Attractiveness versus stickiness: Behavioural preferences of *Drosophila melanogaster* with competing visual stimuli

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ABSTRACT

In nature, animals often encounter various competing stimuli and must make choices among them. Although the behaviour under two identical stimuli has been extensively studied for fruit flies, *Drosophila melanogaster*, how the appeal of one stimulus for the animals is influenced by the appeal of the other is not fully understood. In the present study, we systematically investigated this equation using a modified Buridan's paradigm. We focused on the behaviour of fruit flies under asymmetric visual stimuli, i.e., two black stripes of different widths. We characterized two behaviour modes: (1) Attractiveness: moving toward a stripe in the inner area of the platform, and (2) Stickiness: staying around the edge near a stripe. Our results reveal that while Attractiveness of a stripe is primarily influenced by its own width and remains relatively independent of the opposite stripe, Stickiness is significantly affected by the width of the competing stripe. These findings suggest that the behavioural response of fruit flies to visual stimuli involves complex decision-making processes influenced by both intrinsic and extrinsic factors. This study provides new insights into the cognitive and sensory mechanisms underlying visual preference behaviour in *Drosophila* and highlights the importance of considering multiple stimuli in behavioural assays.

1. Introduction

In nature, animals often face various attractive or competing stimuli and need to make choices among them. Previous research has demonstrated that animals exhibit various simple taxis when responding to individual stimulus (Davies et al., 2015; Gepner et al., 2015). However, when multiple stimuli are present, the behavioural responses of animals may involve complex decision-making processes. Fruit flies, *Drosophila melanogaster*, are known to exhibit different types of taxis, including odour taxis, chemotaxis, phototaxis, scototaxis, thigmotaxis (Besson and Martin, 2005), and menotaxis (Gong, 2009; Gorostiza et al., 2020; Heisenberg and Wolf, 1984; Rockwell and Seiger, 1973). However, simple taxis alone cannot fully explain behaviour patterns under multiple stimuli.

The classic Buridan's paradigm is commonly used for studying fruit flies' behaviour under competing stimuli (Colomb and Brembs, 2015; Götz, 1980; Linneweber et al., 2020; Neuser et al., 2008; Strauss and Pichler, 1998; Wehner, 1972). Buridan's paradigm, initially named after the 14th-century French philosopher Jean Buridan, represents a thought

experiment on the concept of free will. In the most commonly told variant of this experiment, a donkey is placed equidistantly between two equally appealing hay stacks. Because the donkey is unable to make a rational decision, it eventually dies of starvation. Buridan's paradigm was first introduced into *Drosophila* visual experiments by Götz in 1980 (Götz, 1980), and subsequent studies further tested and modified the paradigm to study various cognitive behaviours and the underlying neural mechanisms (Colomb et al., 2012; Colomb and Brembs, 2015; Gorostiza et al., 2020; Guo et al., 2015; Han et al., 2021a, 2021b; Linneweber et al., 2020; Neuser et al., 2008; Strauss and Pichler, 1998; Yen et al., 2019). In the classic setting, a single fruit fly with shortened wings is placed on a circular platform with two equally wide black stripes on the wall, separated by 180 °. The fly may randomly fixate on one of the stripes by moving toward it. Additionally, after reaching the edge of the platform, the fly may stay at the edge, characterizing the fly's preference for the boundary and tendency to avoid the central zones, particularly in open-field scenarios (Besson and Martin, 2005). Typically, the fly does not stay in the edge region for long and may resume moving toward the opposite stripe (Götz, 1980; Strauss and Pichler, 1998; Wehner, 1972).

The classic Buridan's paradigm uses two identical stripes. However, in real life, organisms rarely encounter two identical or equally appealing stimuli. Does the saliency deterministically decide the behaviour and the flies always choose the more salient stimulus? Do the flies make their choices stochastically? If they do, whether the preference for one stimulus depends on the saliency of the other? To answer the questions, we investigated the response of fruit flies in an asymmetric visual stimulus environment by modifying the classic Buridan's paradigm. We quantified the behaviour in the inner circle and the outer rim of the platform and displayed two dark stripes with different widths, serving as an asymmetric visual stimulus. We analysed the movement trace of the flies and quantified their behavioural characteristics. Furthermore, we analysed the similarities and differences among the wild-type strains. Our results demonstrated behavioural patterns that are more complex with asymmetric visual targets than with symmetric ones.

2. Materials and Methods

2.1. Fly strains

The fruit flies were raised at a temperature of 25 °C under a 12 h light–dark cycle with a humidity of ~ 50 %. The wild-type strain, *Canton-S*, was obtained from the Bloomington *Drosophila* Stock Center in 2018 (Bloomington stock #64349). The w^+ strain was used and obtained from the Brain Research Center (BRC) at National Tsing Hua University (NTHU), Taiwan.

2.2. The behavioural arena and tracking System

The behavioural tracking apparatus used in this study was similar to those used in previous studies (Han et al., 2021b). The arena consisted of a round platform with a diameter of 100 mm, surrounded by water to prevent flies from escaping (Fig. 1A). The platform was surrounded by a screen with six circular fluorescent light tubes (order product name: MASTER YL5 Circular 55 W/840 1CT/10), which had a diameter of 180 mm and a height of 190 mm. A CCD camera, which shoots 15 frames per second, was positioned directly above the platform to record the movement traces of fruit flies. The tracking was done using a Python 3.5 script. All movement traces were then adjusted to have the origin at (0, 0) with a radius of 50 mm, and all movement distances were measured in millimetres.

2.3. Behaviour preference task

We utilised 2- to 3-day-old wild-type flies in the present research. The wings of flies were clipped one day prior to the experiment. The behavioural task was modified from Buridan's paradigm (Götz, 1980; Neuser et al., 2008; Strauss and Pichler, 1998) (Fig. 1A). In the classic Buridan's paradigm, two narrow dark stripes serving as the visual stimulus are placed 180 ° apart on the circular screen. We made changes to the numbers and widths of the stripes. We used all-bright and all-dark conditions and eight different stripe widths in one-stripe condition: 10 °, 20 °, 30 °, 60 °, 90 °, 120 °, 150 °, 180 °, with the actual widths 15.71, 31.42, 47.12, 94.25, 141.37, 188.50, 235.62, 282.74 mm, respectively. We also used nine different stripe widths in two symmetric stripe conditions: 10 °, 20 °, 30 °, 40 °, 50 °, 60 °, 90 °, 120 °, 150 °, and six stripe widths in two asymmetric stripe conditions: 10 ° and 30 °, 10 ° and 60 °, 10 ° and 90 °, 30 ° and 60 °, 30 ° and 90 °, 60 ° and 90 °. The actual widths of the 40 ° and 50 ° stripes are 62.83 and 78.54 mm, respectively. The stripe centred at 0 ° in one-stripe conditions, while the stripes centred at 0 ° and 180 ° in two-stripe conditions. In each trial, only one fly was placed on the platform, and the task duration was 90 s.

2.4. Simulated fly traces

To simulate the traces of the fruit flies, we implemented a random-walk model based on the power-law distribution of the rotation angle and the Cauchy distribution of the moving distance per video frame (Fig. 1). Both distributions were derived from the traces of real flies under all-bright and all-dark conditions. When the rotation angle is positive (counterclockwise) or when calculating the movement distance, the power-law distribution can be described by the following equation:

$$y = a * x^{-n} \tag{1}$$

and when the rotation angle is negative (clockwise), the power-law distribution is described by:

$$y = a * (-x)^{-n} \tag{2}$$

where x is the rotation angle of the fruit fly, and y is the proportion of this rotation angle. Parameters a and n were determined by the curve that fit the corresponding distribution of real flies. We considered the rotational angle smaller than 0.33 ° per frame (~5 ° / s) as a straight movement and only fit the power-law distribution to the data that exceeded this value. Based on the criterion, we can also determine the probability of rotational movement per frame, which is an important parameter for the simulation.

On the other hand, we use the Cauchy distribution to fit the moving distance per frame of fruit flies. The Cauchy distribution is described by:

$$f(x; x_0, \gamma) = \frac{1}{\pi \gamma \left[1 + \left(\frac{x - x_0}{\gamma} \right)^2 \right]} \tag{3}$$

where x_0 is the location parameter, representing the peak position of the distribution, and γ is the scale parameter, representing the half-width of the distribution. Considering the noise caused by the small wobbling or none-walking movement exhibited by the flies, we neglected the movement that is smaller than two pixels (~0.44 mm) per frame in the data. By fitting the Cauchy distribution to the data, we can obtain these parameters that describe the statistics of the fruit flies' moving distances.

In each time step (corresponding to one video frame in the experiment), we first determined whether the agent performed a rotation based on the probability calculated from the data. If a rotation was needed, we drew a rotation angle from the power-law distribution and rotated the agent. Next, we determined a moving distance from the Cauchy distribution described above and moved the agent. When the agent approached the edge of the platform, if the next movement caused the agent to move out of the platform, the movement was neglected, and a new movement was drawn from the distributions again until the new movement stayed within the platform.

2.5. Data analysis

The present research aimed to explore the preference behaviour in different asymmetric visual conditions. Therefore, we designed behavioural measures and analytical methods to quantify the index of the preference behaviour. We first analysed the movement trace density, which showed the relationship between the distance of the fruit fly from the centre of the platform and the time spent by flies. The movement trace density was calculated by dividing the platform into 20 concentric circles with equal radius increments, and then dividing the time spent by the area of each circle.

Based on the previous research (Han et al., 2021b), we defined the border of the inner circle and the outer rim of the platform as a radius of 0.85. The percentage of time spent in the inner circle and the outer rim was calculated by all time spent within the 0.85 radius and between the 0.85 radius and the edge, respectively. Next, to analyse the behaviour
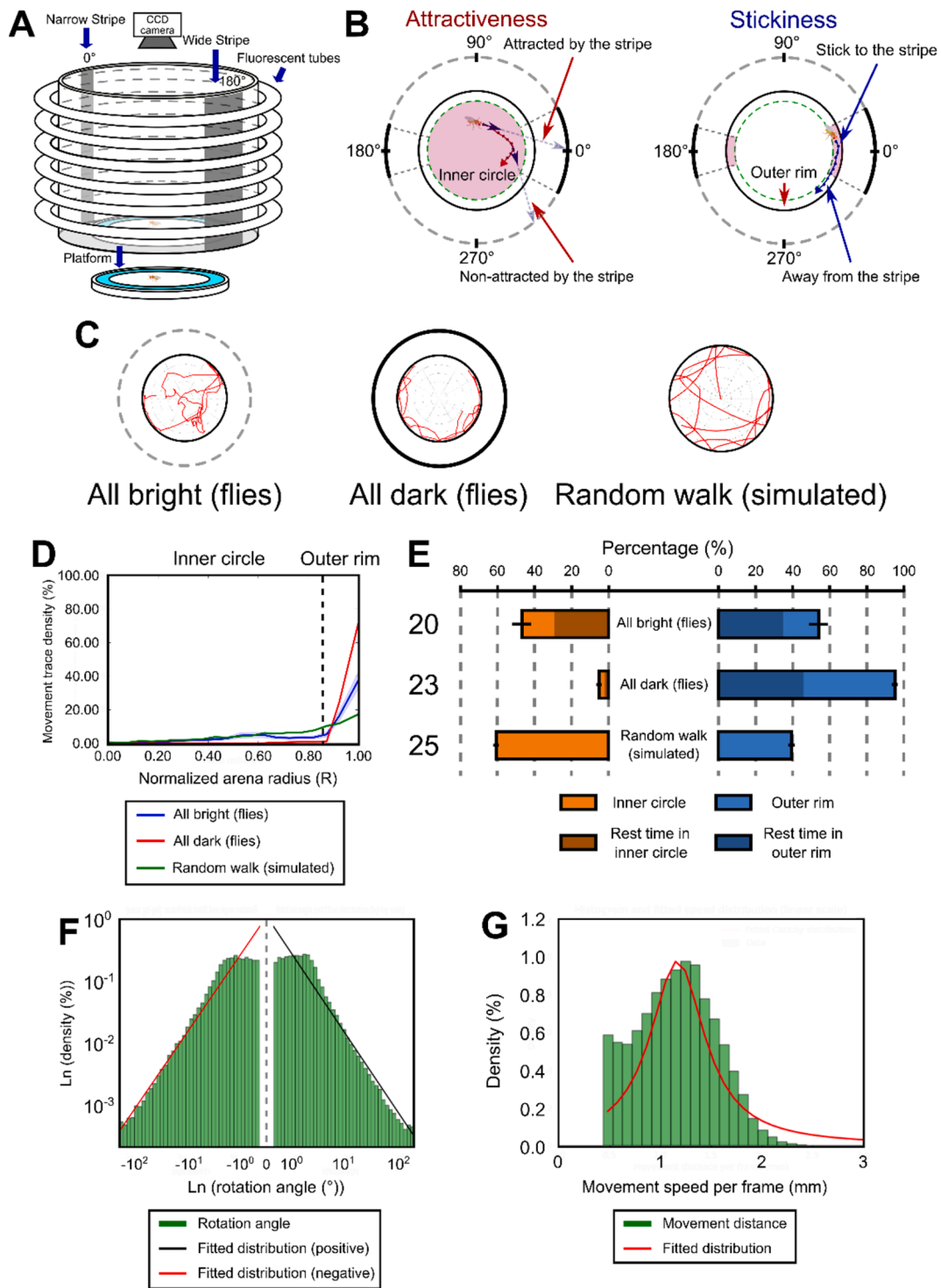
Fig. 1. Experimental setup and the baseline behaviour. (A) The behavioural arena. A circular platform was surrounded by water and a screen illuminated by six circular fluorescent tubes. Dark vertical stripes made of black cardboard were placed on the screen to serve as the visual stimulus. In each trial, a single wing-shortened fruit fly was placed on the platform and allowed to move freely for 90 s. (B) Left: when the fly was in the inner circle (within 0.85 of the platform's radius, the dashed green circuit), we recorded the head direction in each video frame and computed the attractiveness index for each stripe. Right: when the fly was in the outer rim (outside 0.85 of the platform's radius), we recorded the fly's location in each video frame and computed the index of the stickiness for each stripe; see Materials & Methods for details. (C) Typical traces of single fruit flies in the all-bright (left), all-dark (middle) conditions, and a random-walk simulation (right). (D) The movement trace density (per unit area) for the flies in the all-bright condition (the blue line), the all-dark condition (the red line) and the random-walk simulations (the green line). The shaded area indicated the standard error of the mean. (E) The percentages of time flies spent in the inner circle (orange bars) and the outer rim (blue bars) in the observed all-bright and all-dark conditions, and random-walk simulations. The shaded area in each bar indicates the proportion of time at rest in each condition. Numbers to the left of the orange bars indicate the number of flies in each group. (F) Distribution of the rotation angle per video frame and the power-law curve fitting. (G) Distribution of the moving distance per frame and the Cauchy curve fitting.

preference in the inner circle and the outer rim, we defined the attractiveness A, which quantifies how well the flies preferred to move toward the visual stimulus in the inner circle, and the stickiness S that quantifies how well the flies preferred to stay near the visual stimulus in the outer rim (Fig. 1B). The attractiveness represented the proportion of the movement traces in which fruit flies moved towards the stripes in the inner circle, and the stickiness represented the proportion of time spent by fruit flies staying close to the stripes in the outer rim. We defined the methods for calculating the attractiveness (A) and stickiness (S) as follows:

A is calculated as the time spent by the fruit flies move towards the visual stripes (T_A), divided by the total time spent in the inner circle (T_{total_inner}, which is the total number of the time spent in the inner circle, θ_A is the stripe width in radians), and then subtracting the value of A (random) when the fruit flies move completely randomly ($\frac{\theta_A}{2\pi}$). This is the standardised method for calculating attractiveness A Eq. (4):

$$A = \frac{T_A}{T_{total\_inner}} - \frac{\theta_A}{2\pi} \qquad (4)$$

Notably, in the two-stripe conditions, A is calculated separately for the two stripes, not combined. For the symmetric two-stripe conditions, we reported the average A for the two stripes in the manuscript.

Similarly, the stickiness S was calculated as the time spent by the fruit flies staying near the visual stripe in the outer rim (T_b), divided by the total time spent by fruit flies in the outer rim (T_{total_outer}, which is the total number of the time spent in the outer rim, θ_A is the stripe width in radians), and then subtracting the value of S (random) when the fruit flies move completely randomly ($\frac{\theta_A}{2\pi}$). S showed the proportion of fruit flies that prefer to stay near the visual stripes in the outer rim relative to random movement Eq. (5).

$$S = \frac{T_b}{T_{total\_outer}} - \frac{\theta_A}{2\pi} \qquad (5)$$

2.6. Statistical models

To quantify how the behaviour preference (attractiveness and stickiness) for one stripe is statistically affected by the opposite stripe, we proposed a statistical model based on the idea of Phenotypic Response Surface (Zarrinpar et al., 2016), in which the response of an organism to multiple stimuli is modelled by a multi-dimensional quadratic function. In the present paper, the response z is the attractiveness or stickiness, and the two stimuli, x and y, are the widths of the measured and the opposite stripes, respectively. The quadratic model is described by the following equation:

$$z = ax^2 + by^2 + cxy + dx + ey + f \qquad (6)$$

where a-f are the coefficients that can be determined by fitting the model to the observed data. The coefficients a and b describe the quadratic dependency of z on the widths of the measured and opposite stripes, respectively, while d and e are for the linear dependency. The coefficient c describes the multiplicative effect of the widths of the two stripes. The coefficient f is a constant bias. We also test an alternative model which has only linear terms:

$$z = dx + ey + f \qquad (7)$$

Moreover, we also test the cubic model:

$$z = ax^3 + by^3 + cx^2y + dxy^2 + ex^2 + fy^2 + gxy + hx + iy + j \qquad (8)$$

2.7. Statistical analysis

Approximately 700 flies were used in all conditions. Data from flies that did not move within the 90-second observation period were removed from the analysis. Therefore, generally, 15–25 flies were included in each group for all tested conditions.

Statistical analysis was conducted using Statistical Product and Service Solutions 22.0 (SPSS 22.0). Descriptive statistics were used to analyse the movement trace density and the percentage of time spent in the inner circle and the outer rim. The differences between various stripe conditions of A and S were analysed using mixed variance analysis.

2.8. Ethical Note

Our study did not require any licenses/permits. Fruit flies had their wings shortened under low-temperature anaesthesia to minimize pain. They were allowed 48 h of recovery before behaviour test. Euthanasia was administered by placing them in a freezer.

3. Results

3.1. The arena and trace distribution

We first recorded the baseline behaviour of fruit flies in a homogeneous environment without landmarks. This was done by using the all-bright (light on) and all-dark (light off) conditions (Fig. 1 and Video 1). The density of the movement traces of flies indicated that flies in both conditions exhibited a stronger preference for the outer rims than the inner circle. Moreover, this behaviour preference was much stronger in the all-dark conditions than in the all-bright condition (Fig. 1C – E). This finding is consistent with the notion of darkness preference exhibited by the wing-shortened flies (Han et al., 2021a, 2021b; Neuser et al., 2008; Strauss and Pichler, 1998; Yen et al., 2019).

Notably, not only the percentage of time spent in the outer rim was different between the all-bright and all-dark conditions, but the movement patterns were also different. We found that the flies exhibited higher average speed (total trace length / total trial time) and movement speed (total trace length / total moving duration) in the all-dark condition than in the all-bright condition. The total rest time of the flies was less in the all-dark conditions compared to the all-bright conditions (Table 1).

For comparison, we build a simple random-walk model to simulate the behaviour of fruit flies based only on the statistics of their traces under all-bright and all-dark conditions. We calculated the proportion of straight movements (9.87 %) and other movements (83.63 %) in the data, and incorporated these proportions into the simulation of the fruit fly traces (see Materials and Methods). We found that the rotation angle of the fruit flies can be described by power-law distributions with $a = 0.199$ and $n = 1.194$ (see Materials and Methods) (Fig. 1F). The R^2 values are 0.924 and 0.934 for the clockwise and counterclockwise rotation, respectively. We also found that the movement distance can be described by Cauchy distribution with $x_0 = 1.168$ and $\gamma = 0.326$ (see Materials and Methods) (Fig. 1G). The R^2 values is 0.884. We performed random walk simulations based on these parameters (see Materials and Methods) and found that the simulated agent spent a significantly smaller percentage of time in the outer rim than real flies in the all-bright and all-dark conditions (Fig. 1D – E). The result suggests that instead of exhibiting a single behavioural pattern as the random walk model, the real files displayed two different behavioural modes between the inner circle and the outer rim. The result is also consistent with the

Table 1
Motor functions and rest time in all-bright and all-dark conditions.

Conditions	Average speed (mm / s)	Movement speed (mm / s)	Rest time in inner circle (%)	Rest time in outer rim (%)	Total rest time (%)
All-bright	5.39 ± 0.17	15.82 ± 0.10	29.99 ± 0.95	36.59 ± 1.33	66.57 ± 1.03
All-dark	9.07 ± 0.17	17.53 ± 0.08	3.33 ± 0.28	45.48 ± 0.79	48.81 ± 0.83

widely reported border preference of fruit flies (Besson and Martin, 2005; Hughson et al., 2018; Soibam et al., 2012).

3.2. Behaviour preference in symmetric two-stripe conditions

Next, we tested the flies by presenting two stripes of the same widths (symmetric two-stripe conditions). By putting two stripes on the opposite side of the screen, the setup resembled the classic "Buridan's paradigm," and we observed the visual fixation behaviour reported in the paradigm (Fig. 2A – B and Video 2) (Götz, 1980; Han et al., 2021a, 2021b; Strauss and Pichler, 1998; Yen et al., 2019). The attractiveness *A* increased proportionally with the stripe width until width = 30 °, reached a plateau between width = 30 ° and 90 °, then decreased when width ≥ 120 ° (Fig. 2C).

The stickiness *S*, following a similar trend with *A*, also increased in the small stripe-width region (≤ 50 °) but decreased when the width was large (≥ 90 °) (Fig. 2D). Besides, the time fruit flies spent in the outer rim increased with the stripe width (Fig. 2E).

3.3. The behavioural preference in the asymmetric two-stripe conditions

The most interesting question is how fruit flies make decisions between two stripes with different widths (the asymmetric condition) (Fig. 3A). Does a wider stripe imply greater attractiveness or stickiness? To address this question, we compared the behaviour preference with two stripes of different widths selected from four possible values, 10 °, 30 °, 60 °, and 90 ° (Fig. 3B and Video 3). We evaluated the attractiveness and stickiness for each stripe individually. The results showed that the attractiveness *A* of a given stripe width was not significantly affected by the width of the opposite stripe in most of the cases (Fig. 3C). By contrast, the stickiness *S* of a given stripe width strongly depended on the width of the opposite stripe (Fig. 3D). Furthermore, the stickiness of a given stripe tended to reduce when the opposite stripe became wider, suggesting a competition effect between stripes. We also observed interesting effects on the time spent in the inner circle versus the outer rim. When the width difference between the two stripes was greater, fruit flies tended to spend slightly more time on the outer rim of the platform (Fig. 3E).

To better visualise the competing effect between stripes of different widths, we plot heat maps for Attractiveness *A* and Stickiness *S* with
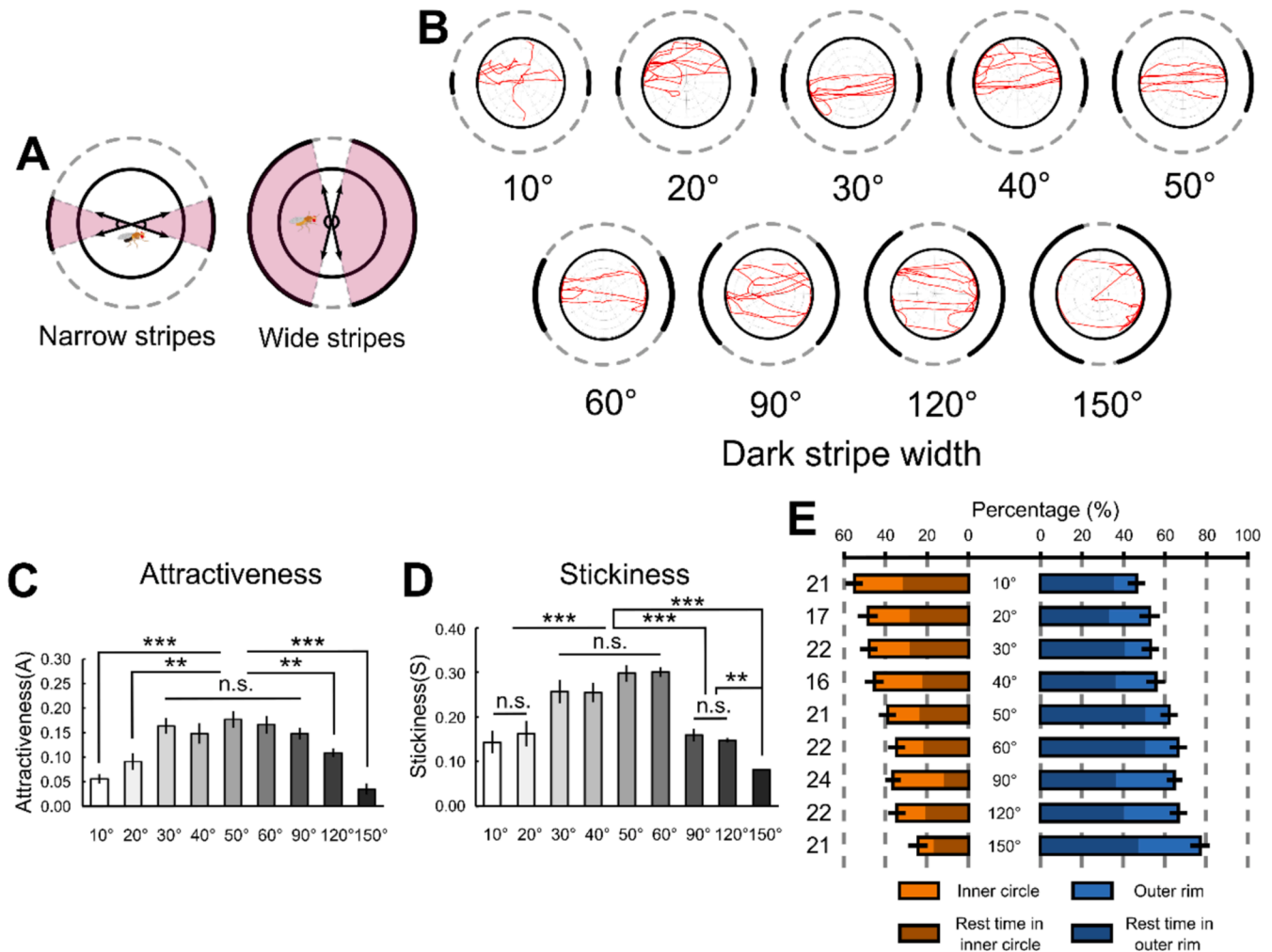

Fig. 2. Behavioural preferences for the symmetric two-stripe conditions. (A) Schematics of the setup for the symmetric two-stripe conditions (left, stripe widths = 30 °; right, stripe widths = 150 °). (B) Example traces of *CS* flies for different stripe widths of symmetric two-stripe condition, showing that the flies were attracted to both stripes and tended to stay in the outer-rim areas closer to the stripes. (C) The population-averaged attractiveness for all symmetric two-stripe conditions. The strongest attractiveness occurred when the stripe widths ranged from 30 ° to 90 °. (D) The population-averaged stickiness for all symmetric two-stripe conditions. The strongest stickiness occurred when the stripe widths were in the range of 50 ° to 60 °. (E) The percentages of time flies spent in the inner circle (the orange bars) and the outer rim (the blue bars) in all symmetric two-stripe conditions. The shaded area in each bar indicates the proportion of time at rest in each condition. Numbers to the left of the orange bars indicate the number of flies in each group.
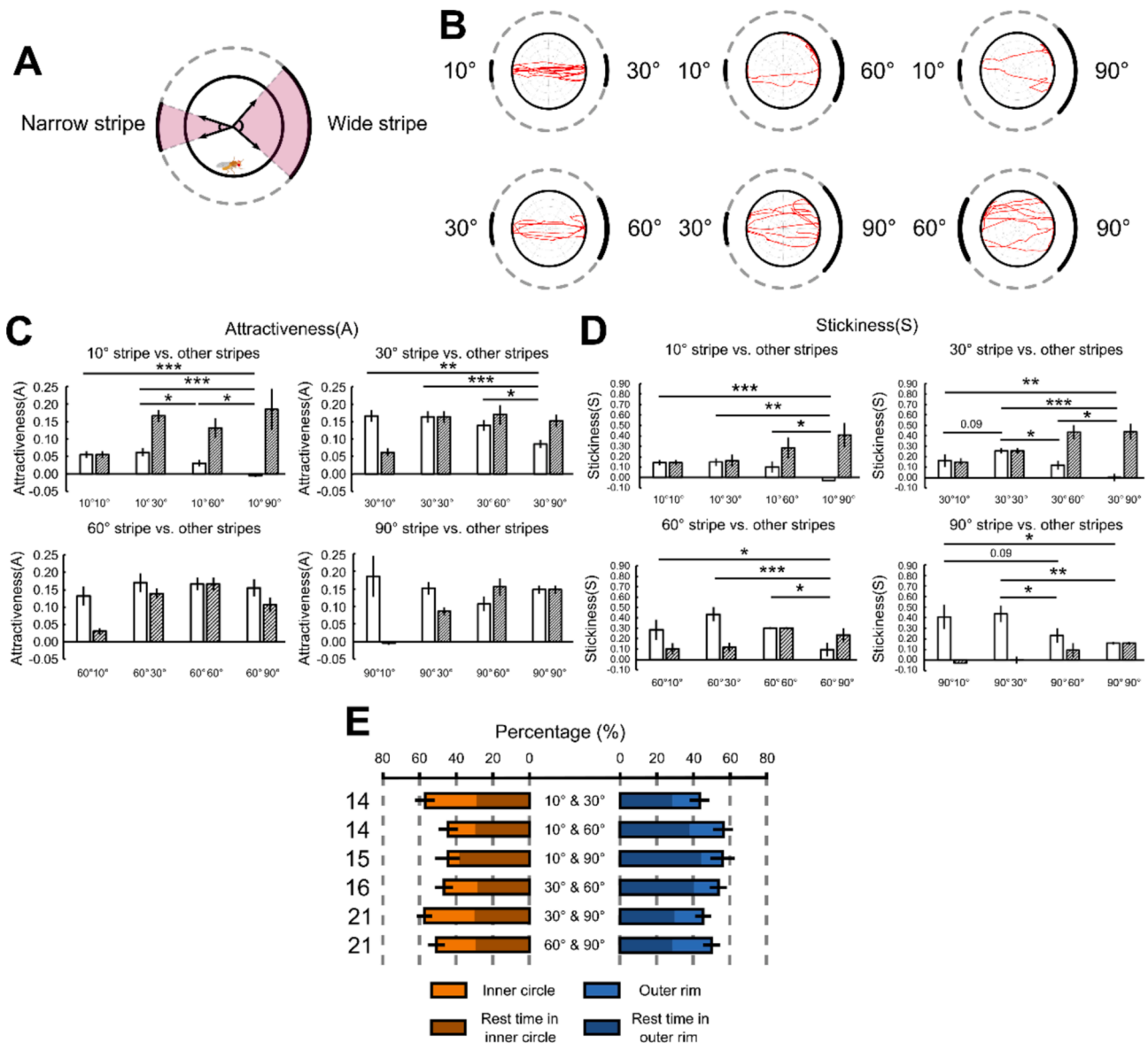
Fig. 3. Behavioural preferences for the asymmetric two-stripe conditions. (A) Schematics of the setup for the asymmetric two-stripe condition (stripe widths are 30 ° and 90 °). (B) Example traces for different stripe widths. (C − D) The population-averaged attractiveness and stickiness, respectively, for all asymmetric two-stripe conditions. Note the general trend of decreased stickiness for one stripe when the width of the other stripe increases. (E) The percentages of time flies spent in the inner circle (the orange bars) and the outer rim (the blue bars) in all asymmetric two-stripe conditions. The shaded area in each bar indicates the proportion of time at rest in each condition. Numbers to the left of the orange bars indicate the number of flies in each group.

rows representing the measured stripe and columns representing the opposite stripe (Fig. 4A). If *A* or *S* of a given stripe is independent of the width of the opposite stripe, we would expect that each row in the heat map is of the same colour. Otherwise, the colour would change in the same row. Our results revealed that the Attractiveness (*A*) roughly remained at the same level for each measured stripe regardless of the width of the opposite stripe (Fig. 4B). But if the width of the measured stripe increased, *A* also increased. By contrast, the heat map clearly showed that Stickiness (*S*) for each measured stripe was strongly affected by the width of the opposite stripe (Fig. 4C). To quantify the different trends between *S* and *A*, we fit a quadratic model to the heat maps (see Eq. (5) in Materials and Methods) (Fig. 4D − G). The quadratic model has six coefficients, *a − f*, with two (*a* and *d*) quantifying the effect of the width of the measured stripe, two (*b* and *e*) for the effect of the width of the opposite stripe, one (*c*) for the effect of the interaction between the measured and opposite stripes and one (*f*) as the bias, or an effect not related to the width of any stripe. The model fitting indicated

that *A* is mainly influenced by the effect from the measured stripe (large *a* and *d*), while *S* is influenced by both the measured stripe (large *d*) and the opposite stripe (large *b* and *e*) (Table 2).

In addition to observing the absolute values of attractiveness and stickiness, it is also informative to investigate their relative values with respect to the baseline. Here, we took the symmetric two-stripe conditions as the baseline and asked how much the attractiveness or stickiness changes for a stripe when the opposite stripe has a different width. To this end, we subtracted the value of attractiveness (or stickiness) of a stripe with a given width in the symmetric condition, e.g. 10 ° vs. 10 °, from those of the same stripe width in the asymmetric conditions, e.g. 10 ° vs. 30 °, 10 ° vs. 60 ° and 10 ° vs. 90 ° (Fig. 5). Our analysis showed that the relative attractiveness *A* in asymmetric two-stripe conditions is similar to that in the symmetric conditions (Fig. 5A, 5C). These findings indicated that when in the inner circle, the preference of fruit flies for approaching one of the two stripes was independent of each other.

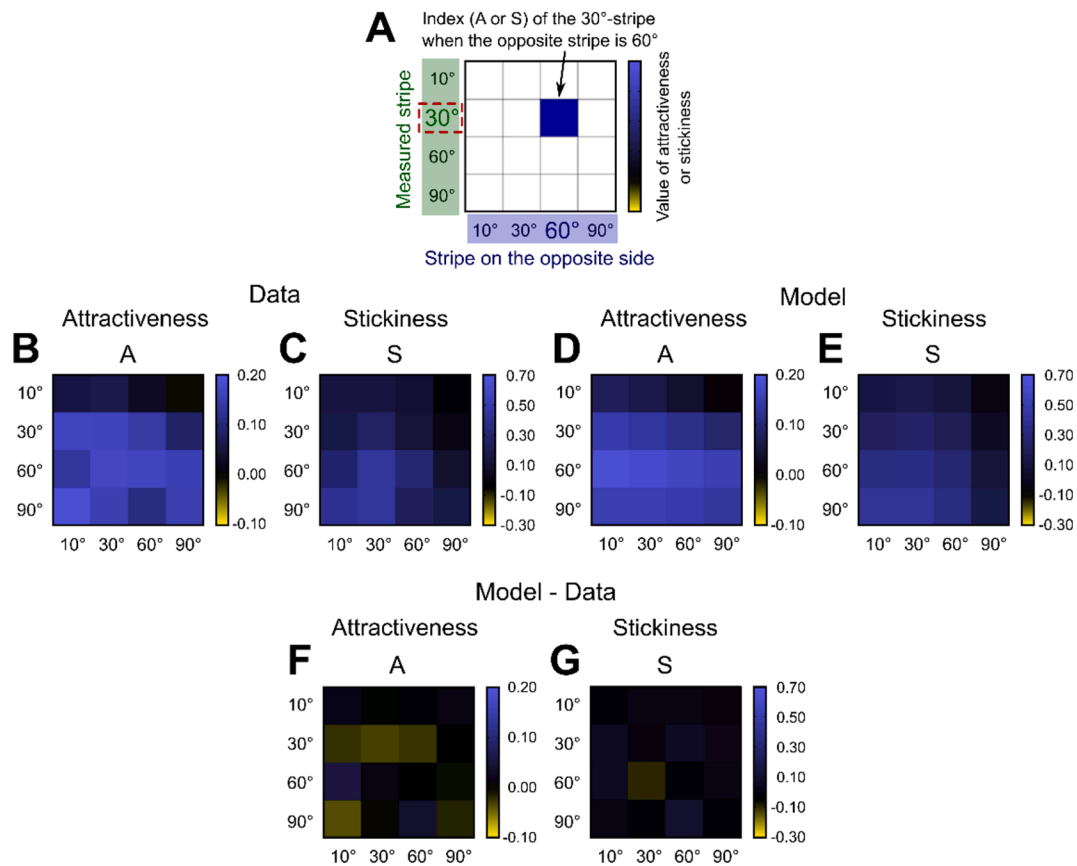On the other side, the relative stickiness *S* in the asymmetric two-

Fig. 4. Effect of the opposite stripe on attractiveness and stickiness in two-stripe conditions. (A) Schematics show how to read the colour maps shown in panels (B − G). The colour indicates the value of A or S of a measured stripe for each width of the opposite strip. The colour maps visualise how A or S is affected by the width of the opposite stripe. If each grid in a row is of the same colour, the attractiveness (or stickiness) of the measured stripe is unaffected by the opposite stripe. (B − C) The values of A and S, respectively, in all two-stripe conditions. (D − E) The values of A and S, respectively, from the quadratic model (see Materials and Methods) after fitting to the observed values shown in (B) and (C). The model suggests that the stickiness of a stripe is strongly affected by the opposite stripe, while the attractiveness is not. (F − G) The residual values of A and S, respectively. The values represent the differences between the model and the observed data.

Table 2
The coefficients of the quadratic model for attractiveness A and stickiness S.

Parameter	A	S
a	−0.141	−0.064
b	−0.011	−0.193
c	0.025	−0.059
d	0.298	0.330
e	−0.033	0.229
f	0.030	0.029
R^2	0.830	0.901

stripe conditions was different from those in the symmetric conditions (Fig. 5B, 5D), indicating that the relative stickiness of fruit flies for a stripe is influenced by the opposite stripe. Furthermore, as the width difference between the two stripes increased, the deviation of relative stickiness S from the symmetric condition increased.

We fit the quadratic model to the relative Attractiveness and found that all parameters are extremely small (Table 3), indicating that the data is best described by a nearly flat surface. This result suggests that the Attractiveness in the asymmetric conditions is similar to that of the symmetric conditions with the same width of the measured stripe. By contrast, the model fitting to the relative Stickiness yielded a different result. Compared to the symmetric conditions, the measured stripe tends to be stickier with a larger width (large a) and is less sticky if the opposite stripe becomes larger (large b). The coefficients for the linear terms (d and e) are also large. However, the effect is dominated by the quadratic terms (a and b).

Finally, we also tested alternative models, including simpler linear and more complex cubic models. The linear model led to a worse fit in all cases (Table 4). The cubic model produced better fits as expected. However, by examining the model coefficients, we found that the quadratic and linear terms still dominate the effect, while the cubic terms are relatively minor (Table 5). Therefore, the quadratic model is more suitable for describing the data than the linear and cubic models.

3.4. Behaviour preference in different wild-type strains

It is informative to investigate whether similar behaviour traits are also observed in different wild-type strains of fruit flies. Therefore, we compared the behaviour patterns of the CS and w^+ wild-type strains (Supplementary Figs. S1 - S5, Supplementary Table S1, Supplementary Videos S1 − S3). We found that CS flies spent more time staying in the outer rim than w^+ flies under the same visual stimulus conditions, consistent with previous research (Qiu et al., 2017). Regarding Attractiveness and Stickiness, both CS and w^+ flies exhibited similar trends, indicating that the behavioural preferences of the visual stimuli we reported in this study are not specific to one type of wild-type strain.

4. Discussion

In the present study, we examined the behavioural preferences of fruit flies in Buridan's paradigm under different visual landmark conditions. The test conditions included one stripe, two stripes with the same width (symmetric), and two stripes with different widths
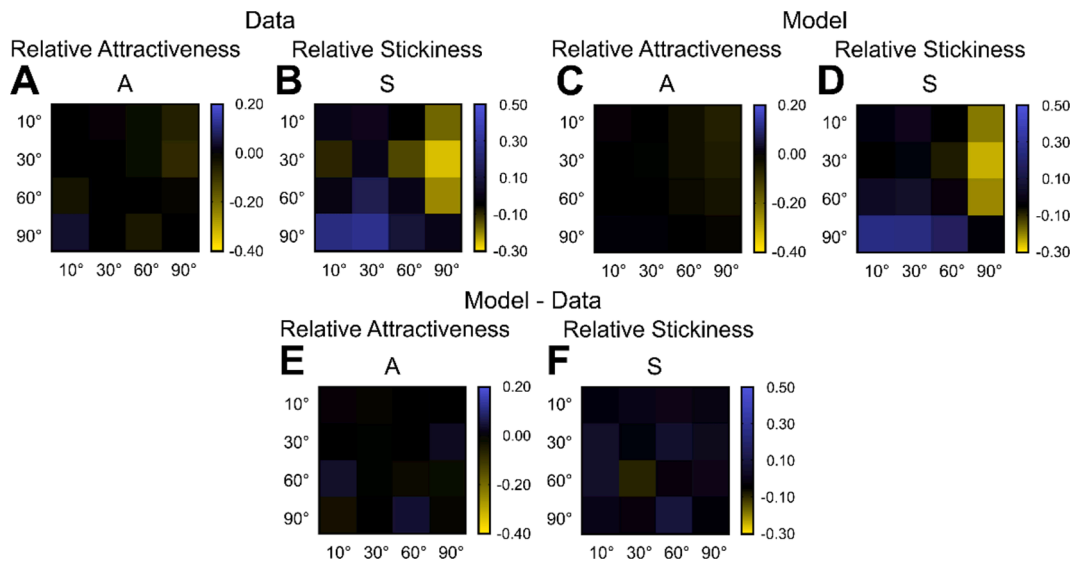
Fig. 5. The relative attractiveness and stickiness. The values indicate the differences between the asymmetric and the symmetric two-stripe conditions. (A) The relative attractiveness, as computed by subtracting the attractiveness of the measured stripe in the symmetric conditions from the asymmetric conditions. (B) Same as in (A) but for relative Stickiness. (C − D) The values of relative *A* and *S*, respectively, from the quadratic model (see Materials and Methods) after fitting to the observed values shown in (A) and (B). (E − F) The residual values of relative *A* and *S*. The values indicate the differences between the model (as shown in (C) and (D)) and the observed data (as shown in (A) and (B)).

Table 3

The coefficients of the quadratic model for relative Attractiveness *A* and Stickiness *S*.

parameter	relative *A*	relative *S*
a	−0.014	0.250
b	−0.011	−0.193
c	0.025	−0.059
d	−0.029	−0.230
e	−0.032	0.229
f	0.017	−0.025
R^2	0.520	0.913

Table 4

The parameters of the linear model for the Attractiveness *A* and Stickiness *S*.

parameter	*A*	*S*	relative *A*	relative *S*
d	0.070	0.169	0.016	0.159
e	−0.031	−0.159	−0.031	−0.158
f	0.087	0.195	−0.002	−0.012
R^2	0.530	0.793	0.439	0.689

Table 5

The parameters of the cubic model for the Attractiveness *A* and Stickiness *S*.

parameter	*A*	*S*	relative *A*	relative *S*
a	0.183	−0.277	−0.040	−0.230
b	0.062	0.367	0.063	0.368
c	−0.058	−0.018	−0.058	−0.017
d	0.092	0.028	0.091	0.027
e	−0.573	0.676	0.165	0.866
f	−0.249	−1.179	−0.253	−1.179
g	−0.036	−0.077	−0.034	−0.076
h	0.598	−0.195	−0.142	−0.668
i	0.188	0.941	0.190	0.941
j	−0.056	−0.002	−0.004	−0.069
R^2	0.935	0.955	0.739	0.958

(asymmetric). We quantified the behaviour preferences of the flies using two metrics: Attractiveness, which measures how a fly moved toward any visual stripe, and Stickiness, which measures how a fly stays at the outer rim area adjacent to a visual stripe. Although the Attractiveness and Stickiness increased with the width of a stripe as expected, we found several interesting effects. Based on the result of the asymmetric two-stripe condition, the effect of the opposite stripe is positively correlated with its width. Furthermore, the effect of the opposite stripe is limited to stickiness, whereas attractiveness is not affected by the width of the opposite stripe. Additionally, the observed difference in the probabilities of staying in the inner circle and outer rim indicated two behavioural modes exhibited by fruit flies in the two regions. The result validates the necessity of using two metrics (Attractiveness and Stickiness) because they quantify these different behaviours, which were not considered on other existing metrics.

We suggest that the effects of the asymmetric stripe widths on the Attractiveness and Stickiness can be explained by the "visual attention" that a fly may allocate to its field of view (FOV) of the flies during taxes (Fig. 6A). Behavioural and neural mechanisms underlying the visual attention of fruit flies have been studies in various studies (Kirszenblat et al., 2018; Palermo and Theobald, 2019; van Swinderen, 2011). We hypothesize that although a fruit fly's FOV is around 320 ° (Heisenberg and Wolf, 1984), most of the fly's attention is allocated to the front part of the FOV. When a fly moves toward a stripe, the opposite stripe is in the back half of the FOV and receives less attention. Therefore, the opposite stripe is less likely to affect the fly's movement toward the stripe in front of the fly (Fig. 6B). In consequence, the attractiveness of one stripe is independent of the opposite stripe. After the fly reaches the edge of the platform proximal to a stripe, the fly turns its body and walks along the edge. This movement places the proximal and the opposite stripe to the front part of the FOV. Therefore, both stripes receive strong attention, and the fly may choose one that is more attractive, i.e., the bigger stripe. In consequence, the stickiness of a stripe is affected by the width of the opposite stripe (Fig. 6C).

In the symmetric two-stripe condition, the attractiveness and stickiness decrease when the width is larger than a certain value (120 ° for attractiveness and 90 ° for stickiness). We hypothesize that when the stripe width is very large in the symmetric two-stripe condition, the stripes take up most of the screen space, and the remaining bright space (now more like stripes) becomes very salient. The bright stripes attract the flies and reduce their attractiveness and stickiness to the dark stripes. Alternatively, the attraction of the narrow bright stripes may be partially
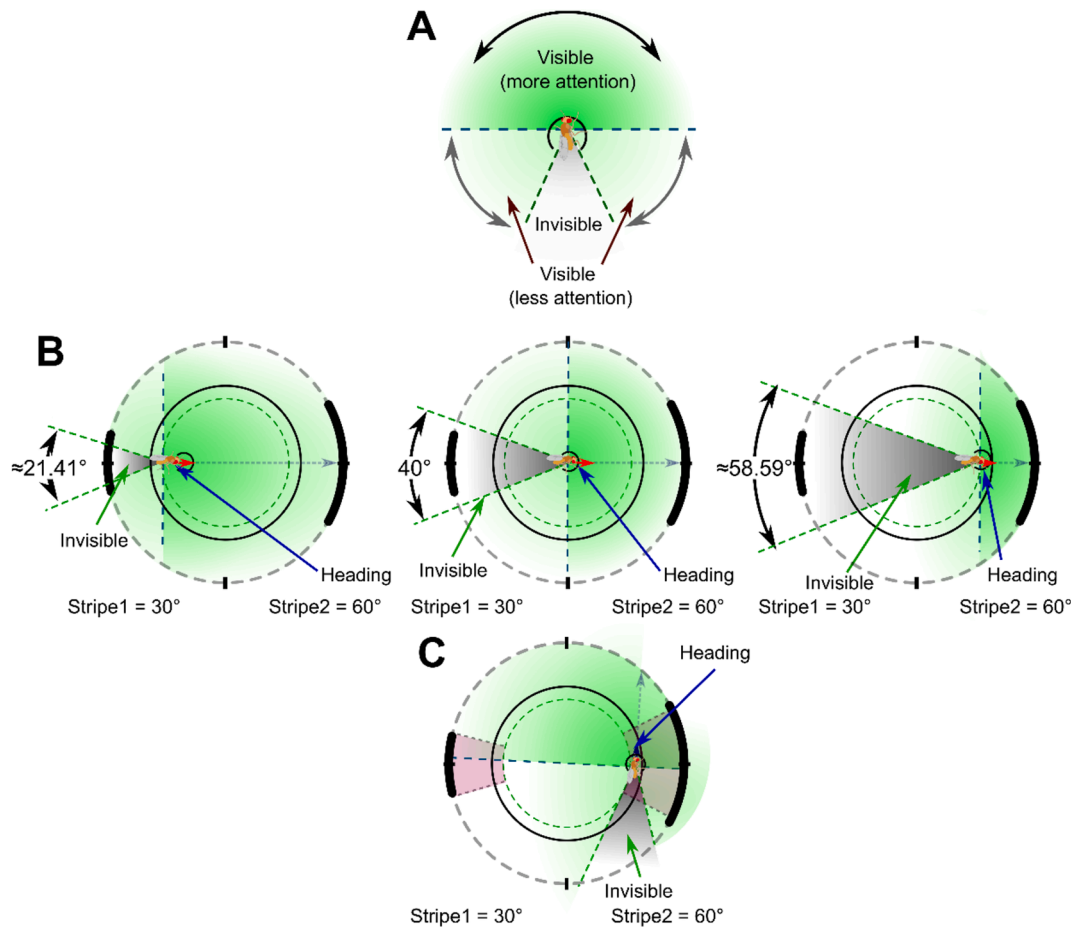
Fig. 6. Hypothetical visual attention can be used to explain the observed behaviour. (A) We hypothesize that although fruit flies possess approximately a 320 ° field of view, they allocate more attention to the front part of their field of view while paying less attention to the area behind. (B) When a fruit fly is in the inner circle of the platform and moving toward one stripe, depending on the fly's location (as shown in the three examples from left to right), the opposite stripe may or may not visible to the fly. We hypothesize that the fly pays more attention to the stripe in front, but less attention to the stripe behind, even if it is visible to the fly. Therefore, the attractiveness of either stripe is relatively independent of each other. (C) Both stripes may enter the high-attention area when the fruit fly moves along the platform's edge. The fly may be attracted by the stripe on the other side if it is larger than the proximal stripe. Thus, the stickiness of a stripe is influenced by the width of the other stripe.

explained by the sensitivity to contrast and edges reported in a study (Keller, 2002). Further research is required to identify the actual cause.

Our study also showed faster movement speeds and shorter rest time for fruit flies in the all-dark condition than the all-bright condition. Moreover, the flies stay in the outer rim in both conditions more than predicted by the random walk model. This is particularly interesting because it favours the active dynamics perspective of the animal behaviour over the pure sensorimotor theory (Brembs, 2021). During the all-dark condition, the flies receive minimal visual stimuli, but they still exhibited active locomotion with a pattern different from the all-bright condition and the random walk model. The sensorimotor hypothesis posits that behaviour is a direct response to external stimuli, but our experiments suggest that fruit fly behaviour is influenced not only by external stimuli but also by intrinsic cognitive processes and spontaneous activity. The results highlight the need for more complex models that integrate internal states, perception, and cognitive processes to more accurately predict behaviour under varying environmental conditions.

It worth noting that several studies have tested asymmetry stimuli in various experimental setups. One study used different numbers of black squares as asymmetrical visual stimuli and found that untrained wild-type *Canton-S* fruit flies showed a greater preference for the wider visual stimulus, which aligned with our conclusions (Bengochea et al., 2023). However, in another study, the flies exhibited no significant

difference in preference for the 10 ° stripes versus the 60 ° ones (Keller, 2002). We suspect that the inconsistency may stem from the different metrics and protocols. Keller's study reported the fixation efficiency, which was calculated based on the number of entrances to the 30 ° segment on the edge near the stripe. In the present study we calculated the attractiveness based on the percentage of time a fly heading toward the stripe. Moreover, in Keller's study, a trial was ended once the fly reached the edge. In our study, we allowed the flies freely moving on the platform until the fixed-length trial (90 s) ended. In other words, we observed a continuous behavior while Keller's study observed the one-shot decision. In another study (Colomb et. al. 2020), the flies exhibited a stronger fixation for 11 ° stripes than the 20 ° stripes. However, our study indicated a higher attractiveness for 20 ° stripes than 10 ° stripes. This inconsistency may stem from the different ways in calculating the fixation and the attractiveness. In Colomb et al 2020, fixation was represented by stripe deviation, which was measured by the deviation angle for a fly's heading from the center of a stripe. The Attractiveness used in our study counts the percentage of the time the fly heading toward a stripe. For a large stripe, a fly may head toward any part of the stripe but not necessarily toward the exact center of the stripe. In this scenario, the movement would still count in the Attractiveness, but the stripe deviation would increase. Therefore, we do not think that the two studies contradict to each other. Rather, the stripe deviation and the Attractiveness measure slightly different behaviour

properties. The former indicates how precise a fly moves toward the center of a stripe while the latter indicates how often the fly moves toward the area spanned by a stripe. The core value of our study is that we provide a systematic evaluation of visual stimulus competition in fruit flies, highlighting their nuanced responses to varying degrees of asymmetry.

It should be noted that when the fly is not at the centre of the platform, even the same stripe appears to have different angular widths. When the fly is at the edge of the platform, such differences become very pronounced (left panel in Fig. 6B and right panel in Fig. 6B). Previous research has shown that the fly's brain can process depth information from the photoreceptors, allowing for precise depth perception (Kemppainen et al., 2022). Bees are also shown be able to perceive the distance of an object regardless of its apparent angular size (Lehrer et al., 1988). Given that insects, including fruit flies, possess depth vision, it is possible that they can estimate the absolute size of stripes. Therefore, it is necessary to discuss whether the flies make behavioural decisions based on the absolute size of the stripe or its apparent angular width? Our experiments and analyses were conducted based on the former, but further studies are required to validate this hypothesis.

It is worth mentioning that wild-type strains, *Canton-S* and w^+ flies, with distinct genetic backgrounds, did not exhibit differences in their preference behaviour. This suggests that attractiveness and stickiness are not specific to one genetic strain. Despite of our finding, it is known that some behaviours of fruit flies does depends on the genetic background and physical conditions. Previous studies have indicated significant effects of different genetic backgrounds on the phototaxis of fruit flies, particularly when wings are removed or in mutant wingless flies, which show altered phototaxis (Benzer, 1967; McEwen, 1918). Moreover, when the wings of fruit flies are temporarily glued, their phototaxis decreases, but it returns to normal once the wings are restored. Additionally, phototaxis behaviour is mainly driven by external stimuli as well as internal decision-making processes, and this effect is not limited to fruit flies walking on the platform (Gorostiza et al., 2020; Wehner, 1972). Therefore, to acquire a more comprehensive understanding of the flies' behaviour, it would be interesting to extend the study to more genetic strains with various conditions in the future.

CRediT authorship contribution statement

Rui Han: Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Yi-Heng Tan:** Data curation. **Chung-Chuan Lo:** Writing – review & editing, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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On behalf of co-authors of the submission "Attractiveness versus Stickiness: Behavioural preferences of *Drosophila melanogaster* with competing visual stimuli" I declare no involvement of AI-assisted technologies in the writing process. The original data used in the manuscript as well as the manuscript itself have not been submitted for publication to other journals.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2024.104716.

Data availability

The data set for this paper is available at https://figshare.com/articles/online_resource/Journal_of_Insect_Physiology_Drosophila/24996854.

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