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# RESEARCH ARTICLE

# The yellow specialist: dronefly *Eristalis tenax* prefers different yellow colours for landing and proboscis extension

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## ABSTRACT

Droneflies, imagoes of the hoverfly *Eristalis tenax*, are known to possess a preference for yellow flowers, i.e. they prefer to visit yellow flowers and prefer to extend the proboscis to yellow colours. In this study we disentangle these colour preferences by investigating the landing reaction and proboscis reflex with particular reference to intensity, spectral purity and dominant wavelength of colour stimuli and their UV reflection properties. In multiple-choice tests, naïve and non-trained flies prefer to land on yellow colours independent of their UV reflection characteristics, but also accept blue, white and pink colours if they absorb UV and are of sufficient brightness. Flies trained to land on colours other than yellow still prefer yellow colours to some extent. Moreover, the flies prefer bright over dark yellow colours even if trained to dark yellow ones. The flies refuse to land on dark colours of all hues. Naïve flies exhibit the proboscis reflex only to pure yellow pollen. These experiments show for the first time that landing in droneflies is triggered by a yellow colour independent of its UV reflection properties, but proboscis extension is triggered by yellow colours strongly absorbing blue and UV. The ability to discriminate colours is better than predicted by the categorical colour vision model. The colour preferences in *E. tenax* represent a fine-tuned ability to visit yellow flowers displaying a UV bull's-eye pattern.

KEY WORDS: Syrphidae, Hoverfly, Colour preference, Yellow colour, Flower colour, Colour pattern

## INTRODUCTION

Visual cues and signals are important for flies to find food sources, hosts and mating partners (Kelber et al., 2003; Woodcock et al., 2014). The compound eyes of flies are exceptional among insects (Lunau, 2014; Stavenga et al., 2017). Like other insects, the flies' compound eyes are composed of several hundreds to several thousands of ommatidia, resulting in a spatial resolution comparable to that of bees (Sukontason et al., 2008). However, the anatomy of the ommatidia and the neuronal processing of photoreceptor input in a fly's compound eye are outstanding due to a unique neural superposition subsystem and a tetravariant colour vision subsystem, as outlined below. Each ommatidium contains eight photoreceptor cells, the six outer retinula cells, R1–R6, which encircle the two inner retinula cells, R7 and R8, which are arranged as a tandem pair. The retinula cells R1–R6 are sensitive to a broad range of wavelengths ranging from ultraviolet (UV) to green (Lunau, 2014). The pale type of ommatidia consists of a UV photoreceptor (R7p) and a blue photoreceptor (R8p), whereas the yellow type of ommatidia consists of a UV photoreceptor with an additional sensitivity in the blue range of wavelengths (R7y) and a green photoreceptor (R8y) as confirmed by electrophysiological studies with *Musca domestica* (Hardie and Kirschfeld, 1983), *Calliphora erythrocephala* (Hardie, 1985), *Eristalis tenax* (Bishop, 1974; Horridge et al., 1975; Tsukahara and Horridge, 1977a,c) and *Drosophila melanogaster* (Stark et al., 1979; Yamaguchi et al., 2010). Functionally, the compound eyes of flies constitute two different visual systems that are processed in parallel (Strausfeld and Lee, 1991). R1–R6 retinula cells build an achromatic (colourblind) high-acuity neural superposition system for motion detection (Yamaguchi et al., 2008; Zhou et al., 2012). The broad sensitivity range of the R1–R6 retinula cells is caused by green-sensitive photoreceptors that are coupled with UV-sensitive antennal pigments (Lunau, 2014). The relatively narrow and partially overlapping spectral sensitivities of the four types of R7 and R8 photoreceptors build a tetrachromatic colour vision system that mediates chromatic vision and colour discrimination. The outcome of colour discrimination experiments with *Lucilia* flies, prompted Troje (1993) to propose a categorical colour vision model for flies, in which two opponent mechanisms are based on the relative excitation of the two pale-type and the two yellow-type photoreceptors. The categorical colour vision model assumes only four colour categories based on the difference in excitation of the R7 and R8 receptors in the pale- and yellow-type photoreceptors (see below). According to this model, all colour stimuli with colour loci of the same category are considered to be indistinguishable to flies. However, Troje (1993) admitted that there might be more than four colour categories, but experimental evidence was lacking. The fly colour vision model of Troje (1993) has been successfully used in recent ecological studies (Arnold et al., 2009; Bergamo et al., 2018; Gray et al., 2018).

Flower-visiting insects are known to exhibit innate colour preferences, including those for distinct colour hues, colour saturation and colour contrast (Lunau and Maier, 1995), whereas preferences for distinct brightness of colours seem to play a less important role (Chittka and Menzel, 1992; but see Kelber, 2005; Koshitaka et al., 2011). Most studies on innate colour preferences in pollinating insects focus on bees (Lunau et al., 1996; Hempel de Ibarra et al., 2000) and lepidopterans (Weiss, 1997; Goyret et al., 2008), whereas studies on colour preferences in flies are sparse, even though flies are considered to be important pollinators (Arnold et al., 2009; Shrestha et al., 2016; Tiusanen et al., 2016; Lefebvre et al., 2018).

Field studies found that many flies predominantly visit flowers of distinct colour hues (Kugler, 1950; Kay, 1976; de Buck, 1990; Proctor et al., 1996; Goldblatt et al., 2001; Kastinger and Weber, 2001; Manning and Goldblatt, 1997; Willmer, 2011) or are caught

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in pan traps of distinct colour hues (Ssymank and Krause, 2007; Vrdoljak and Samways, 2012; Lunau, 2014; Lucas et al., 2017). Colour preference tests under controlled conditions using artificial flowers have been done with the hoverfly *E. tenax*. Findings include an unspecified preference for yellow in the landing of experienced droneflies (Ilse, 1949) and an innate proboscis reflex to yellow, UV- and blue-absorbing, colours (Lunau and Wacht, 1994, 1997). Recently, the inability of conditioning the proboscis reflex to other colours has been demonstrated (Lunau et al., 2018).

This study aims to analyse the colour preferences in the flower-visiting hoverfly *Eristalis tenax* (Linnaeus 1758). This species is also known as the dronefly because of its similarity to honeybee drones (Golding and Edmunds, 2000) and is used for greenhouse pollination management (Jarlan et al., 1997). The experiments in this study focus on testing the hypothesis that the preference for yellow colours in the landing response and proboscis extension differs with particular reference to additional reflection in the UV range of wavelengths. For this purpose, multiple-choice tests for the landing response were performed for different colours with and without UV reflection using naïve flies and colour-learning experiments with dual-choice tests using trained flies. In order to study the fine-tuned colour preferences of *E. tenax* flies, the innate proboscis reflex to the natural pollen stimuli of various shades of yellow was tested. In addition, we compared the colour preference between the landing reaction and the proboscis extension in naïve and untrained *E. tenax* flies for identical colour stimuli. This study is thus intended to fill the gaps of knowledge about colour preferences in *E. tenax*, namely the innate colour preference for the landing response, the colour learning and the proboscis reflex towards natural stimuli (i.e. floral pollen).

## MATERIALS AND METHODS
### Test animals
Larvae of the hoverfly *E. tenax* were collected in June and July, 2015 and 2017 on a farm in Düsseldorf, Germany. Only larvae of the third (last) larval stage and already pupated animals were collected. Some pupated animals were separated and placed in an acrylic glass box (width×depth×height: 15×15×9 cm). The box was stored in a refrigerator for some days or up to 3 weeks to prolong the pupal stage. The other larvae and pupae were placed in metal boxes (36×25×20 cm), which had a top window made of glass and were kept in the laboratory. Freshly emerged imagoes were transferred into another metal box, kept at room temperature (≍20°C) with a 12 h:12 h light:dark cycle. Flies were maintained on diluted honey, but never on natural flowers or pollen, in order to avoid colour learning. In the tests, flies were fed with sugar water.

### Colour stimuli
Natural hand-collected pollen and artificial flowers were used as colour stimuli and offered on artificial flowers made of coloured foamed rubber. In other experiments, artificial flowers made of glossy photographic paper (UV reflecting) or Whatman No. 1 filter paper were offered as colour stimuli. The colours were printed onto the filter paper or photographic paper using a Canon MX925 inkjet printer and original inks. In some experiments the artificial flowers were covered either with a UV-transparent or with a UV-absorbing foil in order to modify the UV-reflection properties. The spectral reflectance of the colours were measured with a USB4000 spectrophotometer (Ocean Optics, Inc., Ostfildern, Germany) using the program SpectraSuite. The details of the spectrophotometric measurements are described by Papiorek et al. (2014). The colour loci of colour stimuli are shown in the colour

vision model of Troje (1993) using the spectral sensitivity curves of the photoreceptor types of *E. tenax* (Bishop, 1974; Tsukahara and Horridge, 1977a,b; Horridge et al., 1975).

The model of categorical colour vision in flies (Troje, 1993) was used to analyse how flies perceive different colours. The model includes R7 and R8 retinula cells of the pale-type ommatidia and of the yellow-type ommatidia. Owing to the assumed subtraction of the R7 and R8 receptor cell excitations, both the yellow-type and pale-type systems produce a positive or a negative signal, allowing for the assignment of four colour categories: UV, p+ y+; purple, p+ y−; blue, p− y+; green; p− y−. It was assumed that the photoreceptors are adapted to the background colour and the excitation of the photoreceptors is consistently half-maximal (Laughlin, 1981; Laughlin, 1989).

The calculation of the receptor excitations from quantum catch values $P$ is described with: $E=P/(P+1)$, where $E$ can be any value between 0 and 1 (Naka and Rushton, 1966; Lipetz, 1971; Backhaus and Menzel, 1987; Chittka and Menzel, 1992). In the case when the receptor is stimulated by the adaptation light, $P=1$ and $E=0.5$. The applied light conditions and background colours were considered. The daylight was approximated by the D65 daylight function (Wyszecki and Stiles, 1982), laboratory illumination was provided by flicker-free Osram Biolux T8 fluorescent tubes. The background of the green meadow was approximated by the standard green leaf background AV 400; the background used for laboratory experiments was grey cardboard, which reflected light by approximately the same amount in the UV and visible range of wavelengths. The quantum catch values for all stimuli and all receptor types is given in Table S1. The calculation of colour loci is explained in detail in Table S2.

### Multiple-choice experiments with colour hues
The rationale of these experiments was to study innate colour preferences of the landing behaviour in *E. tenax*. The parameters varied were colour hue, colour brightness and UV reflection. All experiments were done outside in the Botanical Garden of Heinrich-Heine-University under a mosquito net (Fig. 1A). In the multiple-choice experiments, 12 'reward-less' artificial flowers printed on photographic paper were presented simultaneously. In one experiment, a white, a pink, a yellow, a blue, a red and a green artificial flower were presented twice both covered with either a UV-transparent or with a UV-absorbing foil. The artificial flowers were discs (3 cm diameter) attached to a 10-cm-long wooden stick and arranged in a circle of ~60 cm diameter (Fig. 1B). In another experiment, 12 darker colours of similar hues were presented and covered likewise with a UV-transparent or with a UV-absorbing foil. About 30 flies were placed under the net and all flies landing on one of the artificial flowers were recorded. Each fly had only one choice and afterwards was set free. These tests were time-consuming; in all cases, only one single fly was performing a choice and there was thus no necessity to exclude social stimulation. To determine the significant detection level, a two-sided Chi-square test was conducted.

### Colour learning and discrimination
The rationale for these experiments was to study colour learning, the influence of innate preference on colour learning and the impact of the categorical colour vision on colour discrimination. The artificial flowers (3 cm diameter) used for the colour learning experiments were printed on photographic paper and presented on a 10-cm-long stick to which they were attached by means of a hook-and-loop fastener against a grey and UV-reflecting background made of
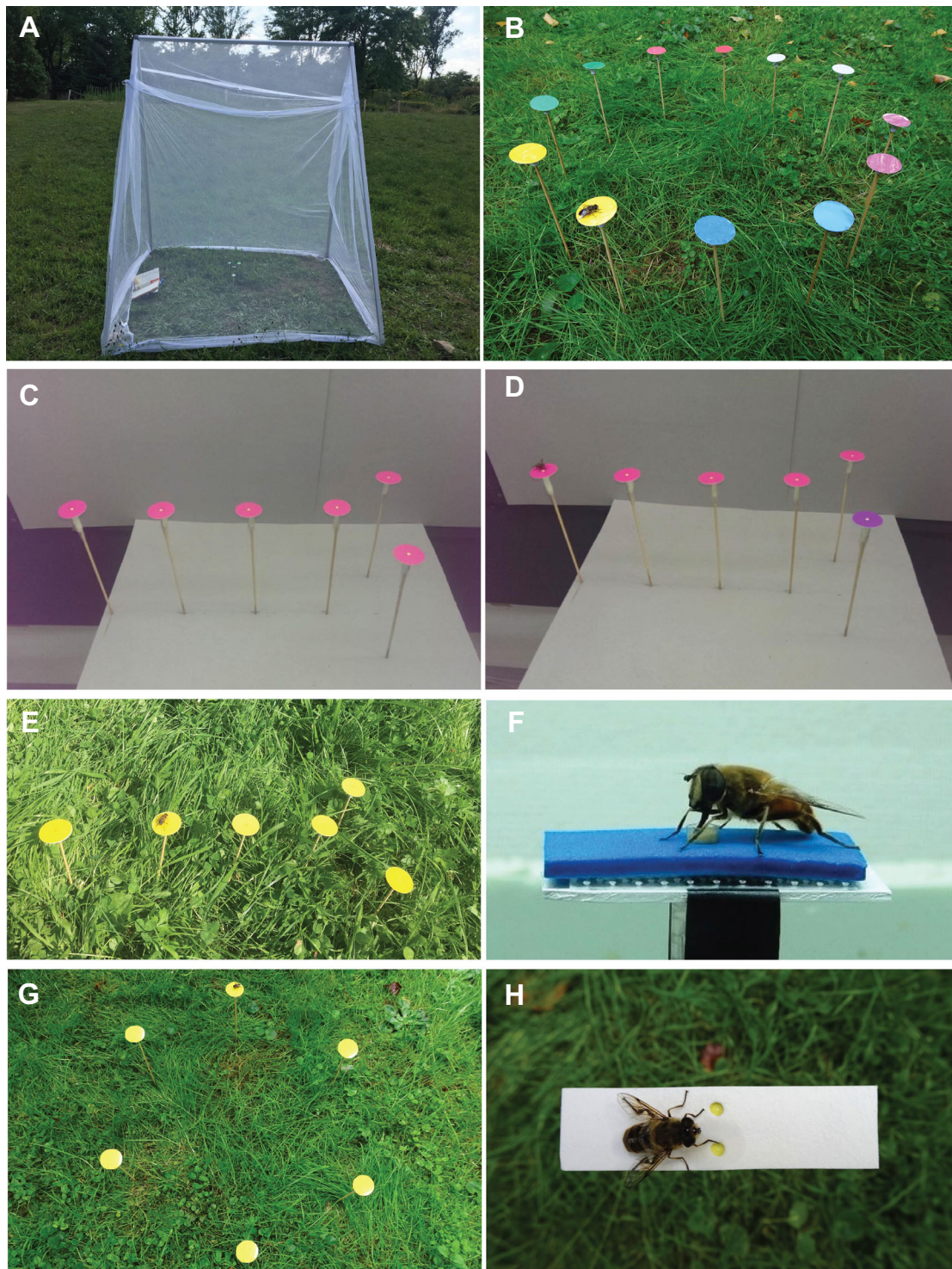
**Fig. 1. Experimental set-up.** (A) Some experiments were performed outdoors under a mosquito net. (B) Multiple-choice experiment testing preference for different colour hues. (C) Colour learning and discrimination test: training procedure. (D) Colour learning and discrimination test: testing procedure. (E) Dual-choice experiment with yellow colours varying in brightness. (F) Innate proboscis reflex to nature pollen stimuli. (G) Dual-choice test with landing response of naïve flies to β-carotene with or without UV. (H) Dual-choice test with proboscis reflex of naive flies to β-carotene colour with or without UV.

styrofoam. A training and test procedure with a reciprocal design was used; each tested fly was individually trained to one of two colours and tested in a dual-choice test with these two colours, the treatment was repeated at least twice, while a similar number of flies were trained to the other colour and tested similarly (Fig. 1C,D). In contrast to bees, flies do not carry the imbibed sugar water to the nest and then return to search for further rewards. Thus the number of rewarded visits has to be limited since the flies no longer cooperate if satiated. The rationale of this experimental design was to study the ability of colour learning and colour discrimination and to test how much the choice of colours in trained flies is still influenced by innate preferences.

For intense training, 6 similar artificial flowers were presented with 10 cm distance between them; of these, 4 flowers were presented in a row and the 2 remaining offered as a dual choice. All artificial flowers had a small yellow spot in the middle (diameter 2 mm) on which a droplet of a 30% sugar water solution was offered. For training, a fly was placed on the first artificial flower. The fly normally visited the remaining three artificial flowers one by one in a row and finally chose between two identical artificial flowers presented in an angle of 45 deg. For testing, immediately thereafter, the fly was put on the first artificial flower of another set-up of artificial flowers, in which one of the two artificial flowers for dual choice was replaced by one displaying another colour. A choice was noted for which of the two flowers was visited first, independent of the number of artificial flowers visited during the second trial. Most flies visited 4 artificial flowers before choosing one of the two presented at an angle of 45 deg; however, some flies visited fewer flowers. The experiments were conducted in a laboratory illuminated with flicker-free Osram Biolux T8 fluorescent tubes.

### Dual-choice experiment with colours varying in brightness or saturation

The rationale of these experiments was to study the impact of the brightness and saturation of colour stimuli on colour preference. The intensity and spectral purity of the stimuli were varied to represent perceptual dimensions of brightness and colour saturation in the flies. The stimulus brightness was calculated as quantum catch of the R1–R6 retinula cells. The stimulus saturation was defined as the relative distance between the colour loci of background and stimulus and the colour loci of background and corresponding spectral locus in analogy to bees (Rohde et al., 2013). In order to test the preference for the brightness of colour stimuli, a series of outdoor dual-choice experiments was set up; the training and test procedures were similar to the experiments on colour learning in *E. tenax* hoverflies (see above), but the preceding training trial was omitted. The flies thus visited four rewarded identical artificial flowers and then were given a dual choice between two rewarded artificial flowers, of which only one was identical to the trained artificial flowers (Fig. 1E). In one series of tests, five yellow colours differing in brightness were tested as follows. A number of flies were trained to one of the two tested colours; in a reciprocal test, other flies were trained to the other of the two tested colours; both groups of flies were given the same dual choice. This was repeated for all colour combinations resulting in 20 tests.

Preference Index was calculated by using the formula:

$$PI = (N_1/N_t) + (N_2/N_t) - 1, \qquad (1)$$

where PI is preference index; $N_t$ is the total number of flies; $N_1$ is the total number of flies trained to the bright colour visiting bright colours; $N_2$ is the total number of flies trained to the dark colour visiting bright colours. The brightness of the colours was estimated by the quantum catch to the R1–R6 receptors: PI is 1 if flies visit only bright colours; PI is −1 if flies visit only dark colours; 0<PI<1 means flies prefer brighter colours; −1<PI<0 means flies prefer darker colours.

The rationale for this experiment was to study the impact of the saturation of colour stimuli on colour preference. In order to test the preference for the saturation of colour stimuli, a dual-choice experiment was set up similarly to that testing the preference for the brightness of colour stimuli. The stimuli were produced using a mixture of blue, white and black powders in order to disentangle saturation and brightness (for details, see Koethe et al., 2016). The flies thus visited four rewarded identical artificial flowers and then were given a dual choice between two rewarded artificial flowers, of which only one was identical to the trained artificial flower (Fig. 1E). In the test, two blue colours similar in brightness but different in saturation were tested as follows. A single fly was trained to one of the two tested colours; in a reciprocal test, other flies were trained to the other colour; both groups of flies were given the same dual choice. In total, 30 flies were tested. The preference index was applied as above.

### Innate proboscis reflex to natural pollen stimuli

This experiment aimed to determine a precise description of the natural colour stimuli eliciting the proboscis reflex in *E. tenax*. The artificial flowers used for testing the flies' response to natural pollen were made of a 3×1 cm piece of blue foam rubber with a round hole in the middle which had a diameter of 3 mm. A coiled piece of UV-transparent foil stuck to the hole formed a cylinder protruding 2 mm above the level of the foam rubber. The pollen was placed in this cylinder filled level with the top edge (Fig. 1F). This artificial flower ensured that the pollen was visible to the tested flies, but the flies did not touch the pollen with their tarsi which are equipped with taste receptors for pollen (Wacht et al., 2000). A minimum of 10 flies were tested for each pollen. Each fly was placed with a piece of white cardboard to one end of the artificial flower. Each fly was tested three times as to whether it extended its proboscis towards the pollen. A full response consisted of proboscis extension each time; no response means no proboscis extension during the three trials. Hand-collected pollen of the following species were tested: *Helianthus annuus*, *Taxus baccata*, *Juglans nigra*, *Corylus avellana*, *Typha latifolia*, *Typha angustifolia*, *Narcissus pseudonarcissus*, *Bellis perennis*, *Centaurea jacea*, *Malva sylvestris* and *Papaver orientale*, as well as mixtures of pollen from different species in order to produce distinct shades of yellow. The following four mixtures (percentage by weight) were used: (1) 39% *T. baccata* and 61% *C. avellana*; (2) 34% *H. annuus* and 66% *T. baccata*; (3) 57% *H. annuus* and 43% *T. baccata*; (4) 21% *H. annuus* and 79% *T. baccata*. The experiments were conducted in a laboratory illuminated with flicker-free Osram Biolux T8 fluorescent tubes.

### Dual-choice test of proboscis reflex and landing response with identical colours

This experiment was designed to test the flies' preference for distinct yellow colours with proboscis extension and the landing reaction using identical colour stimuli. For this purpose, dual-choice tests offering two different yellow colours were used. The proboscis extension of naïve and untrained flies was tested using a 1.5×5.0 cm rectangular artificial flower offering the two different yellow spots in the middle next to each other (Fig. 1H). This set-up allowed us to assess which yellow colour the flies extended their proboscis first. The landing responses of flies were tested in two ways. First, the landing response of naïve and untrained flies was tested using β-carotene-coloured artificial flowers differing in UV-reflection properties (one was covered with UV-transparent foil and the other with UV-absorbing foil) (Fig. 1G). The artificial flowers (3.0 cm diameter) were presented on a stick and each of the two different β-carotene colours was presented three times. This set-up allowed us to determine which yellow colour the flies landed on first. Second, the landing response of trained flies was tested using the β-carotene-coloured artificial flowers with and without UV. The training and testing were the same as above (Fig. 1C,D). The

proboscis extension of naïve and untrained flies was tested using a 1.5×5.0 cm rectangular artificial flower offering the two β-carotene-coloured spots differing in UV reflection side by side. This set-up allowed us to determine which yellow colour the flies extended their proboscis to first. Both experiments were performed outdoors under a mosquito net.
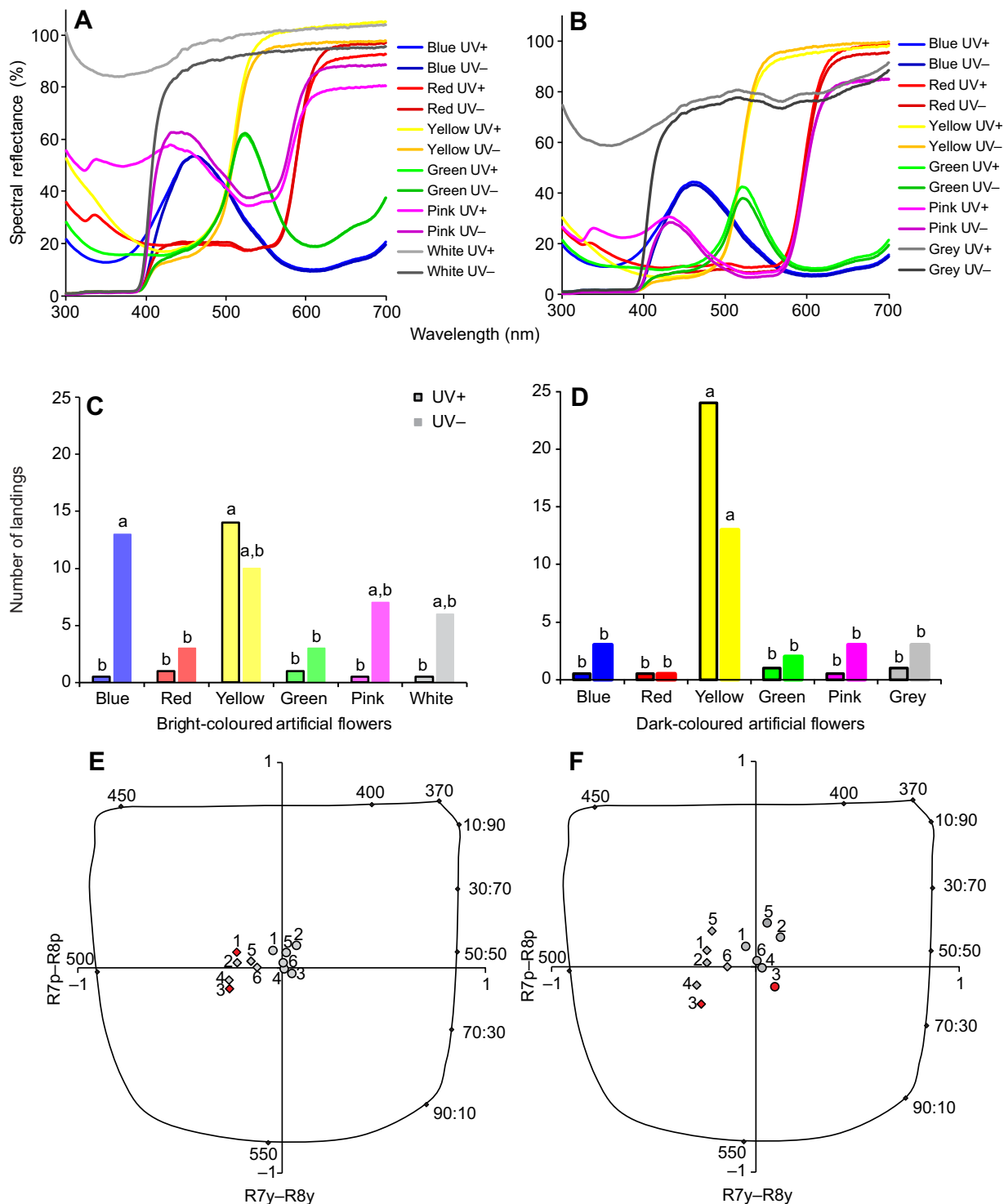


Fig. 2. Multiple-choice experiments of colour hues. Spectral reflectance of (A) bright-coloured or (B) dark-coloured artificial flowers covered with either UV-absorbing (−) or UV-reflecting (+) foil. Innate colour preference for landing of *Eristalis tenax* flies on six kinds of (C) bright-coloured (*n*=58) or (D) dark-coloured (*n*=50) artificial flowers covered with either UV-absorbing or UV-reflecting foil. Bars with black border indicate artificial flowers with UV-transmitting foil (UV+) and bars without black border indicate artificial flowers with UV-absorbing (UV−) foil. Different letters denote significant differences in two-sided Chi-square test (*P*<0.05). (E) The loci of bright-coloured artificial flowers. 1=blue, 2=red, 3=yellow, 4=green, 5=pink, 6=white. (F) Colour loci of dark-coloured artificial flowers. 1=blue, 2=red, 3=yellow, 4=green, 5=pink, 6=grey. Circles represent UV-reflecting (+), rhombuses represent UV-absorbing (−) artificial flowers. Circles and rhombuses in red indicate colours for which the flies have a significant preference, those in grey indicate colours that are significantly less attractive.

## RESULTS

### Multiple-choice experiments of colour hues

Naïve *E. tenax* flies given a choice among 12 bright artificial flowers (Fig. 2A) preferentially visited the yellow colours, with or without UV, the blue colour without UV and, to a lesser extent, the pink and white colours without UV. All other colours (red with and without UV; green with and without UV; blue, pink and white with UV) were significantly less attractive (Fig. 2C). The finding that the flies mostly prefer UV-absorbing over similar but UV-reflecting colours indicates that the preference is not determined by brightness alone. By contrast, given a choice among 12 dark artificial flowers (Fig. 2B), naïve *E. tenax* flies preferred the yellow colours with and without UV over all other colours, with all other colours significantly less attractive (Fig. 2D). Again, preference according to colour brightness as indicated by the quantum catch of one of the photoreceptors (Table S1), cannot predict the choice behaviour. Evaluation of the preferred and non-preferred bright and dark colours in the colour vision model of Troje (1993) shows that both of the colour loci of the preferred colours fall into more than one single colour category and that the colour loci of preferred as well as of not-preferred colours fall into the same categories (Fig. 2E,F).

### Colour learning and discrimination

Twelve pairs of colours were used for experiments on colour preference in *E. tenax* flies following training (Fig. 3A). If trained to grey colours (bright grey and dark grey), the flies were found to land exclusively on bright grey. Remarkably, all flies except three individuals refused the training to the dark grey artificial flowers. If trained to blue colours (bright blue and dark blue), the flies absolutely preferred bright blue; we completely failed to train flies to land on dark blue artificial flowers. If the flies were trained to one colour of a pair of bright green and bright yellow (or colour pair of bright green and blue-green), the flies significantly preferred the trained colour over the alternative colour. If the flies were trained to one colour of a pair of mustard yellow and red-orange (or colour pair of bright yellow and bright green), the flies significantly preferred the yellow colour hue even when they were trained to the alternative colour. If using a colour pair of yellow green and olive green to test the flies, the flies exclusively landed on yellow green even if they had been trained on olive green artificial flowers. For the remaining colour pairs, the flies did not show any significant preference (Fig. 3C). The colour loci of all the colours of the artificial flowers were calculated (Fig. 3B). The position of the colour loci of the two colours presented in one test, i.e. whether the colour loci are situated in the same colour category or in different categories, did not predict the flies' ability to discriminate between the colours.

### Dual-choice experiment with colours varying in brightness or saturation

The preference for colour brightness and saturation in *E. tenax* flies was tested in a series of dual-choice tests with five brightness levels (Fig. 4A). The trained flies preferred brighter over darker yellow artificial flowers even if trained to the darker colour. In 16 out of 20 dual-choice tests, the flies preferred the brighter colour irrespective of the training. In 8 of 10 cases, even the flies trained to darker colours preferred the brighter artificial flowers over the trained darker ones (Fig. 4C). The preference for colour saturation in *E. tenax* flies was tested in the dual-choice tests with two saturation levels. The trained flies did not prefer a more-saturated colour over the less-saturated colour irrespective of previous training to the more- or less-saturated colour (Fig. 4C). The evaluation of the reciprocal tests of the choice behaviour of flies trained to the darker

**Table 1. Preference index for *E. tenax* of five yellow colours differing in brightness and to a lesser extent in saturation, and of two blue colours differing predominantly in saturation**

| Artificial flowers | $N_1$ | $N_2$ | $N_t$ | PI |
|---|---|---|---|---|
| Y 255 vs Y 245 | 12 | 12 | 20 | 0.20 |
| Y 255 vs Y 230 | 11 | 10 | 20 | 0.05 |
| Y 255 vs Y 200 | 18 | 16 | 20 | 0.70 |
| Y 255 vs Y 150 | 19 | 16 | 20 | 0.75 |
| Y 245 vs Y 230 | 9 | 9 | 20 | −0.10 |
| Y 245 vs Y 200 | 14 | 16 | 20 | 0.50 |
| Y 245 vs Y 150 | 19 | 17 | 20 | 0.80 |
| Y 230 vs Y 200 | 12 | 9 | 20 | 0.05 |
| Y 230 vs Y 150 | 18 | 16 | 20 | 0.70 |
| Y 200 vs Y 150 | 11 | 12 | 20 | 0.15 |
| L-SB vs H-SB | 9 | 10 | 15 | 0.27 |

PI, preference index; $N_t$: total number of flies; $N_1$: total number of flies trained to the bright colour visiting bright colours; $N_2$: total number of flies trained to the dark colour visiting bright colours. For a full description of codes, which represent R:G:B values, see legend to Fig. 4.

and those trained to the brighter colour of each colour pair shows that all but one preference index are above zero, indicating a preference for the brighter colour (Table 1). The colour loci of the artificial flowers were calculated (Fig. 4B).

### Innate proboscis reflex to natural pollen stimuli

Naïve flies were tested on artificial flowers with 11 kinds of pure natural pollen and four mixtures of two kinds of pollen (Fig. 5A). *E. tenax* flies extended the proboscis towards six out of the 15 tested probes. The proboscis reaction in *E. tenax* flies was significantly triggered by pollen of *H. annuus*, *Co. avellana*, *T. latifolia*, *T. angustifolia*, *B. perennis* and mixture 3 (57% *H. annuus* and 43% *T. baccata*). The proboscis extension in *E. tenax* flies was not triggered by pollen of *T. baccata*, *N. pseudonarcissus*, *Ce. Jacea*, *M. sylvestris*, *P. orientale* and mixture 4 (21% *H. annuus* and 79% *T. baccata*). The other kinds of pollen and pollen mixtures sometimes triggered the proboscis reflex (Fig. 5B). The colour loci of the pollen probes that triggered the proboscis extension were the most distant from the background colour and thus indicate – besides the key yellow hue parameter – a role of colour saturation or/and colour contrast for the extension of the proboscis reflex. The positions of the colour loci of accepted and rejected pollen in the flies' colour space indicate that the relative quantum catch of R8y and R7y seems to be more relevant than that of R8p and R7p (Fig. 5B).

### Dual-choice test of proboscis reflex and landing response with identical colours

The experiments on the colour preference in the landing response and proboscis reflex in *E. tenax* flies showed that different yellow colours are preferred for the particular behavioural reactions. Naïve *E. tenax* flies landed on yellow β-carotene-coloured artificial flowers (Fig. 6A) with similar frequency irrespective of whether they were covered with a UV-transmitting or a UV-absorbing foil. The flies significantly preferred the trained artificial flower if trained to β-carotene-coloured flower with UV reflection, but showed no preference in the reciprocal experiment. *E. tenax* flies extended their proboscises to β-carotene yellow significantly more often if covered with a UV-absorbing foil compared with a UV-transmitting foil (Fig. 6C). The loci of the stimuli fall into different colour categories (Fig. 6B).

## DISCUSSION

Besides olfactory cues, colour is one of the most important floral attributes used by pollinators to identify flowers as potential food
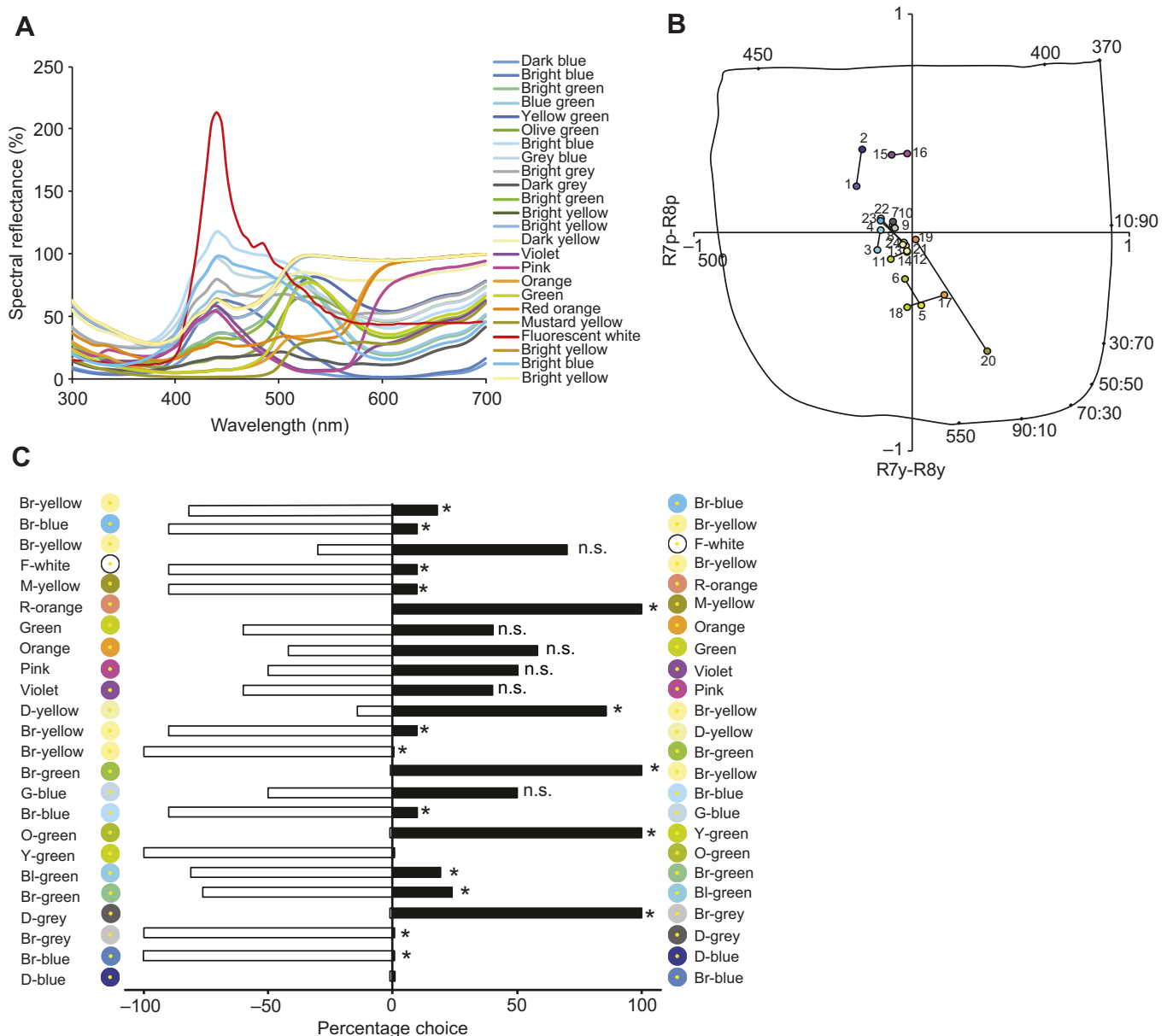
**Fig. 3. Colour learning and dual-choice tests of *E. tenax* flies trained to one colour of a pair of different artificial flowers.** (A) Spectral reflectance of different colours. (B) Colour loci of 12 colour pairs of artificial flowers. The colour loci of each colour pair are connected by a line. 1=bright yellow, 2=bright blue, 3=bright yellow, 4=fluorescent white, 5=mustard yellow, 6=red orange, 7=green, 8=orange, 9=pink, 10=violet, 11=dark yellow, 12=bright yellow, 13=bright yellow, 14=bright green, 15=grey blue, 16=bright blue, 17=olive green, 18=yellow green, 19=green blue, 20=bright green, 21=dark grey, 22=bright grey, 23=bright blue, 24=dark blue. (C) Response to the training colour (left) and response to test colour presented together with the training colour as a dual choice (right; *n*=252). At least 10 replicates were performed for each trial except grey and blue colour pairs. Br-yellow=bright yellow, Br-blue=bright blue, F-white=fluorescent white, M-yellow=mustard yellow, R-orange=red orange, D-yellow=dark yellow, Br-green=bright green, G-blue=grey blue, O-green=olive green, Y-green=yellow green, Bl-green=blue green, Br-grey=bright grey, D-grey=dark grey, D-blue=dark blue. *$P<0.05$; n.s., not significant; two-sided Chi-square test.

sources. Flower-visiting insects use flower colours to find flowers (Lunau and Maier, 1995). Colour learning represents a common attribute of flower-visiting insects. Since the kind and amount of floral rewards differ among species as does the handling time to exploit flowers, flower visitors benefit from learning which plants offer the best reward food (Neumeyer, 1981; Chittka and Menzel, 1992; Giurfa, 2004; Dyer and Arikawa, 2014). From various studies it is known that honey bees and bumblebees can learn to associate flower colours, odours, shapes, electric fields, textures and locations with nectar rewards (Clarke and Braun, 2013; von Frisch, 1967; Gould, 1993; Kevan and Lane, 1985; Menzel and Muller, 1996;

Sherry and Strang, 2015). The results of our study do not confirm that visual orientation of droneflies and western honeybees might be similar due to shared ecological niche (Srinivasan and Guy, 1990). Bees can even simultaneously learn to associate pollen rewards with one flower colour and nectar rewards with another colour (Muth et al., 2015, 2016). Hoverflies also take nectar and pollen from flowers (Holloway, 1976) and are able to use floral scent for finding flowers; the marmalade hoverfly, *Episyrphus balteatus* can learn floral scent to visit non-yellow flowers and thereby overcome the innate preference for yellow colours (Sutherland et al., 1999; Primante and Dötterl, 2010).
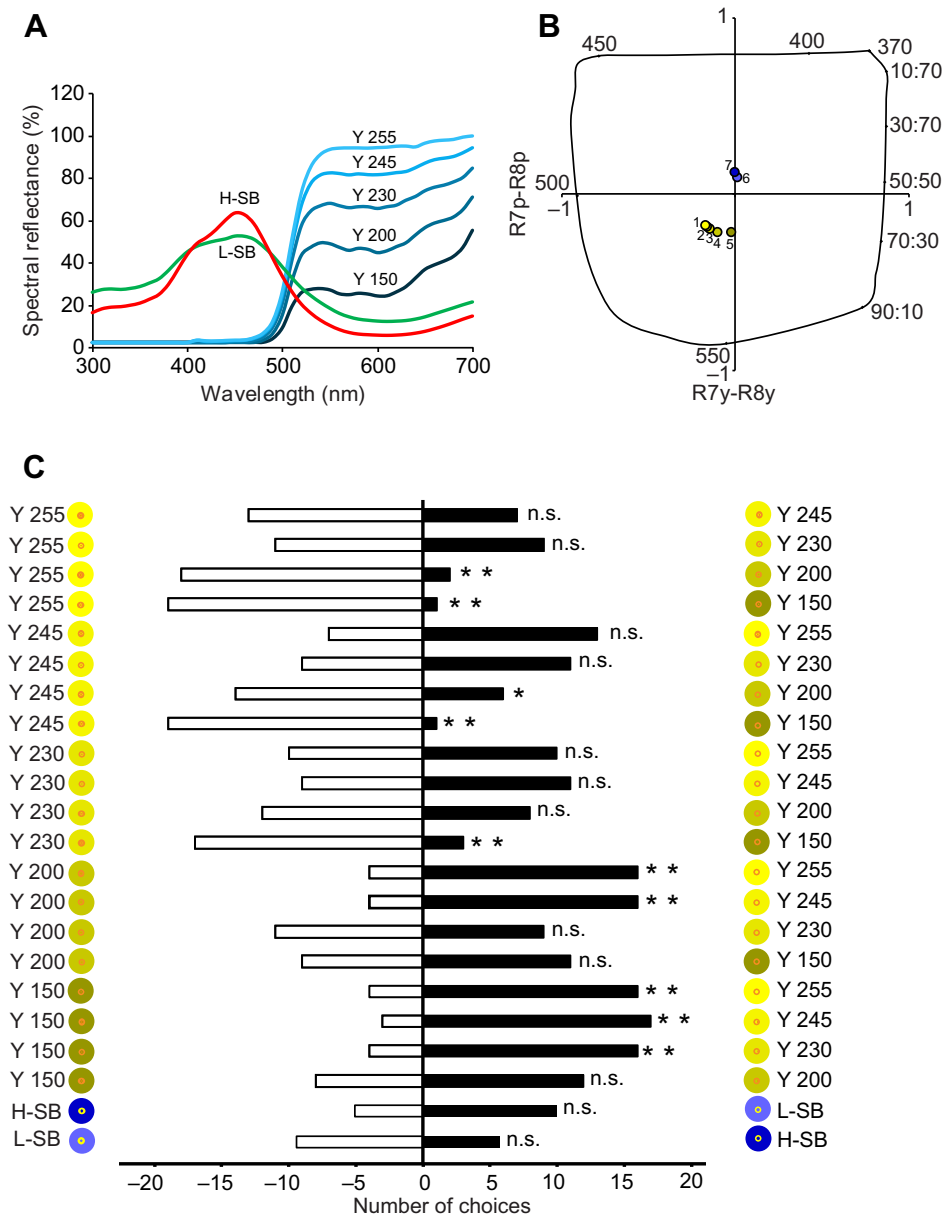
**Fig. 4. Colour learning of *E. tenax* flies trained to colours varying in brightness and saturation.** (A) Spectral reflectance of different colours. Codes correspond to colours shown in C. (B) Colour loci of artificial flowers tested in dual-choice tests. 1=bright yellow (Y 255; R:G:B=255:255:0), 2=bright yellow (Y 245; R:G:B=245:245:0), 3=mid-yellow (Y 230; R:G:B=230:230:0), 4=dark yellow (Y 200; R:G:B=200:200:0), 5=dark yellow (Y 150; R:G:B=150:150:0), 6=low-saturated blue (L-SB), 7=high-saturated blue (H-SB). R:G:B values refer to the amount of red, green and blue when printing colours. (C) Dual-choice tests of flies trained to colours varying in brightness and saturation. Each left column shows the response to the training colour; each right column shows that to the test colour presented along with the training colour as a dual choice (n=430). *P<0.05, **P<0.01; n.s., not significant; one-sided Chi-square test.

Many flower-visiting insects possess innate colour preferences (Lunau and Maier, 1995). These innate colour preferences can be task-specific and thus differ for distant approach, landing (Lunau et al., 1996), walking on the flower and extension of the mouthparts (Lunau et al., 1996; Dinkel and Lunau, 2001). Bees use green contrast to find flowers as long as the flowers appear under a small visual angle (Giurfa et al., 1997). Although such a colourblind phase in flower detection has never been described for hoverflies, it is assumed that hoverflies use the colourblind neural superposition system for motion detection, independently of the colour vision system (Zhou et al., 2012; Lunau, 2014).

Innate colour preferences of flower-visiting insects may be fixed, persistent or replaced by learnt preferences. *Eristalis* flies possess a fixed proboscis reflex towards yellow and UV-absorbing colours that cannot be modified by conditioning (Lunau et al., 2018). The honeybees' and bumblebees' preference for saturated colours persists despite training, whereas they rapidly learn colours of different hues (Rohde et al., 2013). Hawkmoths have an innate preference for blue colours facilitating the naïve individuals'

probability of finding flowers that is overwritten by white colours advertised by most of the nocturnal flowers (Goyret et al., 2008). Haslett (1989) observed that specialist hoverflies like *Cheilosia albitarsis* exhibit very strict colour (hue) preferences, whereas generalist hoverflies like *Eristalis pertinax* visit flowers of different colours, prefer abundant flowers and perform flower constancy (Kugler, 1950).

Based on discrimination of monochromatic colour stimuli in the Green Bottle Fly, *Lucilia* sp., the categorical colour vision model of Troje (1993) assumes that discrimination is limited to colours, of which the colour loci belong to different of only four colour categories and thus predicts poor colour discrimination. The analysis of the colour choice behaviour of *E. tenax* by means of the colour vision model of Troje (1993) shows that the preference of *E. tenax* cannot be predicted by the position of the respective colour loci in one distinct colour category. This holds for the detection of natural pollen and for the multiple colour choice experiments. The tendency that the colour loci of the preferred colours are positioned more distant from the centre of the colour space, i.e. the locus of the
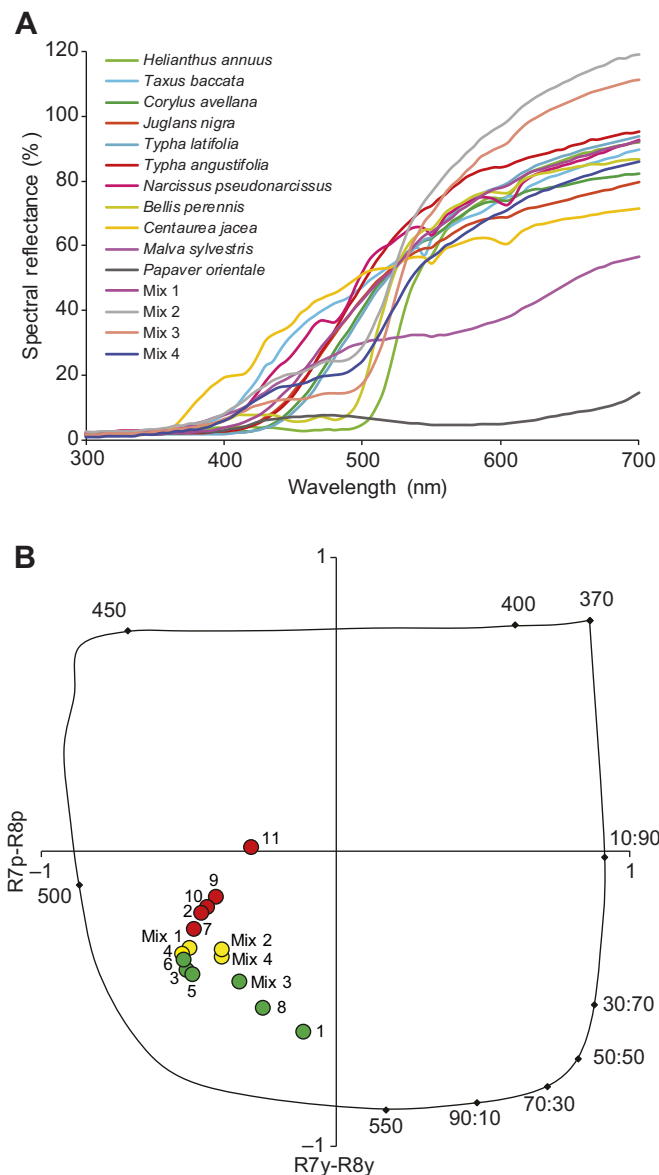
**Fig. 5. Innate proboscis reflex to natural pollen stimuli.** (A) Spectral reflectance of pollen and mixtures. (B) Colour loci of pollen probes in tests of the innate proboscis reflex in *E. tenax* flies. The data were analysed using a one-way ANOVA test. Green represents colour loci of pollen probes that trigger the proboscis extension, red those that do not trigger the proboscis extension, and yellow those that do trigger the proboscis extension, but not significantly. 1, *Helianthus annuus*; 2, *Taxus baccata*; 3, *Corylus avellana*; 4, *Juglans nigra*; 5, *Typha latifolia*; 6, *Typha angustifolia*; 7, *Narcissus pseudonarcissus*; 8, *Bellis perennis*; 9, *Centaura jacea*; 10, *Malva sylvestris*; 11, *Papaver orientale*; Mix 1–4=mixture 1–4 (see Materials and Methods for full details of mixes).

background colour, is, however, not consistent. Moreover, our results of the learning experiments demonstrate that the colour categories do not predict colour discrimination abilities, since the flies discriminated between colours of the same category. The results of this study reveal other limitations: *E. tenax* flies strongly prefer to land on yellow targets, can learn to land on other colours than yellow, but still prefer the yellow colour to some extent. They also prefer UV-absorbing over UV-reflecting non-yellow colours for landing. In addition, the flies prefer to approach and land on colours of high brightness and avoid landing on dark colours of all colour hues except yellow. The avoidance of dark colours is very

evident from the dual-choice test following training, but contradicts the finding that UV-absorbing colours are preferred over similar but UV-reflecting ones. The results provide no evidence that the flies prefer more-saturated over less-saturated colours of similar brightness as some bees do (Lunau et al., 1996; Koethe et al., 2016). There is also no evidence the hoverflies possess a perceptual dimension for colour saturation, which in analogy to bee colour vision might be evaluated by the distance between the colour locus of a test colour and that of the background colour divided by the distance between the corresponding spectral and that of the background (Koethe et al., 2016). The findings of this study suggest that the hoverflies might possess perceptual dimensions for the yellowness and for the blueness of colours instead, which would fit with the 'yellow and pale' system. Colour learning of *E. tenax* flies is thus limited; the flies can learn some colours, but fail to learn to land on dark colours and on non-yellow colours in the presence of yellow colours.

The results of the experiment about the innate proboscis reflex to natural pollen stimuli show that *E. tenax* flies responded to natural yellow pollen with proboscis extension. This result confirms previous studies in which the proboscis reflex of naïve and untrained *E. tenax* flies was tested using monochromatic light stimuli (Lunau and Wacht, 1994), mixtures of two monochromatic light stimuli (Lunau and Wacht, 1997) and artificial flowers with coloured spots (Lunau, 1988; Dinkel and Lunau, 2001; Lunau et al., 2005; Riedel and Lunau, 2015), which showed that *E. tenax* flies possess a fine-tuned preference for yellow and UV-absorbing colours. Although all tested pollen probes in this study exhibit the maximal reflectance in the green/yellow range of wavelengths, the *E. tenax* flies were actually very fastidious and accepted only pollen with colours displaying steeper slopes of reflectance curves and low reflectance in the UV and blue wavelength ranges. The position of the colour loci of the preferred colours indicates that the stronger stimulation of R8y compared with R7y might trigger the proboscis response (Fig. 5B), which is also supported by the quantum catch data (Tables S1 and S2). From previous studies, it is known that release of the proboscis reflex towards yellow spots is largely independent of the artificial flower colour (Sternke-Hoffmann and Lunau, 2015); thus a preference for bright and saturated yellow colours rather than colour contrast between target and surrounding seems plausible.

The reflectance properties of floral colours are characterized by the typical wavelength of marker points, i.e. inflection points of the spectral reflectance curves, which cluster at ∼400 nm and ∼500 nm (Chittka and Menzel, 1992; Arnold et al., 2009; Dyer et al., 2012; Shrestha et al., 2013). Among the types of pollen we tested, only dark yellow pollen showed such reflectance properties with steep flanks; many types of yellowish pollen have spectral reflectance curves with only small changes of reflectance for given wavelengths. Bukovac et al. (2017) found that the position of the marker points may explain how flower detection and flower discrimination by colour cues interact. In the case of the proboscis reflex in *Eristalis* flies, better detection must be responsible for the flies' choice, since the flies did not exhibit any conditioning to other colours for the proboscis reflex (Lunau et al., 2018).

This study provides several lines of evidence in support of the preference of *E. tenax* for bright colours. The colour learning and discrimination experiments demonstrated that the flies refuse to land on dark colours and prefer bright colours over dark colours irrespective of training. The multiple-choice tests for colour hues demonstrated that darker colours are less attractive than brighter ones. Moreover, the flies did not exhibit any consistent preference
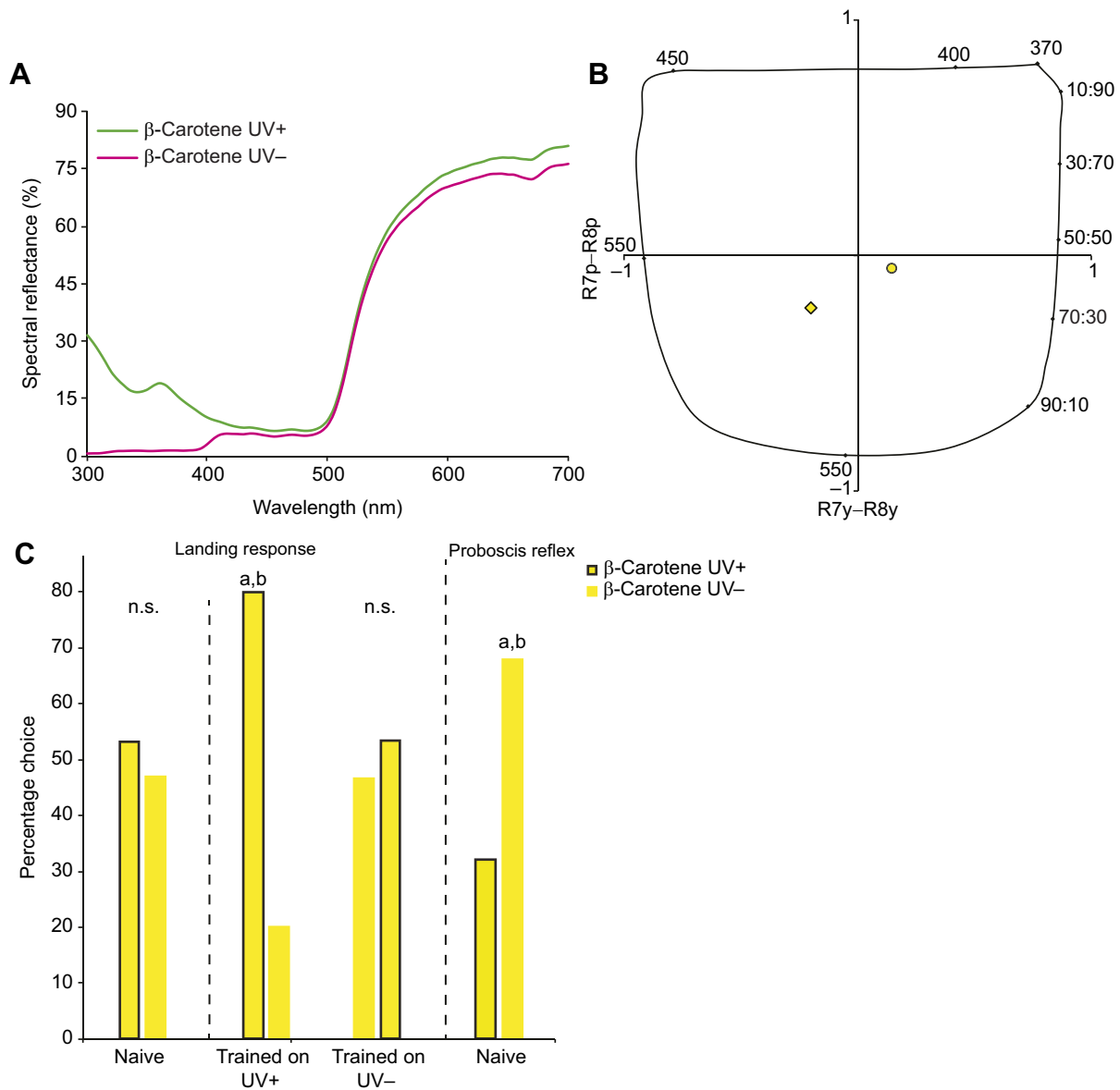
**Fig. 6. Dual-choice test with landing response and proboscis extension for identical colours.** (A) Spectral reflectance of β-carotene colours differing in UV-reflection properties. (B) Colour loci of artificial flowers made of β-carotene colour. Circle represents the artificial flower covered with UV-transmitting foil, rhombus represents the artificial flower covered with UV-absorbing foil. (C) Landing response and proboscis extension for identical colours in *E. tenax*. Each experiment is separated by dashed line. Left: Innate colour preference of landing for flies to β-carotene differing in UV-reflection properties (*n*=32). Middle: Colour learning of flies landing colours of β-carotene differing in UV-reflection properties (*n*=30). Right: Colour preference of proboscis extension for naïve flies to β-carotene differing in UV-reflection properties (*n*=42). The bars with black border indicate artificial flowers with UV-reflection and the bars without black border indicate artificial flower with UV-absorption. Different letters denote significant differences (*P*<0.05) and *ns* means 'not significant' due to a two-sided Chi-square test.

for colours of similar brightness but different saturation. However, the flies preferred UV-absorbing colours over similar but UV-reflecting ones indicating that the UV range of wavelengths does not contribute to the evaluation of brightness. In the case of a preference for bright stimuli, the fly colour vision model cannot explain the flies' choice, since stimulus intensity is not fully represented in the fly colour space. The colour loci of some of the preferred bright colours have similar positions in the flies' colour space as those of some of the rejected dark ones. There are two possible explanations for this choice behaviour. Since 12 different colours were presented simultaneously, the relative attractiveness of the colours might have influenced the flies' choice. Alternatively, the choice might be independent of the chromaticity of the colours,

but instead could rely on the intensity, which might be perceived by other receptor types, e.g. R1–R6. However, since the flies responded better to UV-absorbing white, blue and pink colours than similar but UV-reflecting ones, brightness does not seem to be responsible for this choice, because the UV-reflecting colours are brighter than the UV-absorbing ones, particularly if the R1–R6 retinula cells were involved in brightness perception (Table S1). The adaptation of the photoreceptors to colour might play a role, since it is not known whether fly photoreceptors adapt completely to the background colour as assumed here. Moreover, the colour vision model assumes that the relative excitation of R8 and R7 is important, but the absolute excitation of the very same photoreceptors might also play a role. In bees, the use of colour

contrast or green contrast is dependent on the visual angle of the target (Giurfa et al., 1997) and thus dependent on the distance of the bee and a target of given size. Flies are known to use the R1–R6 retinula cells for edge detection (Zhou et al., 2012). If this response holds also for flower detection, droneflies' choice behaviour might actually rely on two subsequent reactions: from a large distance the flies might use the contrast between target and background perceived by the colourblind neural superposition system for a preliminary decision for an approach and only later use colour vision for the landing response, which would strongly affect the interpretation of the multiple-choice experiments. Finally, the dual-choice experiments with colours varying in brightness also demonstrate a clear preference for bright colours even among colours of the preferred yellow hue. This interpretation, however, ignores the fact that the bright colours in this experiment were also the more saturated ones.

Our results show that the flies prefer yellow for landing and for proboscis extension. However, while *E. tenax* flies accept yellow colours irrespective of UV-reflection properties for the landing response, the flies extend their proboscis preferably to yellow and UV-absorbing colour stimuli. Here, we tested these differences in the response to yellow colours explicitly using dual-choice experiments with identical colours for both behavioural reactions. The different responses to identical stimuli, i.e. UV-reflecting as well as UV-absorbing yellow for landing, only UV-absorbing yellow for proboscis extension, indicate that the flies might be able to handle typical yellow flowers displaying a UV-bull's eye (Lunau et al., 2018). The size of the yellow spot might be critical for this response, since *E. tenax* also responded to large-sized yellow and UV-absorbing spots during landing behaviour (Lunau et al., 2018).

As a result of the diverse results on colour preferences in naïve and experienced flies, we conclude that colour preference in *E. tenax* is based on several parameters. For the landing response, flies prefer yellow over all other colours, prefer UV-absorbing over UV-reflecting colours among non-yellow colours and prefer brighter colours over darker ones. Learning can only partly override the innate preferences for landing. The proboscis reaction in *E. tenax* is triggered only by yellow colours, preferably yellow and UV-absorbing colours and cannot be modified by training (Lunau et al., 2018). The selectivity of the proboscis-extending flies for yellow stimuli suggests that colours stimulating the R8y photoreceptor or colour saturation might be an attractive cue. The results of the landing response are consistent with a preference for colours displaying a distinct perceptual colour contrast against surrounding colours and colours that display a threshold amount of brightness and saturation. The interpretation of colour preferences by means of the categorical colour vision model (Troje, 1993) is limited. Recent findings of Gray et al. (2018) that flower colours are more saturated and less bright at high altitudes, where pollination is predominantly carried out by flies (Arnold et al., 2009; Lefebvre et al., 2018), are consistent with the assumption that colour saturation might play a role in colour choice of droneflies.

## Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.184788.supplemental

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Table S1. Quantum catch of all photoreceptors given for all tested colour stimuli and the respective background and illumination (Daylight: D65; fluorescent tubes: Fl. tubes). Name of experiment, figure with the respective results and experimental light conditions and background are given. Moreover the corresponding wavelength (Corresp. λ) and the spectral purity (Spectral purity) of the colour stimuli are given. Colour stimuli which were tested simultaneously are shaded similarly. The last column (Prefer.) presents frequency of choices or indicate strong (+), middle (~) or no (-) preference for the trained colour or the spontaneously chosen colour.

|  | Colour | R7p | R7y | R8p | R8y | R1-6 | Corresp. λ | Spectral purity | Prefer. |
|---|---|---|---|---|---|---|---|---|---|
| **Mutiple-choice experiment Fig. 2 D65; AV400** | Bright blue UV+ | 3.372 | 4.341 | 5.064 | 2.263 | 4.584 | 430 | 0.101 | 0 |
|  | Bright blue UV- | 1.480 | 3.701 | 4.929 | 2.278 | 4.326 | 484 | 0.175 | 13 |
|  | Bright red UV+ | 4.809 | 3.054 | 2.455 | 1.571 | 2.510 | 389 | 0.130 | 1 |
|  | Bright red UV- | 1.099 | 1.916 | 2.352 | 1.610 | 2.185 | 490 | 0.156 | 3 |
|  | Bright yellow UV+ | 5.644 | 4.125 | 3.297 | 5.434 | 3.720 | 573 | 0.062 | 14 |
|  | Bright yellow UV- | 1.080 | 2.385 | 2.640 | 5.100 | 2.862 | 515 | 0.200 | 10 |
|  | Bright green UV+ | 3.246 | 3.031 | 2.851 | 3.277 | 2.979 | 569 | 0.020 | 1 |
|  | Bright green UV- | 1.055 | 2.335 | 2.748 | 3.348 | 2.753 | 508 | 0.200 | 3 |
|  | Bright pink UV+ | 9.685 | 6.974 | 6.229 | 3.160 | 6.097 | 407 | 0.089 | 0 |
|  | Bright pink UV- | 2.146 | 4.696 | 6.145 | 3.360 | 5.493 | 488 | 0.124 | 7 |
|  | White UV+ | 16.350 | 12.621 | 11.493 | 7.679 | 11.388 | 400 | 0.029 | 0 |
|  | White UV- | 2.782 | 7.212 | 9.369 | 6.818 | 8.580 | 497 | 0.112 | 6 |
|  | Dark blue UV+ | 2.779 | 3.538 | 4.121 | 1.789 | 3.722 | 429 | 0.124 | 0 |
|  | Dark blue UV- | 1.284 | 3.005 | 3.970 | 1.783 | 3.484 | 480 | 0.184 | 3 |
|  | Dark red UV+ | 2.948 | 1.787 | 1.380 | 0.926 | 1.438 | 376 | 0.178 | 0 |
|  | Dark red UV- | 0.794 | 1.049 | 1.193 | 0.876 | 1.143 | 480 | 0.109 | 0 |
|  | Dark yellow UV+ | 2.653 | 1.995 | 1.494 | 4.128 | 1.876 | 558 | 0.138 | 24 |
|  | Dark yellow UV- | 0.874 | 1.552 | 1.542 | 4.378 | 1.844 | 531 | 0.200 | 13 |
|  | Dark green UV+ | 2.214 | 1.958 | 1.790 | 2.079 | 1.892 | 582 | 0.042 | 1 |
|  | Dark green UV- | 0.820 | 1.428 | 1.605 | 1.955 | 1.631 | 512 | 0.180 | 2 |
|  | Dark pink UV+ | 4.559 | 3.056 | 2.673 | 0.975 | 2.596 | 410 | 0.263 | 0 |
|  | Dark pink UV- | 1.220 | 1.862 | 2.349 | 0.921 | 2.086 | 440 | 0.193 | 3 |
|  | Grey UV+ | 11.676 | 9.504 | 8.922 | 5.987 | 8.778 | 404 | 0.034 | 1 |
|  | Grey UV- | 2.370 | 5.997 | 7.762 | 5.623 | 7.115 | 497 | 0.125 | 3 |
| **Colour learning and discrimination Fig. 3 Grey; Fl. tubes** | Bright yellow | 1.578 | 1.876 | 1.948 | 2.509 | 1.965 | 526 | 0.063 | + |
|  | Bright blue | 1.200 | 2.283 | 2.669 | 1.669 | 2.500 | 480 | 0.155 | + |
|  | Bright yellow | 1.321 | 1.492 | 1.512 | 2.153 | 1.554 | 536 | 0.080 | ~ |
|  | Fluorescent white | 1.546 | 3.512 | 4.273 | 2.180 | 3.911 | 477 | 0.158 | + |
|  | Mustard yellow | 0.160 | 0.110 | 0.072 | 0.500 | 0.112 | 563 | 0.639 | + |
|  | Red orange | 0.670 | 0.637 | 0.631 | 0.713 | 0.639 | 559 | 0.039 | + |
|  | Green | 0.316 | 0.385 | 0.335 | 1.310 | 0.420 | 546 | 0.408 | ~ |
|  | Orange | 0.373 | 0.303 | 0.254 | 0.724 | 0.301 | 558 | 0.334 | ~ |
|  | Pink | 0.781 | 0.780 | 0.852 | 0.260 | 0.790 | 426 | 0.474 | ~ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Colour learning and discrimination Fig. 3** Grey; Fl. tubes | Violet | 0.716 | 0.881 | 1.010 | 0.287 | 0.923 | 430 | 0.470 | ~ |
| | Dark yellow | 1.172 | 1.385 | 1.430 | 1.823 | 1.448 | 526 | 0.073 | - |
| | Bright yellow | 1.163 | 1.300 | 1.295 | 2.102 | 1.354 | 541 | 0.108 | + |
| | Bright yellow | 1.177 | 1.303 | 1.296 | 2.063 | 1.353 | 541 | 0.103 | + |
| | Bright green | 0.631 | 0.872 | 0.892 | 1.455 | 0.933 | 526 | 0.165 | - |
| | Grey blue | 1.550 | 2.311 | 2.592 | 2.001 | 2.476 | 485 | 0.094 | ~ |
| | Bright blue | 1.753 | 2.822 | 3.216 | 2.274 | 3.046 | 482 | 0.098 | + |
| | Olive green | 0.475 | 0.556 | 0.527 | 1.223 | 0.587 | 544 | 0.255 | - |
| | Yellow green | 0.393 | 0.420 | 0.352 | 1.465 | 0.450 | 549 | 0.390 | + |
| | Blue green | 0.943 | 1.640 | 1.854 | 1.561 | 1.785 | 492 | 0.162 | + |
| | Bright green | 0.571 | 0.931 | 0.997 | 1.327 | 1.011 | 512 | 0.197 | + |
| | Dark grey | 0.325 | 0.390 | 0.414 | 0.341 | 0.404 | 472 | 0.099 | - |
| | Bright grey | 1.132 | 1.492 | 1.624 | 1.383 | 1.575 | 487 | 0.084 | + |
| | Bright blue | 0.507 | 1.077 | 1.302 | 0.510 | 1.186 | 459 | 0.298 | + |
| | Dark blue | 0.375 | 0.639 | 0.767 | 0.211 | 0.691 | 439 | 0.513 | - |
| **Dual-choice Experiment Fig. 4** D65; AV400 | Yellow 150 | 0.519 | 0.629 | 0.563 | 1.481 | 0.692 | 549 | 0.257 | + |
| | Yellow 200 | 0.557 | 0.873 | 0.792 | 2.562 | 1.023 | 542 | 0.266 | ++ |
| | Yellow 230 | 0.592 | 1.102 | 1.017 | 3.497 | 1.328 | 534 | 0.260 | +++ |
| | Yellow 245 | 0.637 | 1.297 | 1.219 | 4.197 | 1.583 | 529 | 0.253 | ++++ |
| | Yellow 255 | 0.658 | 1.398 | 1.314 | 4.679 | 1.719 | 527 | 0.253 | +++++ |
| | Low-saturated blue | 6.409 | 5.596 | 5.639 | 2.272 | 5.294 | 415 | 0.126 | ++ |
| | High-saturated blue | 5.334 | 5.458 | 6.008 | 1.856 | 5.440 | 419 | 0.165 | + |
| **Innate proboscis reflex Fig. 5** Grey; Fl. tubes | *Helianthus annuus* | 0.171 | 0.225 | 0.193 | 1.086 | 0.261 | 545 | 0.571 | + |
| | *Taxus baccata* | 0.367 | 0.953 | 1.072 | 1.545 | 1.078 | 506 | 0.377 | - |
| | *Corylus avellana* | 0.181 | 0.535 | 0.553 | 1.463 | 0.618 | 514 | 0.523 | + |
| | *Juglans nigra* | 0.202 | 0.619 | 0.662 | 1.444 | 0.711 | 512 | 0.507 | ~ |
| | *Typha latifolia* | 0.183 | 0.517 | 0.528 | 1.467 | 0.597 | 516 | 0.512 | + |
| | *Typha angustifolia* | 0.215 | 0.662 | 0.696 | 1.707 | 0.764 | 513 | 0.504 | + |
| | *N. pseudonarcissus* | 0.308 | 0.857 | 0.949 | 1.624 | 0.977 | 509 | 0.420 | - |
| | *Bellis perennis* | 0.211 | 0.348 | 0.324 | 1.355 | 0.404 | 536 | 0.467 | + |
| | *Centaura jacea* | 0.476 | 1.092 | 1.234 | 1.525 | 1.223 | 504 | 0.309 | - |
| | *Malva sylvestris* | 0.266 | 0.611 | 0.686 | 0.884 | 0.683 | 506 | 0.378 | - |
| | *Papaver orientale* | 0.142 | 0.214 | 0.238 | 0.186 | 0.228 | 485 | 0.234 | - |
| | Mixture 1 | 0.242 | 0.688 | 0.741 | 1.539 | 0.787 | 512 | 0.465 | ~ |
| | Mixture 2 | 0.310 | 0.661 | 0.702 | 1.629 | 0.757 | 518 | 0.378 | ~ |
| | Mixture 3 | 0.254 | 0.472 | 0.479 | 1.426 | 0.542 | 527 | 0.397 | + |
| | Mixture 4 | 0.280 | 0.581 | 0.625 | 1.275 | 0.660 | 517 | 0.375 | ~ |
| **Fig. 6** D65; AV400 | ß-carotene UV+ | 3.068 | 1.869 | 1.243 | 2.705 | 1.537 | 578 | 0.184 | 17.27 |
| | ß-carotene UV- | 0.437 | 0.826 | 0.836 | 2.476 | 1.002 | 531 | 0.308 | 15.57 |

Table S2. Complete dataset. Excel worksheets presenting the calculations of the colour loci of the stimuli presented in the results; the worksheet tabs refer to the numbers of the figures in the results section. Excel worksheets are using the spectral reflectance of the colour stimuli and backgrounds and the spectral distribution of the ambient light (referred to as illumination) conditions in 1 nm steps as well as the adapted spectral sensitivity curves of the photoreceptor types of *Eristalis tenax*. The calculations of the spectral locus is done with the normalized spectral sensitivity curves in two steps as follows: monoch.lim1 shows the calculation of the spectral locus from 350nm to 550nm and mixed purple monochromatic stimuli; monoch.lim2 shows the calculation of the purple part of the spectral locus using mixtures of two monochromatic stimuli, which are 350 nm and 550nm. For all calculations it is assumed that the photoreceptors are half-maximally sensitive when stimulated by the background colour. In separate sheets the values for the spectral reflectance of all stimuli, the spectral sensitivity of all photoreceptors and the spectral distribution of illuminations and backgrounds are given. Not filled but yet numbered columns in the worksheets are formatted in order to calculate the colour loci of further stimuli. Note that the illumination and background must be customized for this purpose.

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