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Learning and discrimination of coloured papers in the walking blowfly, *Lucilia cuprina*

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Summary. A new training and testing paradigm for walking sheep blowflies, *Lucilia cuprina*, is described. A fly is trained by presenting it with a droplet of sugar solution on a patch of coloured paper. After having consumed the sugar droplet, the fly starts a systematic search. While searching, it is confronted with an array of colour marks consisting of four colours displayed on the test cardboard (Fig. 1). Colours used for training and test include blue, green, yellow, orange, red, white and black.

Before training, naive flies are tested for their spontaneous colour preferences on the test array. Yellow is visited most frequently, green least frequently (Table 2). Spontaneous colour preferences do not simply depend on subjective brightness (Table 1).

The flies trained to one of the colours prefer this colour significantly (Figs. 5 and 9–11). This behaviour reflects true learning rather than sensitisation (Figs. 6–7). The blue and yellow marks are learned easily and discriminated well (Figs. 5, 9, 11). White is also discriminated well, although the response frequencies are lower than to blue and yellow (Fig. 11). Green is discriminated from blue but weakly from yellow and orange (Figs. 5, 9, 10). Red is a stimulus as weak as black (Figs. 8, 9). These features of colour discrimination reflect the spectral loci of colours in the colour triangle (Fig. 14).

The coloured papers seem to be discriminated mainly by the hue of colours (Fig. 12), but brightness may also be used to discriminate colour stimuli (Fig. 13).

Key words: Fly – Behaviour – Learning – Colour vision – Colour discrimination

Introduction

Visual learning in flies has been studied by several investigators (e.g. Ilse 1949; Quinn et al. 1974; Spatz et al. 1974; Fukushi 1976), and colour learning – and thus colour vision – has been demonstrated in *Drosophila* (Menne and Spatz 1977; Bicker and Reichert 1978; Reichert and Bicker 1979; Folkers and Spatz 1981; Hernandez de Salomon and Spatz 1983), *Protophormia* (Mazokhin-Porshnyakov et al. 1984) and *Lucilia* (Fukushi 1985). However, in almost all investigations the fly's movements were experimentally constrained, or the flies were even tethered. Furthermore, the trained flies were tested by using an alternative choice paradigm or exploiting the fly's proboscis extension reflex. In both cases monochromatic lights or translucent colour screens were used. These experimental conditions are quite different from the environmental conditions the flies encounter within their natural habitats. There, visual surroundings are neither monochromatic nor consist of bright light within a dark surround but colourful and lit by ambient illumination from sun and sky. In addition, the flies can freely walk or fly, depending on their motivation and the external stimuli available.

One of the difficulties in learning experiments of freely moving flies is to make flies motivated to search for food within a restricted place, as the honeybee does when trained to a feeding station. Recently, the searching behaviour elicited in flies whenever they are stimulated by short exposures to a droplet of sugar solution (Dethier 1957; Bell 1985) was used as an olfactory learning paradigm in the housefly, *Musca domestica* (Fukushi 1983). During training and testing the searching fly walked almost freely within an arena, 30 cm in diameter. The same paradigm was used in experiments on the visual learning of blowflies, *Lucilia cuprina*. In this experiment the flies had to discriminate between two different monochromatic light beams projected on to the floor of a dim arena (Fukushi 1985).

In the present account, individual blowflies are trained to a droplet of sugar solution offered on a patch of coloured paper under white overhead illumination. After having consumed the sugar solution an array of colour marks consisting of four colours (including the training colour) is displayed to the fly. This training and testing paradigm allows one to observe the responses of the fly not only to the training colour but also to colours on which the fly never was rewarded. A complete protocol of the fly's search behaviour yields information about colour learning and colour discrimination.

Materials and methods

Material. Sheep blowflies, *Lucilia cuprina*, were used throughout all experiments. The eggs deposited by the flies on a piece of swine liver were grown in a glass jar containing swine liver and insect food material (Oriental Yeast Co, Tokyo) swelled with water. Dry wheat brans were added on top of the food in a 2 cm thick layer to prevent the larvae escaping from the jar. The larvae pupated within the layer of wheat brans, where pupae were left until eclosion. Adults were reared with sugar, a piece of swine liver and water in a wooden rearing box (22 × 22 × 22 cm). Temperature of the rearing jars and boxes were controlled by a thermostat (25 ± 0.5 °C).

Newly emerged female flies were anaethetised in ice for 30 min. Then their wings were partially clipped off. The flies treated this way were starved in a glass vessel (9 cm wide and 9 cm high) but allowed to take water ad libitum. The glass vessel containing the flies was placed in a room, in which the temperature changed diurnally between 10 ° and 15 °C and illumination depended on the natural light sources incident from the windows. After 1 week, the flies were transferred to the experimental room, in which the temperature was kept constant at 25 ° ± 1 °C and fluorescent lamps (30 W) mounted on the ceiling were switched on and off with the natural day/night cycle. Only sufficiently starved flies that walked continously around on the floor of the vessel were used for the experiments. Prior to the experiments, the flies were transferred individually to a fly container (glass tube, 3 cm in diameter and 4.5 cm in height). Each fly was used only once.

Arena. Experiments were carried out in a circular arena (diameter 30 cm). The floor of the arena consisted of a non-glare glass plate (1.5 mm thick), which was placed on the horizontal surface of the experimental table. The whole arena was sheltered by a cylinder of black cardboard (30 cm wide and 15 cm high). Two black cardboards were placed together between the glass plate and the experimental table; the upper one was the 'training cardboard' or the 'black cardboard' (30 × 30 cm), the lower one the 'test cardboard' (35 × 35 cm). The training cardboard contained in its centre a patch of coloured paper (1 × 1 cm), which was missing in the black cardboard. The test cardboard contained a square array of colour patches (marks), each 1 cm^2 in size and exhibiting one of a set of 4 colours. The 4 colours are arranged in such a way that all nearest neighbours are of a different colour (Fig. 1). No colour mark was presented in the centre of the test array. The inter-patch distance was 3.5 cm. Coloured papers used as the training and testing colours were blue (B); green (G), yellow (Y), red (R), orange (O), white (W) and black (K). The standard test array consisted of blue, green, yellow and red marks (BGYR array). The reflection spectra of the coloured papers used are shown in Fig. 2 as thin lines. The arena was illuminated by a circular white fluorescent lamp (Hitachi FCL 20 W) mounted 20 cm above the glass plate. Its intensity was approximately 4500 lux as measured at the position of the fly. The spectral radiant flux of the fluorescent lamp is shown in Fig. 2 as a thick line. The fly's behaviour was observed from above through the central opening of the circular lamp.

Training and test procedures. Prior to training to a specific colour, naive flies were tested for their spontaneous preference to the coloured patches. A droplet of sucrose solution (0.5 mol/l, 1 µl) was offered at the centre of the black cardboard (where no colour mark occurred). An individual starved fly was released by placing its container upside down on the sugar droplet and allowed to contact the droplet and to feed on it. While the fly was feeding, the fly container was removed, and the circular lamp, which was illuminating the arena from about 40 cm height during the previous procedure, was brought down to 20 cm above the arena. After having consumed the droplet, the fly started gyrating around in narrow loops. The fly was allowed to walk about for 10–20 s. Then the black cardboard was quickly withdrawn by carefully raising the glass plate of the floor of the arena. This treatment hardly disturbed

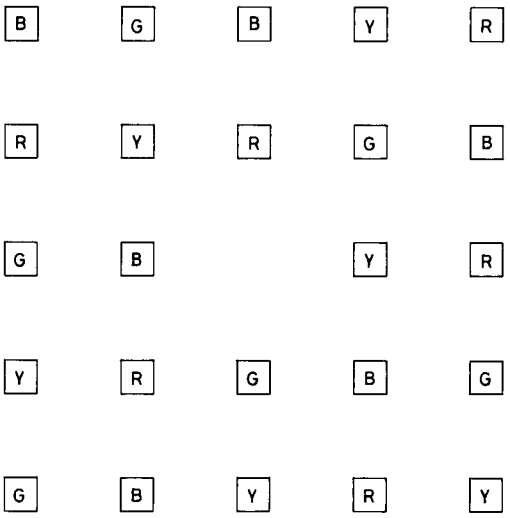

Fig. 1. Test array presenting 4 different colour stimuli (patches of coloured paper). Only the central part of the standard array is shown. *B*, blue; *G*, green; *Y*, yellow; *R*, red

the search behaviour of the fly, which now was positioned in the centre of the test arena of colour marks. The fly continued its searching walks and visited the colour patches one by one without receiving any reward. The colours of the patches visited by the fly were recorded sequentially until the fly had made 10 visits (termed one 'run').

A 'visit' was recorded when the fly intruded into a colour patch at least with its entire head. A new visit was recorded only after the whole body of the fly had left the patch. Even though the fly occasionally happened to ascend on the wall of the arena, recording was continued in almost all cases, because the fly usually descended immediately and started to search again on the floor of the arena. The experiment was broken off only when the fly climbed over the wall or when the fly needed more than 5 min to make 10 visits in a sequence.

Subsequently, the fly was trained to a specific colour by letting it feed on a sugar droplet placed on the colour patch of the training cardboard. Then the fly was tested again as described above. In each fly, this training/testing procedure was repeated 3 times in immediate succession. Thus, each fly performed 4 runs (consisting of 10 visits each). After each run the test cardboard was rotated by 90 ° to avoid positional learning due to visual cues outside the arena. Before a new fly was used in the experiments, the glass plate on the floor of the arena was exchanged by a new one. Unless otherwise mentioned, 50 flies were used in each experiment. Several flies did not manage to complete 4 runs, and incomplete data were rejected.

In the first type of control experiment, the fly was confronted with the colour stimulus without sugar presentation and then tested. In this case, the fly was confined individually within a fly container, of which the outer walls as well as the floor were made of coloured paper (blue or yellow). The fly container was illuminated by a 15 W fluorescent lamp (25 cm above). After having been subjected to the colour stimulus for 1 min the fly was allowed to rest for 30 s within an ordinary fly container illuminated by the same fluorescent lamp. Then a sugar droplet was presented at the centre of the black cardboard to elicit the search behaviour of the fly. After 10–20 s of searching walks, the fly was tested.

The second type of control experiment dealt with memory retention. The fly was trained to blue or yellow by presenting it twice with a sugar droplet, the second reward following 15 s after the first. Afterwards, the fly was kept individually in a fly container (lit by a 15 W fluorescent lamp 25 cm above) for 0, 10 or 60 min and tested in the usual way.

To reduce glare caused by reflection from the coloured paper, sheets of neutral density filters (Sakura ND.15 and ND.3, transmis-

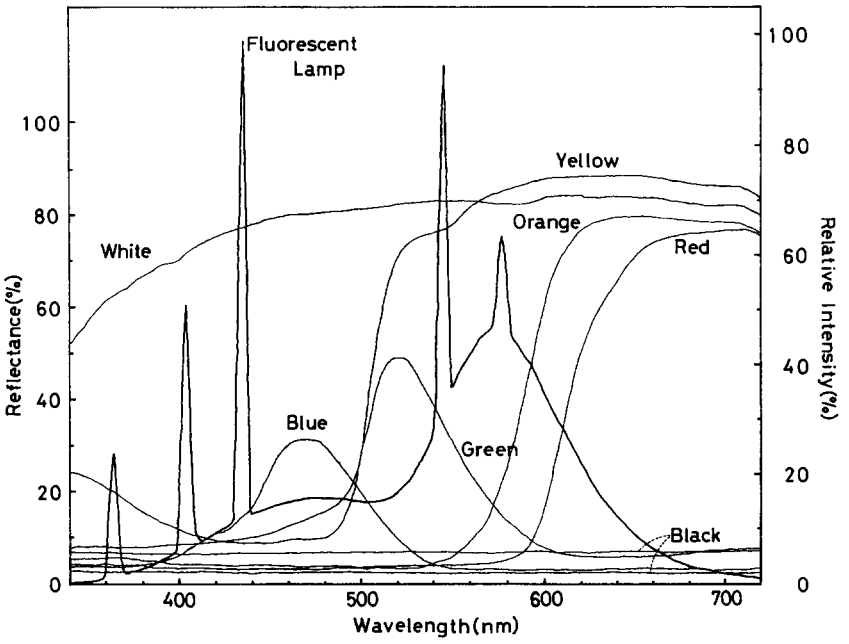

Fig. 2. Spectral reflectance curves of the coloured papers used in the experiments (thin lines; measured by Shimadzu spectrophotometer MPS-5000, using MgO as reference) and spectral radiant flux of the white fluorescent lamp illuminating the array (thick line) (courtesy of Hitachi). The upper reflection spectrum indicated as 'black' is that of the black background (a mount of training and test cardboard) and the lower 'black' spectrum is that of the black paper used as the colour patches

sion 70.5% and 51.2%) were used. The colour patch of the training cardboard was covered with a piece of sheet measuring 2 × 2 cm. In the test array the whole cardboard was covered with a large filter sheet (30 × 30 cm).

Relative fly's subjective brightness of coloured papers. The relative fly's subjective brightness of each coloured paper used was calculated by the equation $B = \int_{340}^{650} R(\lambda)\,\phi(\lambda)\,S(\lambda)\,d\lambda$, where $R(\lambda)$ = spectral reflectance of the coloured paper (Fig. 2), $\phi(\lambda)$ = spectral radiant flux of the illuminating lamp (Fig. 2) and $S(\lambda)$ = spectral sensitivity of the fly. As the spectral sensitivity function of the fly for brightness is not known, it was tentatively calculated by adding that of the 3 types of photoreceptor (R 1–6, R 7 and R 8) obtained from intracellular recordings in *Calliphora* by Hardie (1979; Figs. 3 and 4). This calculation is based on the assumption that the physiological responses from each receptor type are simply summed. For R 7 cells, that of the major subclass, which has a peak at ca. 360 nm and a long tail extending into the green, was used. The curves were weighted with the ratio of absolute sensitivity at peak wavelength (R 1–6, 499 nm; R 7, 358 nm; R 8, 541 nm). Hardie (1979) has obtained the axial peak sensitivities of 3 types of photoreceptor at the 50% level (APS_{50}), which are defined as the reciprocal of the number of quanta·s^{-1}·cm^{-2} of peak wavelength required to generate a 50% V_{max} response when using an axial point source (Laughlin 1976). The relative values of this measure were R 1–6:R 7:R 8 = 1:3.07:2.07. The relative sensitivity of R 1–6 to R 7/8 for an extended source of light caught by a rhabdomere is obtained from multiplying that for a point source by a factor of the ratio of the rhabdomere diameters; d(R 1–6)/d(R 7/8) = 1.6 in *Calliphora* (Hardie 1979). The relative sensitivity of R 1–6:R 7:R 8 = 1:1.92:1.29. In considering neural superposition the relative sensitivity of R 1–6 cells may be multiplied by a factor of 6; thus, the relative sensitivity of R 1–6:R 7:R 8 = 1:0.32:0.22. Using this value the spectral sensitivity function of the fly as shown in Fig. 3 is obtained. Relative values of the fly's subjective brightness of the coloured papers used are shown in Table 1.

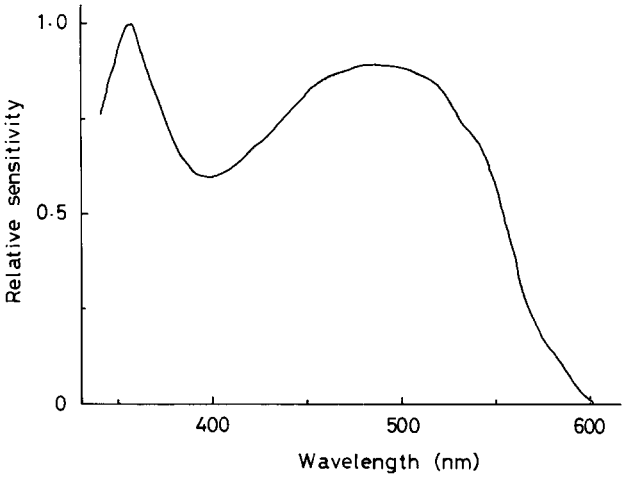

Fig. 3. Spectral sensitivity function of the fly. The curve was synthesised from those of the 3 types of photoreceptor (R 1–6, R 7 and R 8) obtained by Hardie (1979). For more details see text

Results

Training to blue, green, yellow and red

The flies were trained to blue and tested on the standard test array. Results are given in Fig. 4.

In naive flies the response frequencies are highest to yellow in the beginning of a test run but then gradually decrease to a level similar to that for blue and red (Fig. 4A). Throughout the whole run, the green stimulus received the lowest response frequencies.

After the first training to blue, the fly's response frequencies to blue are remarkably high throughout the whole run, although they tend to decrease at later visits (Fig. 4B). Concomitantly, other colours receive very low response frequencies. This is especially true for the green stimulus. The response frequencies do not change significantly after one or more trainings to blue (Fig. 4C, D; ns, χ^2-test).

As changes of the response frequencies to various colours throughout the whole run are relatively small (although the response frequencies between the first and second 5 visits of the test run are significantly different in the second and third trainings but not in the first training), and such treatment of data seems not to pro-

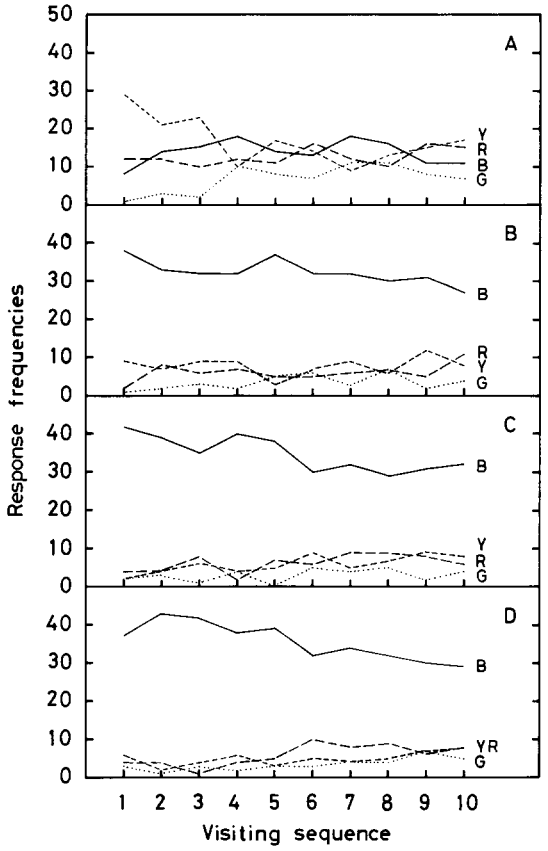

Fig. 4A–D. Response frequencies of flies trained to blue. **A** Naive flies; **B–D** flies tested after having been trained to blue once (**B**), twice (**C**) or three times (**D**). Ordinate, response frequencies to blue (B), green (G), yellow (Y) and red (R). Abscissa, visiting sequences

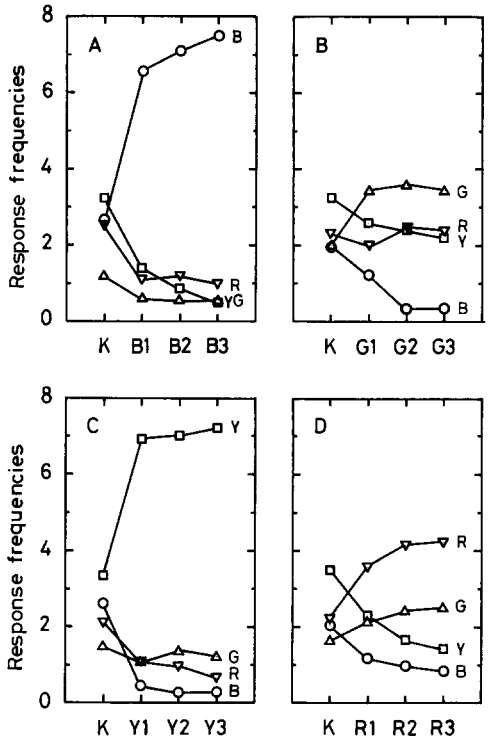

Fig. 5A–D. Response frequencies after the flies have been trained to blue (**A**), green (**B**), yellow (**C**) and red (**D**). Ordinate, response frequencies (medians). Abscissa, *K*, naive flies; *B1–3*, flies trained to blue 1, 2 or 3 times. Same notation is applied for trainings to other colours. For further conventions see Fig. 4

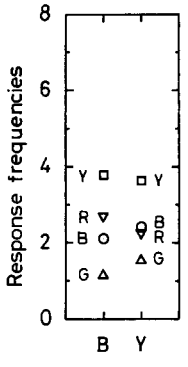

Fig. 6. Control experiment I. Separation of colour stimulus and reward. The flies were presented with the blue or yellow stimulus but not rewarded. Prior to testing, they were presented a sugar droplet and then tested on the standard array

vide much useful information about the degree of learning, the visiting sequence is expediently ignored in the following representations. Instead, the median of the responses of the flies tested is calculated for each colour.

Figure 5 presents the results of experiments in which the flies are trained to blue, green, yellow and red. In naive flies the spontaneous response frequencies always reach maximum values for yellow and minimum values for green.

After training to a specific colour, the response frequencies to the colour stimuli increase significantly and are higher than those to other colours (third training, $P<0.001$, Mann-Whitney *U*-test). The effects of training are especially remarkable in blue and yellow (Fig. 5A, C). The responses to the untrained colours in the test array are also specifically influenced by the training colour. In the training to blue the response frequencies to yellow decrease remarkably and those to yellow and green reach the lowest value (Fig. 5A). In the trainings to green and yellow, blue stimuli receive the lowest response frequencies (Fig. 5B, C). In the training to red the response frequencies to green increase, while those to blue and yellow decrease (Fig. 5D; K vs. R3, $P<0.001$, Mann-Whitney *U*-test).

Two control experiments were performed to decide whether or not the observed changes in the fly's choice behaviour are really due to learning (coupling of the visual stimulus with the reward). In the first control, the presentation of the colour stimulus and that of the food droplet are separated in time. In the second control, training and testing procedures are separated.

First, the flies are presented with an unrewarded blue or yellow stimulus for 1 min. After 30 s of rest, food is offered, and the fly is tested immediately thereafter. The results are shown in Fig. 6. The flies' responses do not differ significantly between the blue and yellow stimulus (ns, Mann-Whitney *U*-test) and between colour-stimulated flies and naive flies (Fig. 5) (ns, Kruskal-Wallis test).

Second, after training to blue or yellow, the flies are kept for periods of 0, 10 or 60 min individually in a fly container under illumination and then tested. The response frequencies to the trained colours are remarkably high at all times after training, but they gradually decrease for longer time intervals (Fig. 7; time interval 0 min vs. 60 min, $P<0.006$, Mann-Whitney *U*-test).

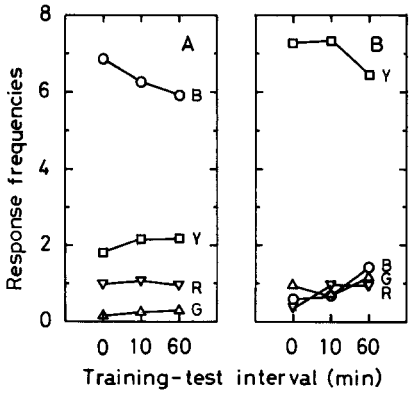

Fig. 7A, B. Control experiment II. Effect of the time interval between training and test on the response frequencies of the flies. After training to blue (**A**) or yellow (**B**), flies were kept individually in a fly container for various time intervals and then tested

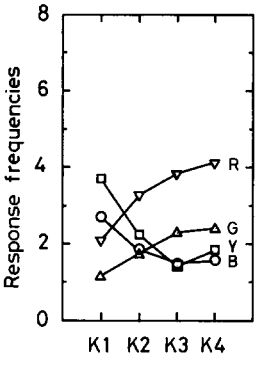

Fig. 8. Training to black. Flies were trained to black and tested on the standard test array. *K 1–4*, 1st to 4th training to black stimulus. 80 flies were used

Both results show that the changes in the flies' responses caused by training are not simply due to some kind of sensitisation by the training colour but to real associative colour learning.

Training to black

When the flies are trained to black (presentation of a sugar droplet on the black cardboard), they later behave as if they had been trained to the red stimulus (Fig. 8). The response frequencies to red and green increase and those to blue and yellow decrease with increasing numbers of reinforcements (K 1 vs. K 4, $P<0.001$, Mann-Whitney U-test).

In another series of experiments, the red stimuli of the standard (BGYR) test array are replaced by black ones (the BGYK test array). The black paper used for the black stimuli is slightly darker than the black cardboard used as background (Table 1 and Fig. 2), so that the experimenter can easily discriminate the black patches from the black background. The flies are trained to blue, green, yellow or black and tested on that test cardboard.

The results are shown in Fig. 9. In naive flies the response frequencies to black are similar to those to blue. The flies trained to specific colour visit the colour stimuli of the BGYK array in quite similar frequencies to those on the standard test array (Fig. 5; ns in all response frequencies in third training between BGYK and BGYR test arrays except that of green visits in red/black training, Mann-Whitney U-test).

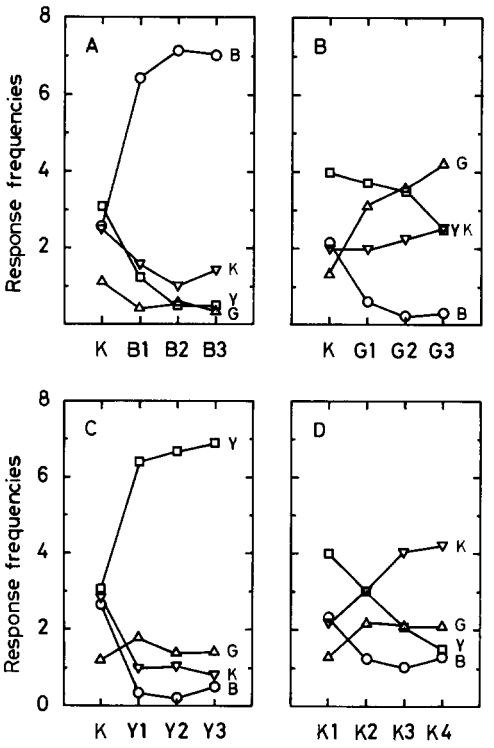

Fig. 9A–D. BGYK test array. The flies were trained to blue (**A**), green (**B**), yellow (**C**) or black (**D**) and then tested on the BGYK test array (in which red was replaced by black)

Table 1. Relative values of the subjective brightness of the coloured papers used in the experiments, taking the value of the black background as 1

Coloured paper	Relative brightness
Blue	1.8
Green	3.8
Yellow	7.3
Red	0.5
Orange	0.9
White	13.1
Black paper	0.3
Black cardboard (background)	1.0

These results show that red and black are treated similarly by the flies and appear to them as weak stimuli (the relative brightness values of red and black papers are 0.5 and 0.3, respectively; Table 1).

Training to orange

The former experiments have shown that *Lucilia* can be trained easily to blue and yellow but that it is difficult to train them to green (Figs. 5B, 9B). One reason could be that the reflection spectrum of the yellow and green stimuli overlap considerably (Fig. 2). Thus, the yellow patches of the standard test array were replaced by orange (BGOR test array). The reflection spectrum of the orange stimulus overlaps with that of the green stimulus only at its marginal region (Fig. 2).

The results shown in Fig. 10 indicate that the flies encounter considerable difficulties in learning orange and green stimuli when both are present in the test array.

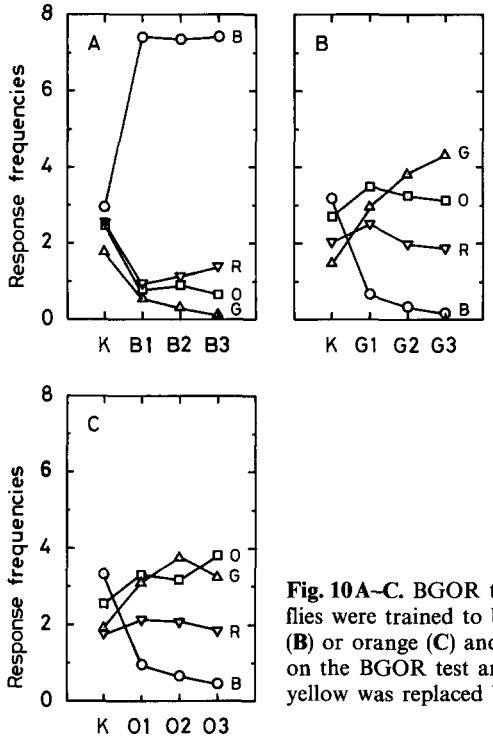

Fig. 10A–C. BGOR test array. The flies were trained to blue (**A**), green (**B**) or orange (**C**) and then tested on the BGOR test array (in which yellow was replaced by orange)

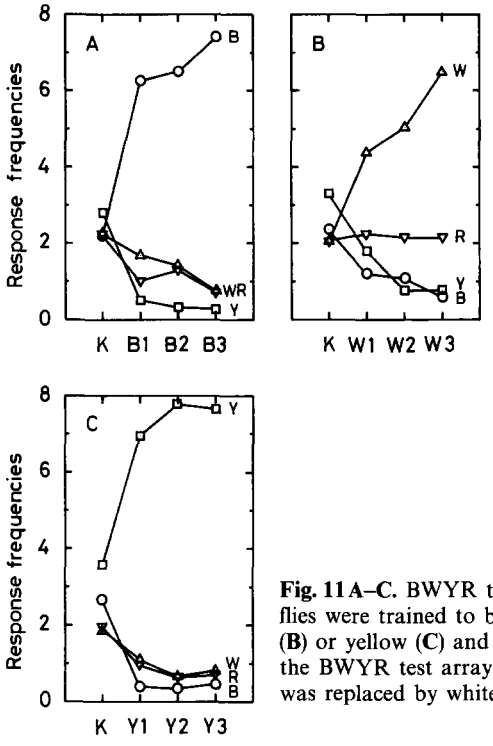

Fig. 11A–C. BWYR test array. The flies were trained to blue (**A**), white (**B**) or yellow (**C**) and then tested on the BWYR test array (in which green was replaced by white)

In the flies trained to green the response frequencies do not change significantly between BGYR and BGOR test arrays (Figs. 5B, 10B; ns, Mann-Whitney *U*-test).

Similar results are obtained when the yellow patches of the standard test array are replaced by black (BGKR test array) (data not shown); for the black marks 1 cm squares are lightly drawn by pencil on the test cardboard.

Training to white

In experiments in which the test array contains white stimuli instead of green ones (BWYR array), and in which the flies are trained to either blue, white, or yellow, the flies learn white well after repetition of training, even though the response frequencies are lower than to blue and yellow (Fig. 11; W vs. B and W vs. Y in third trainings, $P<0.001$, Mann-Whitney *U*-test).

Reduction of reflection rates of the coloured papers

To investigate whether discrimination of the coloured papers depends on their hue or the brightness contrast, flies were trained to the colour stimulus at a reduced intensity (the colour patch of the training cardboard is covered with neutral density filters of either 71% or 51% transmission) and then tested on the standard test array (without the neutral density filters).

The reduction of the intensity of the training stimulus with the 71% transmission filter causes attenuation of the effects of training (compare Fig. 12A, B with Fig. 5A, C; third training, $P<0.001$, Mann-Whitney *U*-test). In using the 51% transmission filter the response frequencies to the trained colour do not increase after training (Fig. 12C, D; K vs. B3 and Y3, ns, Mann-Whitney *U*-test), while those to yellow in blue training and to blue in yellow training still decrease remarkably (K vs. Y3 and B3, $P<0.001$, Mann-Whitney *U*-test).

The flies trained to the colour at reduced intensity by the 51% transmission filter were tested on the BGYR test array covered with the same neutral density filter. In naive flies all 4 colour stimuli of the test array receive similar response frequencies (Fig. 13; ns, Kruskal-Wallis test). After training to blue, the response frequencies to blue increase and those to yellow decrease (Fig. 13A; K vs. B3, $P<0.001$, Mann-Whitney *U*-test). After training to yellow, the response frequencies to yellow increase (K vs. Y3, P 0.001, Mann-Whitney *U*-test), while those to blue are not different from green and red (Fig. 13B; K vs. Y3, ns, Kruskal-Wallis test).

Discussion

Throughout the present work, several kinds of 4 colour test arrays were used, and naive flies were tested for their spontaneous colour preference on them. The results tested on the standard (BGYR) test array are pooled in Table 2. Yellow is visited most frequently and green, least frequently. Blue is visited more frequently than red ($P<0.05$, *t*-test). On the BGOR test array, orange is visited more frequently than green ($P<0.005$, *t*-test) and less frequently than blue ($P<0.02$, *t*-test) (Fig. 10). On the BWYR test array, white is visited similarly to blue (Fig. 11; ns, *t*-test). Spontaneous colour preferences may be $Y>B$, $W\geq O$, $R>G$, while the order rank of the subjective brightness to the fly is $W>Y>G>B>O>R$. Thus, the spontaneous colour preferences of the fly on the test array do not simply depend on subjective brightness. Hecht et al. (1968) demonstrated the selection of resting surfaces of various colours in *Musca domestica*. The attractiveness of colours was on the order of $Y>R\geq W\geq B>G>K$ in the open air, and

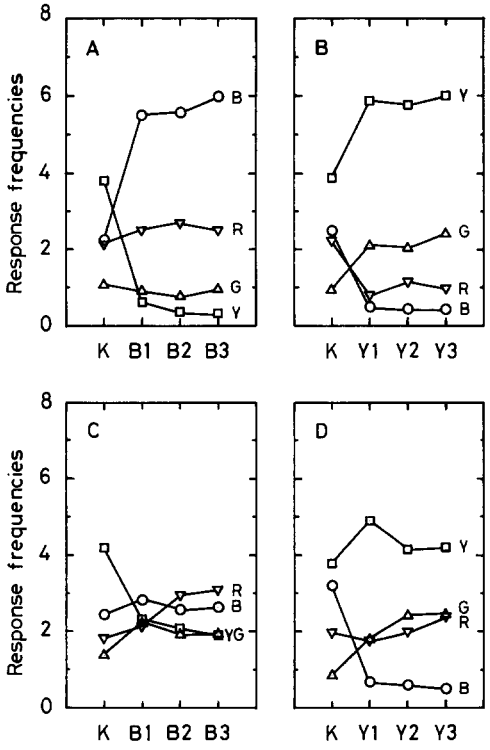

Fig. 12A–D. Reduction of light intensities of the training stimuli. The flies were trained to blue (**A, C**) or yellow (**B, D**) stimuli, in which the training patches were covered by an either 71% (**A, B**) or 51% (**C, D**) transmission neutral density filter. Then the flies were tested by using the standard test array not covered by any neutral density filter

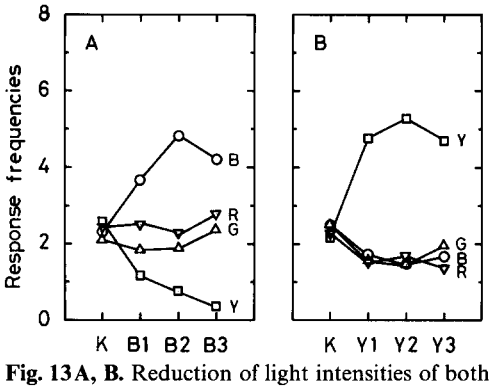

Fig. 13A, B. Reduction of light intensities of both the training and the test colours. The flies were trained to blue (**A**) or yellow (**B**). The 51% transmission filter was used in both cases

Table 2. Spontaneous response frequencies of naive flies to blue, green, yellow and red. Means and standard deviations of nine tests (Figs. 5, 8, 12)

Blue	Green	Yellow	Red
2.48±0.38	1.33±0.36	3.63±0.32	2.15±0.20

K>R>Y>G≥W>B in the laboratory. The rank order of colour preferences obtained in the present experiments resembles that of experiments in the open air by Hecht et al. (1968).

Blue and yellow are the colours the flies learn easily, and they can discriminate well between them (Figs. 5 and 9). White is also a colour discriminated well, although the response frequencies are significantly lower than to blue and yellow (Fig. 11). Green is discriminated clearly from blue, but weakly from yellow and orange; in green training the response frequencies to yellow and orange do not decrease considerably compared with blue (Figs. 5B, 9B, 10B).

The colours used in the experiments determined their loci in the colour triangle, which represent the contributions of the 3 types of photoreceptor (R1–6, R7 and R8) in colour vision (Rushton 1972; Menzel 1979; Neumeyer 1980), although it is not known how many types of photoreceptor participate in the colour vision of flies. Inputs to each receptor were calculated by the equations,

$$X=\int_{340}^{650} ar_{R1-6}(\lambda)\,R(\lambda)\,\phi(\lambda)\,d\lambda,$$

$$Y=\int_{340}^{650} br_{R7}(\lambda)\,R(\lambda)\,\phi(\lambda)\,d\lambda$$

and

$$Z=\int_{340}^{650} cr_{R8}(\lambda)\,R(\lambda)\,\phi(\lambda)\,d\lambda,$$

where $r(\lambda)$=spectral sensitivity of each receptor type, $R(\lambda)$=spectral reflectance of the coloured paper and $\phi(\lambda)$=spectral radiant flux of the illuminating lamp. The factors, a, b and c are the values of the relative peak sensitivities of the R1–6, R7 and R8 cells, respectively (1, 0.32 and 0.22; see Materials and methods). Three coordinates of a colour locus are obtained with $x=X/W$, $y=Y/W$ and $z=Z/W$, where $W=X+Y+Z$ and $x+y+z=1$. Colour loci of the coloured papers used in the experiments are shown in Fig. 14A. All loci gather near the R1–6 corner. It is still an open question whether the influence of neural superposition for inputs of the R1–6 type of photoreceptor in the colour vision system can be arithmetically added. When the factor of neural superposition is ignored (a:b:c=1:1.92:1.29; see Materials and methods), the result shown in Fig. 14B is obtained; the loci are scattered, and the spatial relationship among loci becomes obvious. The loci of blue and yellow are most distant, that of white in the middle between them and loci of green and orange near yellow. Thus, the features of colour discrimination seem to reflect the colour space of the coloured papers. As the spectral loci represent the hue of colour stimuli and ignore the brightness of them, the coloured papers seem to be discriminated mainly by the hue of colours.

If discrimination of coloured papers depends on the brightness of colours, reduction of the intensity of the training stimulus may cause the flies to respond preferably to the colour with lower brightness than the trained colour on the standard test array. The 71% transmission neutral density filter reduces the intensity of reflected lights to approximately 1/2 and the 51% transmission filter, to approximately 1/4. This means that the yellow paper covered with the 71% transmission filter produces a similar brightness to green and that with the 51% filter similar to blue. With reduction of the intensity of

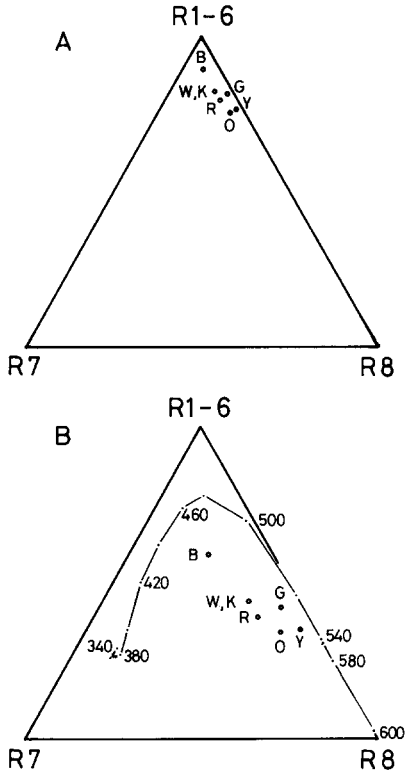

Fig. 14A–B. Colour space in the fly. Each corner of the triangle represents the locus of a colour, by which one type of photoreceptor cell is exclusively excited. The colour loci of the coloured papers used in the experiments are shown in the triangle (*circles*). *B*, blue; *G*, green; *Y*, yellow; *R*, red; *O*, orange; *W*, white; *K*, black. White and black occupy the same locus. **A** Relative sensitivity of R1–6:R7:R8 is 1:0.32:0.22. **B** Relative sensitivity of R1–6:R7:R8 is 1:1.92:1.29. Loci of the pure spectral lights are represented as the *line with dots*

the training stimulus, the response frequencies to yellow decreased, but neither a specific increase of the response frequencies to green or blue nor reversal of preference was observed (Fig. 12B, D). This shows that the brightness of colours is not the major stimulus for discrimination of the coloured papers.

It has been demonstrated that *Drosophila* can be conditioned to discriminate between not only wavelengths but also intensities of light stimuli (Bicker and Reichert 1978; Reichert and Bicker 1979). In the present account, when the flies are trained to blue or yellow with reduced light intensity, the response frequencies to the trained colour increase remarkably on the test array with reduced light intensities as well (Fig. 13A, B), while they hardly increase on the brighter test array (Fig. 12C, D). This suggests that *Lucilia* may also be able to use brightness to discriminate colour stimuli, though it is not the major cue.

Green is brighter than blue (Table 1), but the response frequencies to the trained colour in green training never reached a high value (Figs. 5B and 9B), unlike those obtained in blue, yellow or white trainings (Figs. 5A, C, 9A, C, 11B). This is also true in the experiments using the test array of BGOR (Fig. 10B) and BGKR. Green may be a difficult colour for learning. Colour dependency of learning acquisition has also been reported in honeybees; violet (400–420 nm) is learned fastest and bluish-green (490 nm) most slowly (Menzle 1967, 1985).

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