

Correspondences

Nocturnal bees learn landmark colours in starlight

Hema Somanathan^{1,2,*}, Renee Maria Borges², Eric James Warrant¹ and Almut Kelber¹

Honeybees, like humans and most other vertebrates, are colour-blind in dim light. Bees are primarily day-active and have apposition compound eyes, the typical eye design of diurnal insects. Most bees are trichromats with photoreceptors sensitive in the UV, blue and green [1]. While their diurnal colour vision was established almost 100 years ago, honeybees are known to be colour-blind in moonlight [2]. Here, we present the first evidence that the only known obligately nocturnal bee,

the Indian carpenter bee *Xylocopa tranquebarica* (Fabricius), which flies even on moonless nights [3], uses colour vision to discriminate artificial landmarks at the nest in starlight. Humans, in contrast, are colour-blind at half-moon illumination. This finding, obtained using natural nests under natural illumination, is remarkable because insensitive apposition eyes were thought unable to support nocturnal colour vision. Hitherto, nocturnal colour vision was known only in nocturnal hawkmoths [4] and geckos [5], animals with eyes well adapted to nocturnality.

Outdoor experiments were conducted at natural nests within Bhimashankar Wildlife Sanctuary, Maharashtra State, in the Western Ghats of India (see [3]), between December 2007 and March 2008. Experiments were performed each night when the bees started flying, approximately half an hour after sunset (which was between 18:00 and 19:00 hours). Experiments usually continued until 03:00 the

following morning. Bees exiting and returning to the nest were observed using infrared-sensitive night-vision equipment and recorded on an infrared-sensitive Sony camcorder (TRV130E). At two nests, *X. tranquebarica* were trained to find their nest entrance centred behind a yellow square landmark on a large plywood wall (see Supplemental Figure S1 in the Supplemental Data available on-line with this issue). Each nest housed between one and four foragers at any given time. They were tested with four additional colour landmarks: Test 1: brighter and darker shades of yellow (Y1, Y2) and two shades of grey (Gr1, Gr2); Test 2: two shades of green (Ge1, Ge2) and the same two shades of grey (Figures 1A–D). The colours are named as they appear to the human eye; for example, the greys we chose look grey to humans but do not reflect ultraviolet (see Supplemental Figure S2 for spectral reflectances of all colours). Landmark positions were changed pseudo-randomly

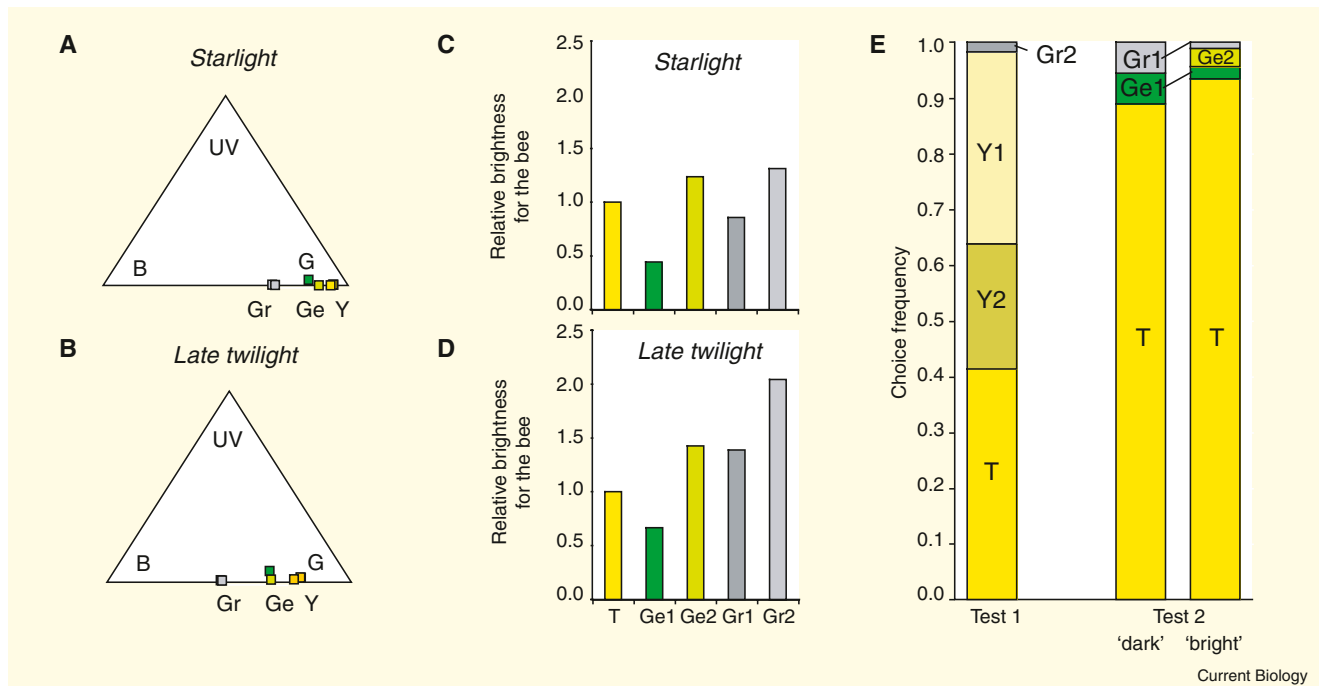


Figure 1. The stimuli used in Test 2 (T, Ge 1, Ge2, Gr1, Gr2) and colour choices by *X. tranquebarica* in outdoor experiments at natural nests. (A,B) Colour triangles illustrate bee colour space, with UV, B and G representing colours that solely excite the UV, blue or green receptors, respectively. We used honeybee photoreceptor spectral absorption curves for modelling, assuming that the photoreceptor spectral sensitivities of *X. tranquebarica* are similar to those of diurnal bees (see [1]). Stimulus colour loci shift between starlight and late twilight but the relative positions of colours remain constant. For details of methods see [6]. (C,D) Quantum catches ('brightness') for bee green photoreceptors looking at stimulus colours under twilight and starlight (see [6]). The quantum catch of the training colour (T) was set to 1. T is brighter than Gr1 in twilight, but darker in starlight. (E) Relative choice frequencies for colours in Test 1 (left) and Test 2 in dimmer (<10⁻³ cd m⁻², n = 54, G = 119.51, P < 0.0001; middle) and brighter light levels (>10⁻³ cd m⁻², n = 91, G = 232.06, P < 0.0001; right) are indicated by the lengths of coloured regions in each bar. In Test 1, bees chose all three yellow shades (T, Y1, Y2) but rarely grey (Gr1, Gr2) or green (Ge1, Ge2) in any test.

following nest exits. Every 2 to 3 days, nest position was changed in the horizontal plane on a 4 m long platform to make positional cues unreliable. The choice criterion for a returning bee was defined as the first colour landmark that the bee hovered in front of, or landed on. Thus, if the bee chose the training colour landmark, the response was considered correct. Data from all bees were pooled, and G-tests were used to determine whether choice distributions differed significantly from chance. With fewer than 20 choices, binomial tests were used.

In Test 1, bees chose darker and brighter yellows (Y1, Y2) as often as the training yellow (T) showing that they neglected intensity-related cues, while grey was chosen only once ($n = 58$, $G = 49.96$, $P > 0.001$; **Figure 1E**). In Test 2, bees discriminated the training yellow from both green (Ge1, Ge2) and grey (Gr1, Gr2; $n = 145$, $G = 350.6$, $P < 0.0001$; **Figure 1E**). Even when Test 2 was performed under the darkest conditions with the sun more than 12° below the horizon, and less than 10% of the moon visible (luminance of white stimuli between 10^{-3} and 10^{-5} cd m⁻²), the bees chose the training yellow (T) (**Figure 1E**, 'dark'). Bees from a third nest trained to a grey landmark, and tested with four yellow and green shades, chose grey in 29 of 31 trials ($G = 79.40$, $P < 0.0001$). When the training grey was substituted with a darker grey (Gr3 in Supplemental Figure S2), bees always chose the correct colour ($n = 8$, binomial test, $P < 0.01$).

Thus, the bees discriminated the landmarks using colour rather than intensity-related ('brightness') cues even at light intensities much lower than the human colour vision threshold. This is the first evidence of nocturnal colour vision in an animal with apposition compound eyes and the first demonstration of nocturnal colour vision in an animal's natural environment. *X. tranquebarica* is an active nocturnal forager on flowers that vary significantly in colour, and may rely on colour vision to distinguish them. Colour vision is useful for reliable object discrimination at night because crepuscular shifts in illumination colour [6] make 'brightness' unreliable: yellow (T) is brighter than grey (Gr1) in starlight but darker in twilight. The colours,

however, remain the same (**Figures 1A, B** and Supplemental Figure S2). Colour vision in starlight by bees with apposition eyes was unexpected — in dim light, diurnal bees apparently pool signals from the different spectral classes of photoreceptors to sacrifice colour vision in favour of an improved signal-to-noise ratio [2]. Exactly how nocturnal colour vision is achieved in apposition eyes remains to be elucidated experimentally, but increasing experimental and theoretical evidence suggests that neural summation mechanisms are likely to be necessary [7].

Supplemental Data

Supplemental data are available at [http://www.current-biology.com/supplemental/S0960-9822\(08\)01100-7](http://www.current-biology.com/supplemental/S0960-9822(08)01100-7).

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¹Cell and Organism Biology, Lund University, Helgonavägen 3, S-22362 Lund, Sweden.

²Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India.

*E-mail: hema.somanathan@cob.lu.se

Female presence is required for male sexual maturity in the nematode *Steinernema longicaudum*

Lemma Ebssa, Ilona Dix and Christine T. Griffin*

Although cheap compared to eggs, sperm are still costly to produce and may deteriorate if stored [1,2]. Therefore, selection should favour the ability of males to adjust the quantity or quality of sperm in response to fertilisation opportunities. The males of some species of insect, for instance, may adjust testis size, sperm number or ejaculate composition in response to the social environment [3]. Males of certain rodents, fish and insects increase sperm production in response to female presence [4–6]. However, males typically have at least some sperm always ready. Here, we show that in the insect-killing nematode *Steinernema longicaudum*, males that develop alone contain no sperm. Only after several hours with a female, but not another male, sperm are produced so that progeny can be sired.

We observed that male *Steinernema longicaudum* dissected from insect cadavers with single-worm infections contained no sperm in their seminal vesicles, while males that developed in insects containing many worms of both sexes all had seminal vesicles filled with sperm. This suggested that males require social experience in order to mature sexually. In a series of experiments carried out *in vitro*, in drops of insect blood, we investigated male sexual maturation (Supplemental data). Males that develop alone *in vitro*, as in insects, contained no sperm, but if they were subsequently paired with a female they contained sperm in the proximal region of the testis and in the seminal vesicle, and had a generally wider reproductive tract than naïve males (**Figure 1** and Supplemental data). When two males of *S. longicaudum* were incubated together for 24 hours, none of the 37 males examined contained sperm, showing that it is specifically female presence that stimulates sexual maturation, rather