First evidence of fine colour discrimination ability in ants (Hymenoptera, Formicidae)

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SUMMARY
In the present study, we report the first evidence that ants discriminate and learn perceptually close colour stimuli. Foragers of the ant species Cataglyphis aenescens and Formica cunicularia were trained in a Y-maze choice apparatus to monochromatic light stimuli of a constant intensity associated with a food reward. Two stimuli, with a mean wavelength of 40 nm perceptual distance, were chosen from the UV (340 nm vs 380 nm) and the green (510 nm vs 550 nm) range because these species are UV–green dichromats. Foragers were trained with two conditioning paradigms [absolute conditioning (AC) and differential conditioning (DC)]. In the UV range, C. aenescens foragers failed to discriminate when presented with a small colour difference in both training procedures. Foragers also failed in the green range when trained with AC but showed significant bias towards the rewarded stimulus when trained with DC. Formica cunicularia foragers achieved the task in the UV range when trained with DC only. In the green range, F. cunicularia foragers showed clear preference for the rewarded stimulus in both training conditioning procedures. Foragers never failed in choosing the rewarded stimulus in DC even when the intensity of the rewarded stimulus was reduced by one log unit. This clearly indicates that DC is of paramount importance to discriminate perceptually close colour stimuli.

Key words: Cataglyphis aenescens, Formica cunicularia, dichromacy, fine colour discrimination, absolute conditioning, differential conditioning.

INTRODUCTION
Fine colour discrimination is defined as the ability of an organism to discriminate between perceptually close colour stimuli. In visible spectrum, wavelength discrimination ability of a person with normal colour vision can be as good as 1 nm (Kruy and Ladunga, 2001). In insects, much of our knowledge on fine colour discrimination comes from the studies performed with the honeybees, bumblebees and moths. Backhaus and Menzel reported that fine colour discrimination ability of honeybees was as good as 5 nm (Backhaus and Menzel, 1987). Vorobyev and Menzel proposed a theoretical analysis showing that a bee’s ability to discriminate similar flowers was fine enough (Vorobyev and Menzel, 1999). However, bees were reported to generalise to similar colours when colour distance was about 0.1 hexagon units (Chittka et al., 1997), and flower constancy was only established when colour distances between stimuli were noticeably different, i.e. greater than 0.2 hexagon units (Chittka et al., 2001). They indicated the importance of simultaneous viewing condition over successive condition in achieving the task correctly, and emphasised that bees were actually faced with the successive condition in nature. Furthermore, Dyer and Chittka reported that errors increased at smaller colour distances when bumblebees were trained to discriminate similar colours (Dyer and Chittka, 2004a; Dyer and Chittka, 2004b; Dyer and Chittka, 2004c). They demonstrated that bumblebees either spent more time (Dyer and Chittka, 2004c) or utilised other cues, such as differences in flower shape (Dyer and Chittka, 2004a), to overcome this problem.

Kelber and Henique stated that hummingbird hawkmoths Macroglossum stellatarum were able to discriminate a difference of 20 nm in UV (360 nm vs 380 nm) and 30 nm between blue and blue-greenish range (470 nm vs 500 nm) (Kelber and Henique, 1999). This moth can also learn to discriminate a difference of 30 nm between the light of 440 nm and 470 nm, which are both in the innately preferred region (Kelber, 2005). Furthermore, recent convincing demonstrations have indicated that training conditions have a great influence on an experimental subject’s ability for discriminating between perceptually close colour stimuli. Differential conditioning seems essential for the task, at least, in honeybees (Giurfa, 2004) and bumblebees (Dyer and Chittka, 2004b; Dyer and Chittka, 2004c). The literature reveals no proper data on fine colour discrimination ability of ants. Very close relatives of the honeybees, the ants, may also have similar sensory capability.

MATERIALS AND METHODS
The ants
Two ant species Formica cunicularia Latreille 1798 and Cataglyphis aenescens (Nylander 1849) were chosen. They live and forage in different habitats; the former mainly in meadows and the latter in semi-desert open areas. Live specimens of F. cunicularia and C. aenescens were obtained from nests in Sazlidere village (41°36’0”/26°40’59”) of Edirne, Turkey. Several large colonies were transferred in open topped containers to a laboratory. Collected colonies of each species were divided into four portions and then transferred to arenas (600 mm × 600 mm) with ‘fluon’ coated Perspex® walls (200 mm high) in which the ants constructed their nests with their original nest material (Fig. 1). They were maintained in a laboratory at 29°C, with uniform artificial illumination and a 12h:12h light:dark regime. A humidifier (Vapac microVap VP-4, Edenbridge, Kent, UK) was used to provide a relative humidity of 50%.

Experimental apparatus
Experiments were performed using a Y-shaped, 30 mm diameter glass maze with the two arms at 120 deg. (Fig. 1). The base of the Y (500 mm long) was connected horizontally to the nest via a hole in the wall of the arena at floor level. Each arm of the ‘Y’ extended horizontally for 200 mm and terminated in a clear Perspex® feeding
box (100 mm width × 150 mm high) from which escape was prevented with a fluon barrier. This arrangement permitted foragers to explore boxes and return to the nest. The foragers had to walk along the floor of the Y-maze towards the decision point, which allowed them to see both spectral stimuli at the same time. The spectral stimuli delivered to the Y-maze was produced by light boxes attached to the backside of the feeding boxes. The light box contained a halogen lamp (Philips Focusline 24 V-250 W, Eindhoven, The Netherlands) and had a built-in ventilator to remove the heat produced by the lamps. Interference bandpass filters with a high stability and very low ripple. Light intensity was measured with a calibrated spectroradiometer (International Light, model # RPS 900, Newburyport, MA, USA). During training and tests, all stimuli were adjusted to have equal physical intensities (I=1.1×10^{11} photons, Fig. 2) at the decision point because absolute spectral sensitivities of the receptors of an experimental subject are not known (see Kelber and Henique, 1999; Kelber et al., 2003a). Absorptive neutral density filter (Thorlabs Inc., ND=1.0) was used to reduce the intensities of the stimuli by 1 log unit. Because all tests were performed in darkness, a digital video camera (Sony TRV520E, Tokyo, Japan) with a nightshot vision was used to monitor the ants.

**Elimination of cues**

Training and tests were performed in darkness to eliminate the use of possible visual cues. This also made the ants positively phototactic. Because ants are known to obtain directional information from magnetic field lines (Camplitepe and Stradling, 1995; Camplitepe et al., 2005; Riveros and Srygley, 2008) and idiothetic cues (Cosens and Toussaint, 1985; Aksoy and Camplitepe, 2005), monochromatic test stimuli were interchanged after every fifteenth forager. Prior to each test, food was removed and the feeding boxes were replaced with clean empty ones. In addition, during tests, a clean Y-maze was fitted after each fifth forager in order to eliminate any kind of possible chemical cues. The experimental set-up was placed on a wooden support and levelled to preclude the use of gravitational cues. On entering the maze, therefore, foragers were denied any point of reference on which to orient.

**Training**

Our preliminary results (Y.C. and V.A., unpublished; V.A. and Y.C., unpublished) have indicated that both species are UV–green dichromats. Therefore, foragers were trained for two weeks with either the absolute conditioning (AC) or differential conditioning (DC) procedure within the Y-maze to monochromatic light stimuli at UV (AC: 340 nm vs darkness, DC: 340 nm vs 380 nm) and green (AC: 510 nm vs darkness, DC: 510 nm vs 550 nm) ranges with a colour distance of 40 nm. The shorter of the wavelengths was always used as the food rewarded one. A small quantity of diluted honey and occasionally field-collected dead insects were placed in the rewarded feeding box to encourage foragers to visit it, while the unrewarded one was left empty. The Y-maze was left undisturbed for seven days, during which time the foragers accustomed to orienting towards the food source associated with both the light stimulus and chemical cue they deposited inside the maze. For the ensuing seven days the maze was removed, washed internally with ethanol and replaced 10 times a day in order to reinforce the memory of spectral colours of the foragers. Fresh feeding boxes, with a food source in the rewarding one, were fitted each day. Each nest was designed to contain approximately 200 foragers to make sure that they all had access to spectral stimuli during training. Training procedure was also resumed between the test periods to maintain...
foraging traffic and provide reinforcement for the directional information from spectral stimuli.

**Tests**

Three types of tests were performed, a control and two critical tests. In the control test, each one of the training groups was tested with their respective training conditions [340 nm vs darkness (AC) and 340 nm vs 380 nm (DC); 510 nm vs darkness (AC) and 510 nm vs 550 nm (DC)]. In critical test I, foragers of each training group was tested with the other group’s training conditions, i.e. ants trained in AC were tested with DC and vice versa. In critical test II, foragers were tested to discriminate the rewarded training wavelength from another one that was perceptually distant, i.e. 340 nm vs 550 nm and 510 nm vs 370 nm [for more information, see Giurfa (Giurfa, 2004)].

Each trial started when a forager entered the maze and continued until she entered one of the feeding boxes. Foragers spending more than 2 min inside the maze without any choice were not included in the analysis. Their possible interpretation as social facilitation is eliminated by the observation that each forager entered and traversed the maze alone. A forager found in either box was recorded, and then gently removed by a paintbrush. Removed foragers were kept in a moist box until the test was completed and they were then returned to the nest. Foragers found in the boxes were accepted as experienced because the total number of ants in the nest was small enough to visit both boxes during the period of the study. To be statistically robust, 30 individuals were used for each test. The distribution of foragers was analysed using G-test.

**RESULTS**

**Fine colour discrimination in UV range**

**Absolute conditioning**

Control results indicated that both *C. aenescens* and *F. cunicularia* foragers showed significant preference for rewarded stimulus of 340 nm than unrewarded darkness (Fig. 3A,D, respectively). However, in critical test I, foragers of both species failed to discriminate the rewarded stimulus and distributed randomly (Fig. 3B,E, respectively). In critical test II, *C. aenescens* foragers discriminated the rewarded stimulus from a novel, unrewarded distant one (Fig. 3C) whereas *F. cunicularia* foragers failed to do so (Fig. 3F).

**Differential conditioning**

In the control test, *C. aenescens* foragers failed by choosing unrewarded 380 nm instead of rewarded 340 nm (Fig. 3G). By contrast, *F. cunicularia* foragers showed significant bias for the rewarded stimulus, indicating a clear discrimination between two

![Graph showing choice frequencies of *Cataglyphis aenescens* and *Formica cunicularia* foragers in the UV range](image)

**Fig. 3.** Choice frequencies of *Cataglyphis aenescens* and *Formica cunicularia* foragers in the UV range; trained with absolute conditioning (A–F) and differential conditioning (G–M). (A) Control test, G=18.02, P<0.001. (B) Critical test I, G=6.79, P<0.05, n.s. (C) Critical test II, G=6.79, P<0.01. (D) Control test, G=4.93, P<0.05. (E) Critical test I, G=11.56, P<0.001. (F) Control test, G=8.99, P<0.005. (K) In a subsequent test where the intensity of the rewarded wavelength was reduced by 1 log unit (I/10), G=0.13, P<0.05, n.s. (L) Control test I, G=18.02, P<0.001. (M) Critical test I, G=0.13, P<0.05, n.s.
close colour stimuli (Fig. 3J). In a subsequent test where the intensity of the rewarded wavelength was reduced by 1 log unit, however, their correct choices were not observed anymore (Fig. 3K). In critical test I, foragers of both species significantly discriminated rewarded stimuli from darkness (Fig. 3H,L, respectively). In critical test II, *C. aenescens* foragers discriminated rewarded stimuli from a novel, unrewarded distant one (Fig. 3I) whereas *F. cunicularia* foragers distributed randomly (Fig. 3M).

**Fine colour discrimination in green range**

**Absolute conditioning**

In control tests, both *C. aenescens* and *F. cunicularia* foragers significantly discriminated rewarded stimuli from darkness (Fig. 4A,D, respectively). In critical test I, *C. aenescens* foragers distributed randomly whereas foragers of *F. cunicularia* showed a clear preference for rewarded 510 nm than unrewarded 550 nm (Fig. 4B,E, respectively). In critical test II, foragers of both species discriminated rewarded stimuli from novel, unrewarded distant ones (Fig. 4C,F, respectively).

**Differential conditioning**

Control test results demonstrated that foragers of both species learned to discriminate close colour stimuli, i.e. rewarded 510 nm from unrewarded 550 nm (Fig. 4G,K, respectively). In a subsequent test where the intensity of the rewarded wavelength was reduced by 1 log unit, *F. cunicularia* foragers still kept on choosing the rewarded one (Fig. 4L). By contrast, the result of the similar test performed with *C. aenescens* foragers indicated a significant failure (Fig. 4H). In critical test I, foragers of both species significantly preferred the rewarded stimulus compared with unrewarded darkness (Fig. 4I,M, respectively). Results of critical test II shown that foragers of both species discriminated rewarded stimuli from novel, unrewarded distant ones (Fig. 4J,N, respectively).

**DISCUSSION**

The present study, for the first time, has demonstrated that ants of *C. aenescens* and *F. cunicularia* foragers learn to discriminate perceptually small colour distances.

In general, *F. cunicularia* foragers were clearly more successful, particularly in the green range. Interestingly, they achieved the task in both conditioning procedures in the green range. Remarkably, they still kept on choosing the correct arm in DC even though the intensity of rewarded stimuli was reduced by 1 log unit. Besides, when green was presented as a novel stimulus in critical tests II of the UV range, *F. cunicularia* foragers were confused and failed to choose the training wavelength of 340 nm. This is also very interesting because *F. cunicularia* foragers can make a good fine discrimination in the UV range with DC. Consequently, for *F. cunicularia*, these findings indicate the importance and dominancy of green channel over UV. However, foragers of *C. aenescens* achieved the task only in green
with DC. They failed in UV with both conditioning procedures, indicating a failure of fine colour discrimination in UV, although they can easily detect and discriminate UV from other ranges of the spectrum. Such contradictions can be explained by the fact that these two ant species use different orientation mechanisms and different strategies to find the location of a goal, and that the mechanism and strategy they use depend on their sensory abilities and the conditions imposed by the environment. For desert ants of Cataglyphis species, compass-orientation based on celestial cues [e.g. the sun, polarised skylight (for review, see Wehner, 1984)] is of paramount importance because they live and forage on sandy habitats with almost no conspicuous landmarks. Therefore, for them, the presence or absence of UV for orientational purposes might be more important than the utilisation of UV for object detection. Formica cunicularia live and forage in a green rich environment; hence, sensory abilities might be adapted to an improvement of fine colour discrimination in the green range of the spectrum in order to detect prey, aphids and extraloral nectarines on foliage more easily and accurately. Menzel reported that green receptors were found in higher numbers compared with UV (6:2) in wood ants of Formica polyctena foragers (Menzel, 1973). Although we found that F. cunicularia foragers have more ommatidia (ca. 1000) than C. aenesecens foragers (ca. 350) more data, especially on fine ommatidial structure, are needed to make better comparison.

Better performance in green is not surprising because reflected light from objects is mainly composed of long wavelengths (Pichaud et al., 1999). Green receptors, found in six in all ommatidia of honeybees, were reported to play significant roles in different visual performances, such as motion-related tasks, perception of achromatic cues and detection of objects in short-range orientation (Lehrer, 1994; Giurfa et al., 1997). Regarding colour constancy in bumblebees, Dyer and Chittka (Dyer and Chittka, 2004a) reported that correction is poorer for flowers that predominantly reflect shorter wavelengths, which was a result of the asymmetrical spectral shape of bee photoreceptor sensitivity functions (Dyer, 1999). He predicted that this could be the reason for the rarity of UV-reflected flowers in nature. Indeed, blue-green flowers were reported to be more common in nature (Chittka et al., 1994), whereas UV-reflecting white flowers were rare (Dyer, 1996; Kevan et al., 1996).

Failure or poor performance in UV fine colour discrimination could be explained with some features of this channel. Firstly, celestial light sources contain high UV whereas light reflected from objects contain almost none. Therefore, for an insect, a high UV content would be interpreted as an open space to escape whereas lower UV would be interpreted as the habitat or a rich food zone [for more information, see Pichaud et al. (Pichaud et al., 1999)]. Secondly, the degree of polarisation, a prominent cue for homeward orientation in desert ants (for a review, see Wehner, 1984), is least affected by atmospheric disturbances in UV range (Barta and Horvath, 2004). Thirdly, UV–green colour contrast mechanism provides the best contrast mechanism for landmark orientation between foliage as foreground and the sky as background (Möller, 2004). Therefore, the most sensitive UV receptors together with green ones are located dorsally looking upwards in the eyes on the dorsal area of the desert ant C. bicolor (Labhart, 1986).

The results also indicate that the training conditions influence the choice success of foragers. Giurfa indicates an experimental subject’s advantage of learning the features of rewarded and unrewarded stimuli simultaneously in DC (Giurfa, 2004). However, although the present work on ants and several other studies on bees (Giurfa, 2004; Dyer and Chittka, 2004b; Dyer and Chittka, 2004c) showed that DC provided a better discrimination performance compared with AC, the results of a more recent work (Niggebrügge et al., 2009) reported no evidence of fine colour discrimination in restrained honeybees with neither DC nor AC.

What might be the benefit of fine colour discrimination for foragers in nature? Could it give foragers extra advantages for distinguishing beneficial food sources faster and more accurately? When bees are in question, it may be plausible to make such a connection owing to a mutual feeding/pollination relationship they share with flowers. Indeed, some works showed that many plants have flower colours that suit the visual system of bees (Chittka and Menzel, 1992; Chittka, 1996). A plausible adaptive explanation for fine colour discrimination in ants is hard to put forward. However, although pollination by ants appears to be rare (Peakall et al., 1991), mutualistic ant–plant interactions mediated by extraloral nectarines are common (for more information, see Chamberlain and Holland (Chamberlain and Holland, 2008)). Our observations on these two ant species in the field have confirmed that both foragers, particularly F. cunicularia, visit such nectar-secreting plants to feed. Foragers could use such an ability to detect fresh and healthy foliage or prey and aphids on plants.

Future experiments will elucidate how fine colour discrimination integrates into the overall orientational capabilities of ant species both in terms of their behaviour, ecology and evolution. In this context, one might not be surprised to find that evolution has been opportunistic in exploiting a wide range of available environmental sources of useful orientation information.

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