of visual perception in an ant with relatively small eyes, *Myrmica sabuleti* MEINERT, 1861. After having analysed several characteristics of visual perception in workers of this ant species (CAMMAERTS 2004a), their sensitivity to light intensity (CAMMAERTS 2005), other visual abilities (CAMMAERTS 2006), and their 3D vision (CAMMAERTS 2007), I now aim to examine their ability to distinguish colours.

Colour vision, an ability that not all animal species have, is a psycho-physiological phenomenon. This means that the presence in an animal's eyes of distinct photoreceptor types sensitive to different parts of the light spectrum is insufficient to affirm its ability to discriminate colours. Behavioural experiments are required to prove so. Colour discrimination has already been studied at the physiological and ethological level, in many invertebrates and vertebrates. It is now widely accepted that many insect species can see colours. This was revealed for the first time by VON FRISCH (1914). It was subsequently demonstrated for many Odonata, Lepidoptera, Diptera, Coleoptera, and Hymenoptera (such as Apidae, Bombycidae) (AUTRUM 1981, BRISCOE & CHITTKA 2001). The evolutionary advantages of colour vision have also been demonstrated (for reviews see MENZEL 1979, KELBER & al. 2003). In ants, colour vision has been investigated in relatively few species. WEHNER & TOGGWEILER (1972) and KRETZ (1979) provided evidence for this capacity in ants. *Formica polyctena* FOERSTER, 1850 for instance has photoreceptors for wavelengths of 350 nm and 510 nm (MENZEL & KNAUT 1973), *Cataglyphis bicolor* (FABRICIUS, 1793) for 350 nm and 510 nm (MOTE & WEHNER 1980, PAUL & al. 1986) and *Myrmecia gulosa* (FABRICIUS, 1775) for 412 nm and 540 nm (LIEKE 1981). *Camponotus abdominalis* (FABRICIUS, 1804) and *Cataglyphis bombycina* (ROGER, 1859) have receptors for green and UV light (in BRISCOE & CHITTKA 2001). All these ants have large eyes. No ants with small eyes, such as the *Myrmica* species, have yet been examined. Here, I therefore apply differential operant conditioning to study the ability of *M. sabuleti* workers to discriminate different colours.

Materials and methods

Collection and maintenance of ants

Several colonies of *M. sabuleti* were collected from Höhes Martelingen (Luxembourg: 49° 49' 30" N, 5° 45' 00" E) and the Aise valley (Belgium: 49° 49' 39" N, 5° 15' 26" E). They were divided into 30 smaller colonies (five series labelled A to E of six colonies numbered 1 to 6), each containing 250 workers, a queen and brood. Each colony was maintained in an artificial nest made of one to three glass tubes half-filled with water; a cotton-plug separated the ants from the water. The glass tubes were placed in a polyethylene tray (43 × 28 × 7 cm) serving as a foraging area where food was delivered. This food consisted of sugared water presented in small glass tubes plugged with cotton and, only when no experiment was performed or planned, of pieces of dead cockroaches (Fig. 1). All colonies were kept in the laboratory at constant temperature (20 °C) and humidity. Light was supplied in one room by five, in another room by eight OSRAM concentra lamps (60 W) attached to the ceiling. The spectrum of the delivered light was measured using a grating spectrograph (an Acton Spectrapro – 500i) with CCD camera (a Princetin Instrument TEA/CCD – 1100 – PF) detection and an optical fibre pointer. The slit opening was 100 µm and the grating was 600 grooves / mm (500 nm blaze). For obtaining (in several parts) the spectrum, the pointer was simply maintained in front of an OSRAM concentra lamp. The entire spectrum, shown in Fig. 2, revealed that the illumination contained all wavelengths of visible light and none (or nearly none) of UV light. The light intensity was measured using a luxmeter, more precisely a Testoterm 0500 luxmeter built

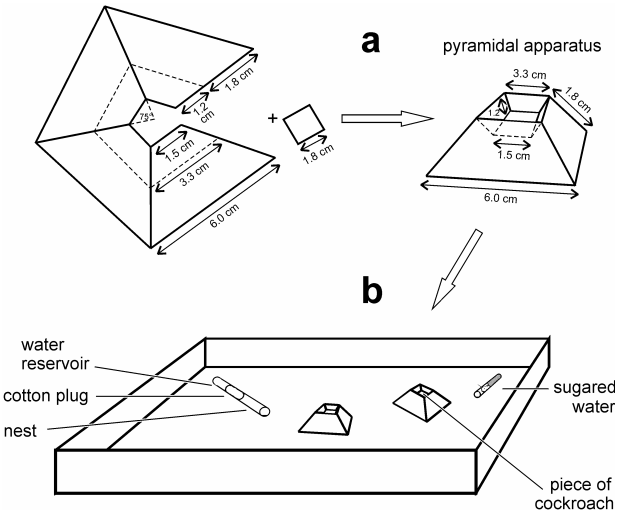

Fig. 1: (a) The experimental apparatus, constructed of thick coloured paper, used to study the ants' discrimination between two colours; (b) the experimental design: a colony, in its laboratory tray, submitted to differential operant conditioning, shown here during a training phase: two differently coloured experimental apparatus are present, one of which is provided with food in order to analyse discrimination between the two colours.

by Testoterm GmbH & Co (D-7825 Lenzkirch, Germany). Two light intensities were used for performing the experiments: 10,000 lux and 600 lux. They were obtained using a dimmer. Doing so, only the intensity of illumination and not the shape of the wavelength spectrum was changed.

Discrimination between two colours

Visual discrimination between two different colours was assessed by operant conditioning. This method has been previously established (CAMMAERTS 2004 b), then used to study the ability of ants to distinguish different numbers of an element, different full or empty shapes, as well as differently oriented cues (CAMMAERTS 2006). It has also been used to analyse *M. sabuleti* workers' sensitivity to light intensity (CAMMAERTS 2005). In the present study, the ants were trained in front of two differently coloured experimental apparatus, only one of which was provided with food. Each experimental apparatus was made out of strong paper (Canson) of the following colours: black, dark red, scarlet, orange, yellow, green, blue-green, blue, blue-violet, and violet (Fig. 2). The spectra of the light either reflected by (or transmitted through) the coloured papers used were measured using the above-described grating spectrograph (with CCD camera detection and an optical fibre pointer) by maintaining the pointer in front of every coloured apparatus, a lighted OSRAM concentra lamp being located either above or, more easily behind, the apparatus. These measurements were rather difficult because only very little light reached the fibre pointer. Nevertheless, it could be observed that the spectrum of the light reflected by or transmitted through the black apparatus did not yield a maximum, whereas that by or through the other coloured apparatus presented maxima of about 655 nm (dark red), 640 nm (scarlet), 615 nm (orange), 550 nm (yellow), 525 nm (green, the more narrow broadband spectrum), 505 nm (blue-green), 425 nm (blue), 450 nm and 375 nm (blue-violet),

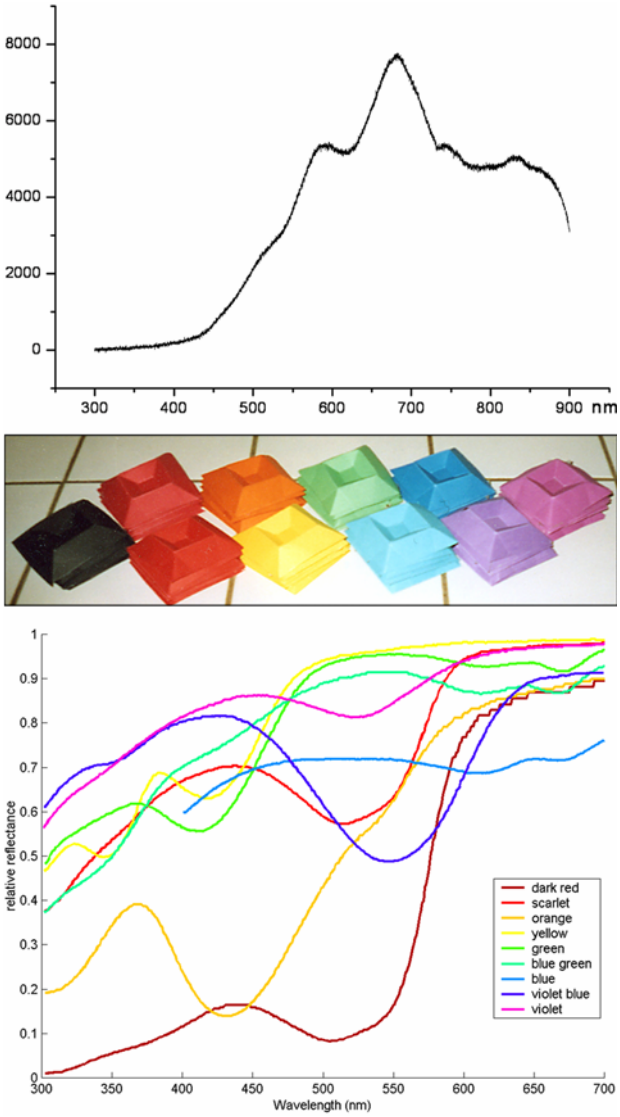

Fig. 2: Spectrum of the light source used (upper graph), coloured experimental apparatus used to study the ants' discrimination between two colours (centre) and spectra of the light absorbed by each coloured paper (lower graph). See Fig. 1 for details about the apparatus and the experimental design. See text and Appendix 1 for precision of the method (differential operant conditioning) and the quantification of responses. Information on the spectra is provided in the text.

445 nm and 375 nm (violet). For checking these measurements, pieces (2 cm × 2 cm) of each coloured paper were boiled in 10 ml of water, for 5 minutes, tingeing the water in the respective colour. It was checked that the colours of the tinged water were exactly the same as those of the corresponding boiled papers. Then, the spectra of the light absorbed by these coloured papers were obtained by Dr. Dibiani and Dr. Azarkan (Laboratory of Prof. Baeyens-Volant) using spectrophotometer. This apparatus was a Cary 50 – Varian UV-VISIBLE spectrophotometer (USA), with a range of sensitivity from 200 nm to 1,000 nm, a maximum scan rate of 24,000 per minute, a Xenon lamp and a Cary WinUV software. After that, on the basis of the spectrum of the entire delivered light (Fig. 2, above)

and of the spectra of the light absorbed by the coloured papers (here above related measurements), the spectra of the light reflected by every coloured papers were calculated using the relation L r (= t) = Lo / 10^{La} in which Lr (= t) is the reflected (or transmitted) light, Lo is the delivered light and La is the absorbed light. These calculated spectra (given in Fig. 2, below the photo of the apparatus) were in agreement with those measured with the grating spectrograph (and of which estimated maxima are given here above). Nevertheless, there were small differences because the lamp located inside of the spectrophotometer was not an OSRAM concentra one as that used while working with the spectrograph. The light intensity existing in front of each coloured apparatus was identical for all the coloured papers used and varied only similarly with the intensity of the light delivered. The latter was therefore standardised for all experiments, being adjusted to either 10,000 lux or 600 lux.

Each experimental apparatus was constructed by drawing a precise shape on the paper, cutting it, then folding it to obtain the correct volume (Fig. 1). This volume was stabilised with a square of similarly coloured paper (Fig. 1) glued inside. Nine experiments were performed (Tab. 1), each one simultaneously on six ant colonies according to the following identical protocol (Appendix 1, Protocol). The six colonies received two differently coloured experimental apparatus, being unrewarded, and a test prior to conditioning (= a control) was performed (see the last sentences of the present paragraph). Then, the apparatus were removed and replaced by identical ones. Those of a given colour were provided with a small piece of dead cockroach (placed on a piece of polyacetate). These apparatus remained on the ants' foraging area for six days, being randomly relocated 6 to 9 times during those six training days (in order to avoid the establishment of a trail and the acquisition of spatial learning). The meat (the reward) was replaced whenever necessary. After that, the training apparatus were removed from the colonies, and those (without reward) used during the test prior to conditioning (= the control) were again presented. A first test was then conducted (see the last sentences of the present paragraph). Following this, the "test" apparatus were removed and those used during the training phase were presented again: the ants' conditioning was pursued for three more days. Once again, during these three training days, the experimental apparatus were relocated 3 to 6 times and meat was replaced as necessary. Following that period, a second test was performed.

The ants' response was quantified as follows (Appendix 1, Quantification, 1). To conduct the control and the two tests, the ants located on each experimental apparatus were counted once every minute over a 15-minutes period and the mean value of the fifteen counts was calculated for each colony and each apparatus. Then, for the control and each of the two tests, the mean of the six mean values (i.e., one per colony) was calculated for each of the two differently coloured apparatus. The difference between the means obtained for the correct and incorrect apparatus was established for each colony. The six differences obtained for each of the two tests were then compared to the six corresponding differences obtained for the control with the non-parametric Wilcoxon test (SIEGEL & CASTELLAN 1989). On the other hand, for the control and both tests, the mean proportion of ants present on the "correct" apparatus (i.e., apparatus of the same colour as that provided with food during training) was determined.

Discrimination between colours and greys

Scarlet, yellow, green, blue and violet were, one by one, tested simultaneously on six colonies in the presence of white, pearl, flannel, slate, pewter and black. These labels are those given by the manufacturer Canson; they do not imply any chromatic or intensity information. Nevertheless, the spectrum of the light reflected by each grey paper types was observed using the grating spectrograph: none of them presented maximum. On the contrary, those of the light reflected by (or transmitted through) the coloured papers presented maxima around 640 nm (scarlet), 550 nm (yellow), 525 nm (green), 425 nm (blue), 445 nm and 375 nm (violet). Moreover, as in the first study (colour discrimination), the spectra absorbed by water (10 ml), in which pieces (2 cm × 2 cm) of coloured papers has been boiled for 5 minutes, were analysed by Drs. Dibiani and Azarkan with the varian spectrophotometer cary 50. Then, on the basis of the obtained spectra and of the spectrum of the entire delivered light (shown in Fig. 2), the spectra of the light reflected by every coloured papers were calculated (Fig. 2, below). These spectra were in agreement with those measured with the spectrograph. Small differences nevertheless appeared, because the lamp located inside of the spectrophotometer was not an OSRAM concentra one. As in the first study, the light intensity (measured using a luxmeter) reflected by each coloured and grey paper was similar.

For performing this study, experimental apparatus were built as follows. Firstly, one equilateral paper triangle of each grey colour was glued onto a hexagonal piece of white paper, yielding a hexagonal assemblage of six grey colours (Fig. 3). Twelve such assemblages were built to perform one experiment (i.e., to test one colour in front of the six greys): six assemblages were used to conduct a test prior to conditioning (= a control) and two tests, while six others were used to train the ants. Furthermore, for each experiment, twelve equilateral paper triangles of the colour to be discriminated from greys were produced: six triangles were used during the two tests, the six other ones during the training phases. Each experiment consisted successively of a test prior to conditioning (= a control), a 6-day training phase, a first test, a 3-day period training phase and a second test (Appendix 1, Protocol). During the test prior to conditioning (= the control), only the hexagonal assemblages with six greys were presented to the ants (Fig. 3, upper drawing). During the two tests, one coloured triangle was laid on each of these hexagonal assemblages (located on a different grey triangle for each of the six tested colonies) (Fig. 3, lower right drawing). During training, the six other assemblages with six greys were presented to the ants, together with a coloured triangle located among the six greys (Fig. 3, lower left drawing). A piece of dead cockroach was deposited on the coloured triangle and was replaced whenever necessary. The assemblages as well as the coloured triangles were randomly relocated 6 to 9 times during the first training phases and 3 to 6 times during the second training phase.

The ants' responses were assessed as follows (Appendix 1, Quantification, 2). During the control and both tests, the ants located on each grey and coloured triangle were

counted every minute over a 15-minutes period, for each colony, and the mean of each fifteen counts was established. Then, six total values were calculated, two for the control and two for each of the two tests. For the control, were summed separately the ants present on the grey covered by a colour during the tests (Nc) and the ants present on the other grey triangles (N5g). For each of the two tests, were pooled separately the ants located on the colour (Nc) and those present on the greys (N5g). The coloured area being five times smaller than the grey ones (Fig. 3), these Nc and N5g values were used as follows to quantify the ants' responses (Appendix 1, Quantification, 2). First, for the control and the two tests, the number of ants that would have been present on a triangle based on random distribution Nr = (5Nc + N5g) / 6 was calculated. Then, non-parametric 2 × 2 table contingency χ^2 tests were applied to Nc, Nr, N5g, 5Nr. On the other hand, the proportion of ants present on the colours was assessed taking into account that the coloured area was five times smaller than the grey one. A variable c = 5Nc / (5Nc + N5g) was calculated (Appendix 1, Quantification, 2).

UV light perception

The experiments were performed inside a hood containing two electric sockets, one connected to an electric incandescence lamp (Osram) and the other to a UV tube. Each socket had its own power switch located outside the hood. The fluorescent UV tube emitted some violet light at low intensity and essentially A, B and C UV light. Such tubes are commonly used for antiseptic purposes. Eye and skin protection were used during the manipulations. The six experimental colonies of series D, then of series E, were successively deposited inside the hood and submitted under UV light to the operant conditioning method previously used to study the ants' sensitivity to light intensity (CAMMAERTS 2005). In fact, the ants were conditioned, under such a UV light, to come under a black hollow cube. The experiment (made on series D as well as on series E) consisted successively of a test prior to conditioning (= a control), a 6-day period training phase, a test under UV light, a 4-hour period training phase, a test under normal light, a 3-day period training phase, a second test under UV light, a 4-hour period training phase and a second test under normal light. As previously, during the training phases, the hollow cubes were relocated several times (6 to 9 or 3 to 6 times) to avoid pheromone deposit and spatial learning, and meat was replaced whenever necessary. During the control and each test, the ants (of the six colonies) located on the correct area (i.e., under the hollow cube) were counted every minute during a 15-minutes period. The mean of the 15 counts was calculated for each colony. Non-parametric Wilcoxon tests were applied to these six mean "test" and "control" values. Moreover, for each control and test, the mean of the six means were established (Tab. 3). After that, because the results obtained using UV and normal light differed, the ants' locomotion (orientation, linear speed, angular speed) under the two lighting systems was analysed using the method explained in CAMMAERTS-TRICOT (1973). Briefly, the orientation of each recorded ant's trajectory was quantified by the mean of the angles, measured after every 0.5 cm of movement, made between the ant direction of movement and the line "ant- centre of the squared bottom of the presented hollow cube". The distributions of the obtained

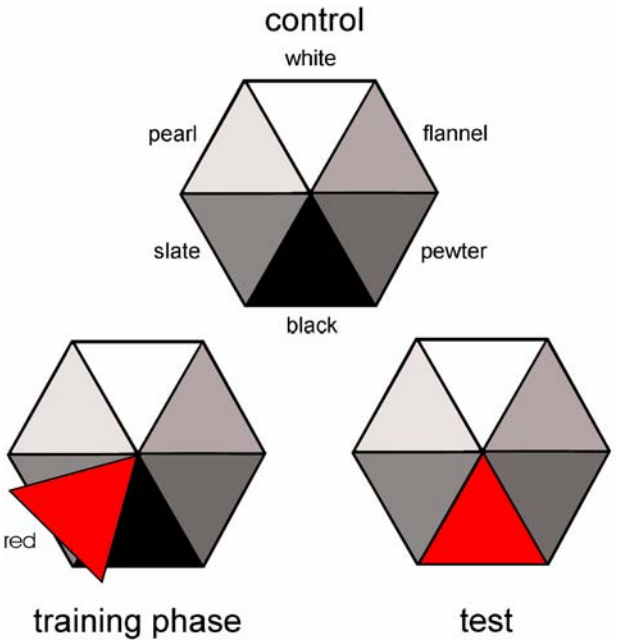

Fig. 3: Experimental apparatus used to study the ants' discrimination between colours and greys. Each experiment consisted successively of a control, a first training phase, a first test, a second training phase and a second test. For details, see text and Appendix 1.

values using UV light on one hand and normal light on the other were characterised by their median and compared to one another using the non-parametric χ^2 test. The linear speed of each ant observed was calculated on the basis of the duration of the movement and the length of the trajectory. Again, the distributions of the obtained values for UV light and for normal light were characterised by their median and compared to one another using the non-parametric χ^2 test. The sinuosity of each registered trajectory was quantified by drawing tangents at every inflexion points, summing the angles made between each tangent and the following one, and dividing the sum obtained by the length of the trajectory. Once more, the distributions of the values obtained using UV light and those obtained using normal light were characterised by their median and compared to one another using the non- parametric χ^2 test.

Results

Discrimination between two colours (Tab. 1)

Under a light intensity of 10,000 lux, the ants weakly discriminated black from dark-red ($P < 0.031$) and clearly discriminated black from scarlet ($P < 0.016$). They were able to distinguish the two colours of all the other tested combinations, that is scarlet from orange, orange from yellow, yellow from green, green from blue-green, blue-green from blue, blue from blue-violet and blue from violet ($P < 0.016$). The ants' best performances occurred in front of orange versus yellow and blue-green versus blue.

At 600 lux, the ants could no longer distinguish black from dark red, nor black from scarlet ($P > 0.05$). However, they continued to discriminate the two colours of all the other tested combinations, that is scarlet from orange, orange from yellow, yellow from green, green from blue-green, blue-green from blue, blue from blue-violet, and blue

Tab. 1: Ants' discrimination between two colours. At either 10,000 lux or 600 lux, the ants (of series A to E, each made of six colonies) were trained to find food on a coloured apparatus (bold in table) versus a differently coloured apparatus. During the control (before training), test 1 (after six training days) and test 2 (after three more training days), the ants were confronted with two identical differently coloured apparatus; the apparatus were new and not provided with food. The ants' response to the two colours was quantified as detailed in "Materials and methods" and summarized in Appendix 1. * indicates ants' best performances.

at 10,000 lux				at 600 lux		
series steps	**mean number on apparatus**		**% correct responses**	**series steps**	**mean number on apparatus**		**% correct responses**
A	Black	**dark red**		**A**	Black	**dark red**	
Control	1.92	0.77	29	Control	1.62	1.72	51
Test 1	1.55	1.32	46	Test 1	1.89	1.00	35
Test 2	1.37	1.03	43	Test 2	2.50	1.72	41
B	Black	**scarlet**		**B**	Black	**scarlet**	
Control	0.93	0.43	32	Control	1.33	0.94	41
Test 1	0.28	1.02	78	Test 1	1.44	1.22	46
Test 2	0.21	1.25	86	Test 2	1.56	1.00	39
C	Scarlet	**orange**		**C**	Scarlet	**orange**	
Control	3.76	2.84	43	Control	2.00	1.00	33
Test 1	0.85	2.08	71	Test 1	0.45	1.67	79
Test 2	0.37	1.82	83	Test 2	0.50	1.72	77
D	Orange	**yellow**		**D**	Orange	**yellow**	
Control	0.71	0.15	17	Control	1.72	1.33	44
Test 1	0.06	0.91	94 *	Test 1	0.61	2.67	81
Test 2	0.22	1.81	89	Test 2	0.89	2.33	72
E	Yellow	**green**		**E**	Yellow	**green**	
Control	0.83	0.51	38	Control	0.83	0.22	21
Test 1	0.33	1.36	80	Test 1	0.44	1.56	78 *
Test 2	0.45	1.91	81	Test 2	0.67	2.33	78
A	Green	**blue-green**		**A**	Green	**blue-green**	
Control	1.72	1.22	41	Control	2.56	0.72	22
Test 1	0.39	2.86	88	Test 1	0.67	2.61	79 *
Test 2	0.22	2.17	91	Test 2	0.66	2.17	77
B	Blue-green	**blue**		**A**	Blue-green	**blue**	
Control	0.89	0.33	27	Control	2.06	0.89	30
Test 1	0.39	3.11	89 *	Test 1	0.84	2.61	76
Test 2	0.22	2.33	91	Test 2	0.95	2.39	72
C	blue	**blue-violet**		**B**	blue	**blue-violet**	
Control	4.02	2.65	40	Control	2.17	0.67	24
Test 1	1.67	5.33	76	Test 1	0.72	2.83	80 *
Test 2	1.06	3.78	78	Test 2	0.38	2.17	85
D	blue	**violet**		**C**	blue	**violet**	
Control	2.16	0.83	28	Control	1.55	1.00	39
Test 1	1.78	3.94	69	Test 1	0.22	2.22	91 *
Test 2	1.72	4.28	71	Test 2	0.50	2.28	82

Tab. 2: Ants' discrimination between colours and greys. The ants (of 6 colonies for each experiment) were conditioned to come onto coloured triangles presented along with six grey ones. Their reactions were quantified, during a control and two tests, by their numbers on the coloured triangle (Nc, sum of 6 means; one mean of 15 counts for each colony) and on the grey ones (N5g, sum of 30 means; 5 means of 15 counts for each colony). A "random" number of ants on a triangle, resulting from a random distribution of the ants, was calculated (Nr = (Nc + N5g) / 6). The ants' response to each colour was statistically analyzed by applying non-parametric 2 × 2 table contingency χ^2 tests to Nc, N5g and Nr. These responses were also evaluated by the proportions of ants on the colours (5Nc / 5Nc + N5g). * indicates ants' best performances.

series colour	steps	number on the 5 greys	 colour	random number	χ^2	P	% on the colour
	At 10,000 lux						
A Scarlet	Control Test 1 Test 2	18.3 6.4 5.9	2.9 11.2 7.8	3.53 2.93 2.28	0.025 6.19 3.22	NS < 0.05 NS	0.44 0.90 0.87
B Yellow	Control Test 1 Test 2	6.1 2.7 1.7	1.4 8.1 12.6	1.25 1.80 2.38	0.32 5.24 11.96	NS 0.05 < 0.001	0.53 0.94 0.97 *
C Green	Control Test 1 Test 2	1.5 2.4 2.3	0.1 8.2 12.1	0.27 1.76 2.40	1.93 5.67 10.51	NS 0.05 0.001	0.25 0.94 0.96
D Blue	Control Test 1 Test 2	6.1 1.1 2.9	0.7 8.5 14.1	2.27 1.60 2.83	1.27 7.27 12.31	NS 0.05 < 0.001	0.36 0.97 * 0.96
E Violet	Control Test 1 Test 2	0.6 3.2 2.6	0.1 8.4 7.3	0.12 1.93 1.65	5.78 5.15 4.51	< 0.05 < 0.05 < 0.05	0.45 0.93 0.93
	At 600 lux						
A Scarlet	Control Test 1 Test 2	3.2 10.0 12.6	0.2 4.2 5.7	0.56 2.36 3.05	0.55 0.14 0.42	NS NS NS	0.24 0.68 0.69
B Yellow	Control Test 1 Test 2	1.6 6.3 8.3	0.3 6.5 13.1	0.31 2.13 3.56	1.40 1.95 7.20	NS NS < 0.01	0.48 0.84 0.89
C Green	Control Test 1 Test 2	2.5 2.7 7.3	0.2 9.4 14.4	0.45 2.02 3.62	0.89 6.72 9.05	NS < 0.05 < 0.01	0.29 0.95 * 0.91
D Blue	Control Test 1 Test 2	4.4 4.2 4.4	0.7 7.1 7.3	0.85 1.88 1.95	0.53 3.31 3.46	NS 0.05 0.05	0.44 0.89 0.89
E Violet	Control Test 1 Test 2	5.0 3.4 2.4	0.5 9.2 7.9	0.92 2.10 1.72	0.28 5.96 5.20	NS < 0.02 < 0.02	0.33 0.93 0.94 *

from violet. Though the responses were weaker than those obtained at 10,000 lux, the level of probability remained high ($P < 0.016$). Here, the ants exhibited their best performances in front of yellow versus green and green versus blue-green, as well as in front of blue versus blue-violet and versus violet.

Discrimination between colours and greys (Tab. 2)

At 10,000 lux, the ants distinguished each of the five colours, tested one by one, from the six greys presented simultaneously. Their best performance occurred in the presence of blue or yellow. Performance was nearly as good for green, still very good for violet, but somewhat weaker

Tab. 3: Ants' reaction under UV and normal light after conditioning under UV light. The ants (of series D and E) were conditioned under UV light to come under a hollow cube. Their reaction was then analyzed under UV and normal light by quantifying their mean number under the hollow cube (twice, after a control counting), their orientation (O, angular degrees) towards the hollow cube, their linear speed (LS, mm/s) and their angular speed (AS, angular degrees / cm) under and near the hollow cube (less than 6 cm away).

	series	**control**	**UV light**			**normal light**		
mean numbers			test 1		test 2	test 1		test 2
of ants	D	0.68	3.08		3.11	2.65		2.61
N = 90	E	0.82	4.00		4.39	3.22		2.66
ants'			O	LS	AS	O	LS	AS
locomotion	D		39.3	9.2	136	56.6	12.2	119
17 < N < 31	E		53.3	8.3	154	60.3	11.7	122

for scarlet. At 600 lux, the discrimination between colours and greys was generally weaker than at 10,000 lux, except in the presence of violet. At this lower light intensity, colour perception was best in the presence of green and violet. Discrimination of blue and yellow from the greys was good. On the contrary, such a discrimination was statistically insignificant for scarlet though there was a clear tendency of an increase choice ratio in favour of the colour. These results agree with those deduced from colour discrimination (first study).

UV perception (Tab. 3)

The ants trained under UV light were clearly conditioned. The mean numbers of ants present under the hollow cube during the tests were statistically larger than those counted during the control ($P < 0.016$). The ants therefore saw the hollow cube under UV light during the training phases and continued to see it during the two tests performed under this light. They also responded correctly ($P < 0.016$), although somewhat weaker, when tested under normal light. This difference reflects the ants' locomotion. Under UV light, they oriented themselves better towards the hollow cube (smaller values of O; $P < 0.05$) and then moved more slowly (smaller values of LS; $P < 0.05$), presenting therefore a higher angular speed (larger values of AS; $P < 0.05$) than under normal light.

Discussion

The present study analysed the visual perception of colours by workers of the ant *M. sabuleti*. These ants are able to distinguish different colours from one another and to distinguish colours from greys. At 10,000 lux, they are slightly sensitive to red, sensitive to green and violet, and very sensitive to yellow and blue. At 600 lux, they are no longer sensitive to red, yet sensitive to yellow and blue, and very sensitive to green and violet. Moreover, they can see objects under UV light. They are thus differently sensitive to the different wavelengths of visible (for humans) light and are sensitive to UV light. Their spectral sensitivity shifts towards shorter wavelengths in response to decreasing light intensity.

The ants' experimental nests, the lamps and all other elements in the laboratory remained stationary during the experiments. In contrast, the coloured objects were relocated 9 to 16 times and variously oriented during the training phases. The ants were thus unable to discriminate the coloured objects based on any characteristics (position, orientation, illumination) other than colour. Note that the ants were tested in front of coloured objects under white light (of which the spectrum was obtained), and not under coloured spots. Only colours present in the natural light spectrum were used. The spectrum of the light reflected by (or transmitted through) the coloured objects was measured with a spectrograph. The spectrum of the light absorbed by water in which each kind of paper was boiled were analysed using a spectrophotometer. Each time, the measured spectra of the light reflected by (or transmitted through) the coloured apparatus were in agreement with those calculated on the basis of the spectrum of the delivered light and of the spectra of the absorbed light. In other words, each coloured paper absorbed part of the incident light and reflected the remainder. The spectrum of the reflected light could be estimated from spectroscopic measurements. Even if some coloured papers appeared brighter than others to the human eyes, the light intensities, measured using a luxmeter, in front of the different coloured apparatus were nearly identical, varying only with the intensity of the delivered light. The same light (lamps, voltage, intensity, distance) and same paper (quality, depth, texture, covering) were used throughout the study.

The present contribution is based on ethology: electrophysiological and histochemical studies would be desirable because colour vision requires that neurons react differently to signals from different colour receptor types. This has been demonstrated in locusts (OSORIO 1986), butterflies (SWIHART 1972), and bees (KIEN & MENZEL 1977, CHITTKA & al. 1992). The mere presence of different photoreceptor types and different electrophysiological reactions, however, is insufficient to prove that an animal can discriminate colours. This ability must be demonstrated via behavioural experiments (as done by VON FRISCH 1914 for bees). This is precisely the type of behavioural experiments I report on here.

Colour vision has been extensively studied in many insects, even from an evolutionary point of view (WEHNER 1981, PAUL & al. 1986, MENZEL & BACKHAUS 1991, BRISCOE & CHITTKA 2001, OSORIO & VOROBYEV 2005). Ants are less well studied in this respect. Nevertheless, among

others, the works of WEHNER & TOGGWEILER (1972), KRETZ (1979), MOTE & WEHNER (1980), LIEKE (1981), PEITSCH & al. (1992) are to be noted.

It is commonly assumed that ants are blind to the colour red, an assumption based on early (LUBBOCK 1882, FIELDE 1904) and more recent studies. Among the latter, MARAK & WOLKEN (1965) found that *Solenopsis saevissima* (SMITH, 1855) has a spectral sensitivity ranging from below 350 nm to 650 nm, while MEYER & DOMANICO (1999) showed that *Cataglyphis bicolor* see wavelengths from 350 nm to 510 nm. Accordingly, these two species cannot see red. More recently, however, DEPICKÈRE & al. (2004) showed that aggregated workers of *Lasius niger* (LINNAEUS, 1758) dispersed when red light was switched on: the brood-tenders then re-aggregated as usual, whereas foragers only gathered in small, unstable clusters. The authors thus deduced some sensitivity of workers to red light. In doing so, they did not want to investigate if the found sensitivity to red was based on achromatic or chromatic perception of red. This led to some confusion between sensitivity to red light (which could be achromatic) and the ability to discriminate the red colour (based on chromaticity). The present paper shows that *M. sabuleti* workers are able to discriminate red at 10,000 lux but not at 600 lux. Thus, ants might be able to distinguish the red colour or red light from black or darkness when these colours are delivered at high light intensity; they might be unable to do so (i.e., to discriminate wavelengths in the red region of the spectrum) when delivered at rather low light intensity, yet may still be able to detect some light. Ants, like many other insects, can adapt to light intensity, being less sensitive under strong and more sensitive under weak light (CAMMAERTS 2005). Therefore, ants maintained under high white light intensity and then suddenly subjected to only red light may not see that red light for some time. Conversely, if maintained in darkness and then suddenly subjected to red light, they may be sensitive to that light for some time. If that red light is delivered at low light intensity, the ants may perceive some light without effectively seeing the red colour.

On the other hand, colour perception in *M. sabuleti* workers may be adapted to light intensity. *M. sabuleti* workers are differently sensitive to certain colours under different light intensities (present work) but also have an efficient light and dark adaptation (CAMMAERTS 2005). These workers may therefore exhibit a wavelength sensitivity adaptation together with their light intensity adaptation, in response to changes in light intensity. In this respect, note that light and chromatic adaptation due to pigment movement have been demonstrated by MENZEL & KNAUT (1973) in *Formica polyctena.* Different spectral sensitivities have also been observed in dark- and light-adapted *Notonecta* eyes by BENNETT & RUCK (1970).

The threshold of light intensity required by *M. sabuleti* workers to detect a black object after having been maintained for 10 days under a given light intensity has been previously assessed (CAMMAERTS 2005). At 10,000 lux, this value is 165 lux. At 600 lux, the threshold, – using the function: $thr = 11.6 \times e^{0.027\sqrt{600}}$ (CAMMAERTS 2005) – is 22,44 lux. The threshold of light intensity required by *M. sabuleti* workers to detect some light or to perceive a colour has never been assessed: the present study indicates that different thresholds or different chromatic and achromatic thresholds may exist for different wavelengths, and that these thresholds depend on the ants' adaptation to a given light intensity. Experiments are being pursued to assess *M. sabuleti* workers' (chromatic and achromatic) thresholds. Working on bees, MENZEL (1981) found a higher threshold for a wavelength of 533 nm, an intermediate one for 440 nm and a lower one for 413 nm. Bees are thus most sensitive to the shortest wavelengths. If the ongoing work on *M. sabuleti* leads to similar results, then the shift of these ants' sensitivities towards shorter wavelengths in response to lower light intensity would be explained.

CAMMAERTS (2004a) previously showed that *M. sabuleti* workers have a stereovision of at least some part of their environment. The present study demonstrates that these ants are differently sensitive to different wavelengths of visible (for humans) light and perceive UV light. Meanwhile, it has been shown that *M. sabuleti* workers probably have their kind of stereovision based on their different sensitivities to several wavelengths of visible and UV light (CAMMAERTS 2007).

The system of vision I tend to propose for *M. sabuleti,* and which I partly and briefly relate here above, would agree with the experimental results of Giurfa on honeybees (GIURFA & al. 1997).

Personal observations (stereomicroscope, 100 ×) reveal that the ommatidia of a *M. sabuleti* worker's eye are not all identical. This points to different sensitivities to light and / or to different wavelengths. Specialised eye regions may also exist. Such a non-uniform distribution of specified photoreceptors has been shown in *Lycaena heteronea* BOISDUVAL, 1852 and *L. rubida* (BEHR, 1866) (BERNARD & REMINGTON 1991), in the honey-bee (MENZEL & BACKHAUS 1991), and in the ant *Cataglyphis bicolor* (LABHART 1986). This fact has also been recently pointed out by WAKAKUWA & al. (2005) for the honeybee and has been shown in an experimental work as well as related in two reviews by Stavenga in the course of his studies on colours vision by butterflies (STAVENGA 2002a, b, STAVENGA & ARIKAWA 2006).

Among the numerous studies conducted on colour vision in insects, that of KRETZ (1979) on the ant *Cataglyphis bicolor* is the most similar to the present one: it was not only performed on an ant species, but the ants also had the choice between two colours and were thus also subject to differential operant conditioning. On the basis of my results, I had deduced a colour diagram, including an "ant-purple colour", for *M. sabuleti*. But this diagram was so similar to that published by KRETZ (1979) that I could then only underline its validity.

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Zusammenfassung

Unter Anwendung der differential-operanten Konditionierung konnte nachgewiesen werden, dass Arbeiterinnen der Ameisenart *Myrmica sabuleti* MEINERT, 1861 Farben voneinander sowie von verschiedenen Grautöne unterscheiden können. Unter hoher Lichtintensität sind diese Ameisen auf Gelb und Blau hoch empfindlich, auf Grün empfindlich und auf Rot schwach empfindlich. Bei geringer Lichtintensität sind sie gegenüber Rot nicht mehr empfindlich, jedoch Grün und Violett gegenüber hoch empfindlich sowie Gelb und Blau gegenüber empfindlich. Die Ameisen vermögen auch eindeutig, schwarzgefärbte Objekte unter UV-Strahlung zu erkennen: Sie sind daher gegenüber Wellenlängen, die kürzer als jene des UV-Lichtes sind, empfindlich.

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Appendix 1:

Experimental protocol:

Performed on 6 colonies, consisted in successively:
- a control
- a 6-day period of conditioning
- a first test
- a 3-day period of conditioning
- a second test

Quantification of the ant responses:

1. Discrimination between two colours.

The ants were presented with two differently coloured apparatus (Figs. 1, 2).

An example: scarlet (sc) versus orange (or), at 10,000 lux:

colonies	1		2		3		4		5		6		mean		statistics		
colours	sc	or	sc	or	sc	or	sc	or	sc	or	sc	or	sc	or	N	T	P
control	13.5	11.7	2.67	2.13	0.93	0.40	0.00	0.00	0.27	0.07	5.13	2.73	3.76	2.84			
test 1	0.93	5.07	0.67	2.67	0.67	1.13	0.07	0.27	0.07	0.47	2.67	2.87	0.85	2.08	6	21	0.016
test 2	0.67	2.73	0.73	2.60	0.47	1.13	0.00	0.27	0.00	0.27	0.33	3.93	0.37	1.82	6	21	0.016

Proportion of correct responses : control: 2.84 / 6.60 = 0.43 (43 %)
Test 1: 2.08 / 2.93 = 0.71 (71 %)
Test 2: 1.82 / 2.19 = 0.83 (83 %)

2. Discrimination between a colour and greys.

The ants were presented with a coloured triangle together with six differently grey-coloured ones (Fig. 3).

An example: blue (b) versus greys (g), at 10,000 lux:

colonies	1		2		3		4		5		6		sum		Nr	P	%
colours	g	b	g	b	g	b	g	b	g	b	g	b	N5g	Nc			
control	0.0	0.0	0.5	0.0	0.3	0.0	4.9	0.7	0.3	0.0	0.1	0.0	6.1	0.7	2.3	NS	0.36
test 1	0.3	1.1	0.4	1.0	0.1	0.8	0.1	3.3	0.2	1.3	0.0	1.0	1.1	8.5	1.6	< 0.05	0.97
test 2	0.0	0.5	0.3	2.0	0.0	1.0	2.6	8.5	0.0	1.8	0.0	0.3	2.9	14.1	2.8	< 0.001	0.96

Nr = number of ants present on a triangle assuming random distribution = (Nc + N5g) / 6
P = results of 2 × 2 table contingency χ^2 tests applied to Nc, N5g and Nr
% = a variable assessing the proportion of ants present on the colour, taking into account that the coloured area was 5 times smaller than the grey ones = 5 Nc / (5 Nc + N5g)