

## RESEARCH ARTICLE

# Picky or Pragmatic? Innate Colour Preferences in Three Pollinating Fly Species

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## ABSTRACT

Vibrant displays are a hallmark of Angiosperm communities, and colour is a primary cue used by pollinators for recognising flowers. Many diurnal pollinators have innate colour preferences which flowers have evolved to exploit, though these preferences are documented in relatively few model species, such as honeybees. In the context of ongoing pollinator declines our need to understand the behaviour of alternative pollinators, like flies, is pressing. Here we investigated the innate colour preferences of three pollinating fly species: the common dronefly (*Eristalis tenax*), the brown blowfly (*Calliphora stygia*) and the common housefly (*Musca domestica*). In laboratory-based choice assays, we presented naive individuals with a simultaneous choice between equally rewarding blue, pink, yellow and white artificial flowers, and quantified key foraging behaviours including visitations, feeding, visit latency and duration. We found significant differences in overall colour preference among species: *E. tenax* exhibited a pronounced attraction to yellow flowers, whereas *C. stygia* and *M. domestica* did not display any significant colour preferences. When considering the underlying colour features (hue, saturation and brightness), however, we found all three species preferred more saturated colours, and both *C. stygia* and *M. domestica* favoured shorter-wavelength hues. We found no between-species differences in either choice latency or the duration of visits. The strong yellow preference of the prolific flower-visitor, *E. tenax*, hints towards its suitability for pollinating yellow-crop flowers. The absence of a strong overall preference in *C. stygia* and *M. domestica*, taken with their preferences for particular colour features, suggests that these flies are more flexible foragers and may be attracted to a variety of visual advertisements. These results highlight the existence of key differences in the nature and impact of innate colour preferences on decision-making in flies and emphasise the importance of broadening our understanding of pollinator sensory biases beyond traditional model species.

## 1 | Introduction

Insect pollinators are the quiet architects behind the world's most colourful plant communities (Frisch and Frisch 1975). Since most flowering plants depend on pollinators for reproduction, plants have evolved complex displays to attract desired insect visitors (Ellis et al. 2021; Garcia et al. 2022; Matouskova

et al. 2023; van der Kooi et al. 2021). Colour is a primary cue used by visually orienting pollinators for recognising flowers, though it often acts in concert with other floral traits, such as scent, to enhance recognition, discrimination and attraction (de Camargo et al. 2019; Matouskova et al. 2023; Woodcock et al. 2014). Colour may also be associated with the quantity and quality of rewards (Giurfa et al. 1995; Matouskova et al. 2023;

Streinzer et al. 2021) and can induce feeding and landing behaviours in visiting insects (Biesmeijer et al. 2005; Chittka and Menzel 1992; Lunau and Maier 1995).

Most diurnal pollinators that have been studied display innate preferences for flowers of a particular colour (Lunau and Maier 1995; Matouskova et al. 2023; Reverté et al. 2016; Willmer 2011). Innate preferences are sensory biases that exist before any experience with flowers; they are instinctive, spontaneous predilections that often help pollinators make foraging decisions (Goyret et al. 2008; Gumbert 2000; Kuenzinger et al. 2019; Lunau and Maier 1995; Muñoz-Galicia et al. 2021). Such preferences may be malleable and modified with experience, though often only up to a limit (An et al. 2018; Kandori et al. 2021), with some pollinators nonetheless visiting flowers of their preferred colour even when more rewarding options are known to be available (Latty et al. 2020). Innate preferences may also vary with sex-specific ecological and evolutionary demands, such as differences in foraging requirements or oviposition behaviour (An et al. 2018; Lunau 2014). Floral colours have repeatedly converged on those that effectively support detection and identification by target pollinators, reflecting adaptation to pre-existing pollinator visual systems, and implying some degree of reciprocal evolutionary change (de Camargo et al. 2019; Garcia et al. 2022; Rodríguez-Gironés and Santamaría 2004).

Most studies of colour preferences in pollinating insects investigate bees (Hymenoptera) of which several species innately favour blue, or butterflies and moths (Lepidoptera) which generally prefer pinks and whites (Deheyn 2017; Dyer et al. 2016; Faegri and Pijl 1979; Giurfa et al. 1995; Nuzhnova and Vasilevskaya 2013; Reverté et al. 2016; Willmer 2011; Yurtsever et al. 2010). Research on fly preferences is comparatively scarce (see Lunau 2014 for a comprehensive review). Existing studies on colour preference tend to favour tests in the field with wild flies, where flower-naïve individuals and innate behaviours cannot be studied (De Buck 1990; Haslett 1989; Lunau and Maier 1995; Mu et al. 2017). Studies undertaken with a pest management goal are also common, these studies test the efficacy of coloured traps and baits (Chen et al. 2015; Kafle et al. 2019; Khan et al. 2013; Mello et al. 2009), and often disregard individual behaviour and motivations (Lunau 2014). An early minority, however, sought to examine preferences directly under more controlled conditions, to great effect (e.g., Lunau and Wacht 1994; Prokopy et al. 1975).

Agricultural pollination is overwhelmingly reliant on the European honeybee, *Apis mellifera* (Hymenoptera: Apidae; Cook et al. 2020; Rader et al. 2020). However, accumulating stressors including the parasite *Varroa destructor* (Mesostigmata: Varroidae), deformed wing virus, and colony collapse disorder, threaten the stability of honeybee populations and their pollination services (Damayo et al. 2023; Karasiński 2018; Latty and Dakos 2019; Phaboutdy and Ward 2024). Flies are effective pollinators of a range of crops and so present a promising alternative (Cook et al. 2020; Inouye et al. 2015; Larson et al. 2001; Rader et al. 2020; Woodcock et al. 2014), yet without fundamental knowledge of their foraging ecology it is difficult to predict which plants they will visit and, hence, evaluate their full potential.

Blowflies (Calliphoridae), hoverflies (Syrphidae) and houseflies (Muscidae) include species which have been identified as effective pollinators, making the colour preferences of these flies of particular interest (Cook et al. 2020; Rader et al. 2020). *Eristalis tenax* is an abundant bee-mimic hoverfly (Howlett and Gee 2019; Willmer 2011) that commonly visits a wide range of open, yellow-flowered species, particularly in the Asteraceae and Apiaceae families (De Buck 1990; Lunau and Maier 1995). Past experimental study suggests *Eristalis tenax* has a strong innate preference for yellow (An et al. 2018; Kelber 2001; Lunau et al. 2018; Matouskova et al. 2023). *Calliphora stygia* is a large necrophagous blowfly found in Australia and New Zealand (Croft et al. 2024; Lang et al. 2006; Levot and Casburn 2016). Although typically associated with carrion, *C. stygia* are generalist foragers and have been observed visiting a variety of flowers, including avocado, onion, bok choy and mango (Cook et al. 2023; Larson et al. 2001; Stavert et al. 2018), though their specific colour preferences are not well established. Finally, while the housefly *M. domestica* is considered a pest, it has shown promise as a complementary pollinator alongside honeybees in crops such as carrots and leeks, as they readily visit small, open flowers with accessible nectar (Hansen et al. 1998; Willmer 2011; Wilson et al. 1991). Past studies have endeavoured to determine the colour preference of *M. domestica* but these have been tied to pest control efforts and yielded mixed results (Diclaro et al. 2012; Geden 2006; Kafle et al. 2019).

Here, we aimed to test the existence and examine the nature of innate colour preferences in *E. tenax*, *C. stygia* and *M. domestica*. To this end, we presented flower-naïve individual flies with a simultaneous choice between equally rewarding blue, pink, yellow and white artificial flowers in laboratory assays.

## 2 | Methods

### 2.1 | Study Species and Animal Husbandry

We used *Eristalis tenax*, *Calliphora stygia* and *Musca domestica* as focal species because these flies possess well-developed colour vision, and because they each bear close relatives that are known effective pollinators (e.g., *Muscina* spp. and *C. vicina*; Clement et al. 2007; Tiusanen et al. 2016), thereby placing them as tractable models for a host of alternatives (Lunau 2014). We obtained fly pupae from separate commercial suppliers: *M. domestica* from ReptileRealm ([www.reptilerrealm.com.au](http://www.reptilerrealm.com.au)), *C. stygia* from Sheldon's Bait ([www.sheldonsbait.com.au](http://www.sheldonsbait.com.au)) and *E. tenax* from SeedPurity ([www.seedpurity.com](http://www.seedpurity.com)). We conducted all experiments between June and September 2024 at The University of Sydney.

We reared *E. tenax* and *C. stygia* pupae in mesh cages (width × depth × height: 55 cm × 55 cm × 90 cm) and allowed batches of approximately 100 g of pupae to eclose within each cage. For *M. domestica*, which are considerably smaller, we placed batches of approximately 50 g in smaller rearing cages (30 cm × 30 cm × 30 cm). All flies took between 2 and 8 days to eclose. We placed the remaining pupae into plastic containers and stored them in a refrigerator for a maximum of 2 weeks to prolong pupal development before use in the experiment.

We housed the pupae and emerging flies in a temperature controlled room set at 23.5°C with a 12:12 light/dark cycle provided by one overhead LED light. Flies were fed ground, desiccated bee pollen and a 10:1 dilution of honey and water *ad libitum*. We applied this solution to fully saturated cotton, lightly dyed green with 3–5 drops of food colouring, and placed it in 2–3 large Petri dishes (90 mm × 15 mm) and 3–5 small Petri dishes (40 mm × 10 mm). We placed the food atop achromatic (black) artificial flowers at a height of 80 mm to familiarise the flies with feeding from artificial flowers before testing (*sensu* Matouskova et al. 2023). We chose black flowers for their high contrast against both the green flower bases and the honey solution, facilitating initial food discovery. The use of an achromatic, non-test-set colour also helped minimise any pre-exposure bias. While feeding behaviour was not systematically recorded, it was routinely observed during daily checks, and we replaced this food every 1–2 days.

## 2.2 | Artificial Flower Design and Presentation

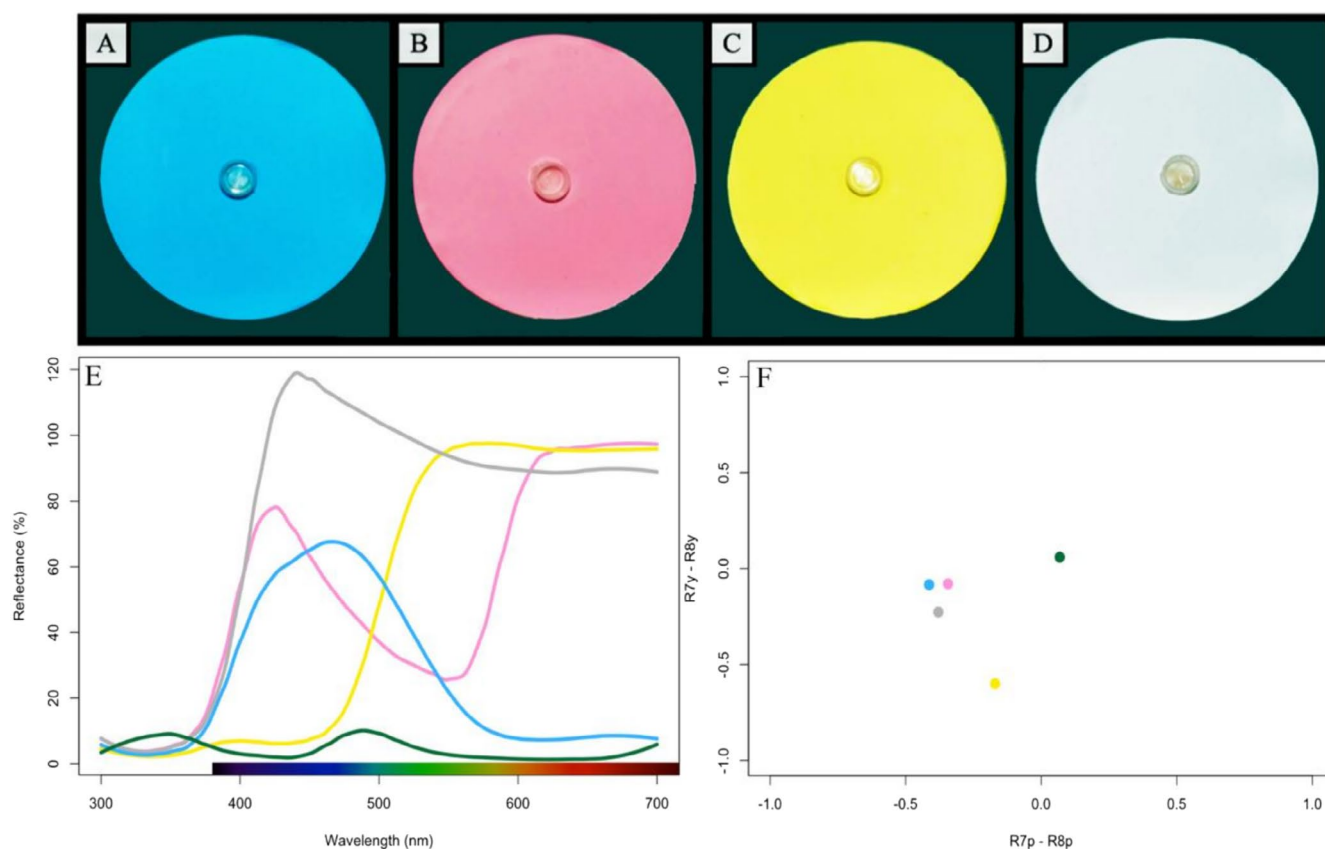
We used artificial flowers for the colour choice experiments for their ease of manipulation (Chapman et al. 2023). We designed three-dimensional (3D) artificial flowers using Tinkercad (<https://www.tinkercad.com/>), with each flower consisting of an 80 mm tall ‘stem’ and a circular ‘flower’ with a diameter of 75 mm and a surface area of 4418 mm<sup>2</sup> (Figure 1A–D). The

artificial flowers were printed using black Polylactic Acid (PLA) filament (eSUN Black PLA+) on a Prusa i3 MK3 3D printer (Prusa Research). To colour each flower, we used matte blue, pink, yellow and white vinyl stickers (ORACAL 631 Removable Vinyl, shades: Light Blue, Soft Pink, Brimstone Yellow, White) cut using a precision cutting machine (Cricut Explore Air 2). We selected the four focal colours to partially span the axes of fly colour space (Figure 1F) and to reflect the general gamut of hues common in floral signals (Chittka and Menzel 1992), while remaining within a biologically realistic and experimentally tractable range.

To motivate flies to forage, each artificial flower contained a well with 4:5 diluted honey as a high-value reward, with the wells constructed from the cylindrical caps of 2 mL microcentrifuge tubes placed in the centre of the flower (Figure 1A–D). We mounted artificial flowers into fixed holes in a square base made with green PLA filament (eSUN Black PLA+) using the same Prusa 3D printer. Each base held four flowers arranged in 2 × 2 rows, with flowers spaced 14 cm apart (Figure S1).

## 2.3 | Artificial Flower Colours

To estimate the subjective visual appearance of our focal colours to fly viewers, we used a combination of reflectance spectrometry and visual modelling (Figure 1E,F). Briefly, we measured



**FIGURE 1** | Photographs of the four artificial flowers used in simultaneous-choices assays of innate colour preferences in three species of fly (A–D), along with their reflectance spectra (E) and locations as modelled in a dipteran colour-space (F) from which measures of hue, saturation and luminance were extracted. Artificial flowers were circles (diameter = 75 mm, surface area = 4418 mm<sup>2</sup>) with a central well to hold the diluted honey reward.

the reflectance of our coloured vinyl with an Ocean Optics Jaz UV-VIS spectrometer with a PX-2 pulsed xenon light source, calibrated against a diffuse Spectralon white standard and black velvet dark standard. We used a bifurcated probe with light and collector held at 45° to the sample plane and set an integration time of 200 ms with a boxcar width of 10.

We then used an early-stage (i.e., retinal-level) model of dipteran colour vision to estimate the subjective hue, saturation and luminance of our artificial flowers. We drew on the visual phenotype of *Musca domestica*, and we assumed the involvement of R7p, R8p, R7y and R8y photoreceptors in chromatic processing and R1–6 receptors in achromatic processing (Hardie 1986; Troje 1993). We drew on the model of Troje (1993) and first estimated receptor quantum catches as the integrated product of receptor sensitivity, stimulus reflectance and a standard-daylight illuminant. For hue and saturation, we then calculated the difference in relative stimulation between R7y–R8y and R7p–R8p receptors as putative opponent channels that define the location of a given stimulus in dipteran colour space (Figure 1F), and took saturation as the Euclidean distance from the achromatic origin to each flower, and hue as the angle between the flower, origin and 12 o'clock (Table 1). For luminance, we simply took the quantum catch of R1–6 receptors, as per convention. All spectral processing was carried out using the packages 'pavo' (ver. 2.2; Maia et al. 2019; Maia and White 2018) and 'lightr' (v. 1.5; Gruson et al. 2019) in R (ver. 3.5.2; R Core Team 2018).

## 2.4 | Experimental Protocol

To increase foraging motivation, we withheld all food for at least 12 h before experimentation (Lunau et al. 2018). We conducted experiments simultaneously in nine arenas, each made from mesh insect rearing cages (30 cm × 30 cm × 30 cm). The floor of each arena was covered with a green base, simulating a natural leaf-green background. Arenas were illuminated by five full-spectrum self-ballasted halogen lights (Exo Terra Solar Glo Lights 160 W), which broadly matched daylight spectral properties. We placed diffusion paper (Rosco E-Colour 216 White Diffusion Sheet) beneath each light to even the intensity of light across the arena and simulate cloud-diffused sunlight. We performed all experiments in a temperature-controlled room set to 23.5°C.

**TABLE 1** | The relationship between categorical flower colours used in our innate-choice assays with three species of fly, and their constituent values of hue, saturation and luminance.

Flower colour	Colour feature		
	Hue	Saturation	Luminance
Pink	−2.91	0.35	0.34
Yellow	−1.84	0.62	0.26
White	−2.60	0.44	0.67
Blue	−2.94	0.42	0.34
Green (background)	0.71	0.09	0.06

*Note:* Colour features are estimated via reflectance spectrometry and dipteran visual modelling so as to broadly account for the visual system of our focal flies (see Methods).

During experimental trials, we released one fly into the centre of each arena and presented it with a simultaneous choice between blue, pink, yellow and white rewarding flowers. Over a 20-min observation period, we recorded the flower first visited (defined as 1 s of sustained contact with the flower surface), the time taken for the fly to land, the duration of the visit (minutes), whether feeding from the central well was observed, and the sex of the fly. After each trial, we cleaned all artificial flowers with water, dried them with a paper towel and sprayed them with isopropyl alcohol (99.8%), before resetting them in a randomised arrangement.

If a fly did not make a choice within 20 min, it was excluded from this and subsequent trials, and all flies participated in only one trial. We performed trials until 120 individuals from each species landed on a flower, with a further 35 *C. stygia*, 78 *E. tenax* and 78 *M. domestica* excluded without making a choice within the 20-min period.

## 2.5 | Statistical Analysis

To examine overall colour preferences, we performed a series of chi-square ( $\chi^2$ ) goodness-of-fit tests on the first choice made by an individual (to best capture innate preferences; Lunau and Maier 1995) for each species. We further used chi-square ( $\chi^2$ ) tests of independence to test for an association between fly sex and flower colour choice, again for each species.

To investigate whether fly foraging behaviours differed between species and colour choices, we analysed two further behavioural metrics: latency (the time taken for flies to land on a flower) and foraging duration (the time spent on a flower). As such, we used generalised linear mixed models (GLMMs) with a Gamma error distribution and log link, with the independent variables being fly species with three levels (*E. tenax*, *C. stygia*, *M. domestica*) and flower colour with four levels (blue, pink, yellow, white), and the dependent variables being latency and foraging duration in their respective models. We included fly identity as a random intercept to account for non-independence among simultaneously presented flowers.

As well as overall colour preferences and foraging behaviours, we examined the influence of subjective colour features—namely fly-perceived hue, saturation and luminance—on first choices for each species. For this, we constructed three GLMMs (one per species) with hue, saturation and luminance as fixed effects and choices coded as a binary response for each of the four flower options in a trial. While hue, saturation and luminance are not strictly independent due to physical constraints on reflectance, they are derived from distinct photoreceptor channels and treated as separable predictors in standard visual models. Our use of four colours reflects a compromise between ecological relevance and experimental tractability, as detailed earlier. We included individual fly identity as a random effect to account for non-independence among simultaneously presented flowers, as earlier, and specified a binomial error family with a logit link function in all models. In addition to reporting the Wald *p*-values from model summaries, we also calculated *p*-values for each fixed effect using likelihood-ratio tests (LRTs) comparing the full model to reduced models omitting each term in turn.



These results were qualitatively unchanged from the main analyses and are presented in Table S2.

We visually confirmed all model assumptions using the DHARMA (v. 0.4.7) and performed all data processing and statistical analyses in R (v. 3.4.3; R Core Team 2013). We used the conventional threshold of  $\alpha < 0.05$  for statistical significance in all tests.

### 3 | Results

#### 3.1 | Overall Colour Preferences

We found that when presented with a choice between equally rewarding blue, pink, yellow and white artificial flowers, the hoverfly *Eristalis tenax* displayed an innate overall preference for yellow (68%), but neither the blowfly *Calliphora stygia* nor the housefly *Musca domestica* showed a pronounced preference for any one colour (Figure 2; see Table 2 for full numerical results). *Calliphora stygia* visited pink flowers slightly more often than the alternatives (33%; though this was statistically non-significant), while *Musca domestica* visited white and yellow flowers most often, accounting for 60% of all visits; though this was also not statistically significant (Figure 2; Table 2). We found no evidence for sex differences among species, with both male and female *E. tenax* preferring yellow (66% vs. 69%; Figure S2; Table 2). Female *C. stygia* chose pink flowers slightly more often than male flies did (Figure S2; 42% compared to 24%); however, this difference was not statistically significant (Table 2).

#### 3.2 | Foraging Behaviours

We found no effect of flower colour on the time taken for flies to land for any of our focal species (Tables 3 and S1). *Eristalis tenax* tended to take longer to land on flowers overall compared to the other flies, with an average latency of  $6.61 \pm 5.92$  min compared to  $4.57 \pm 4.56$  min for *C. stygia* and  $4.90 \pm 5.22$  min for *M. domestica* (Figure 2; Table 3), though this difference was not statistically significant. The duration of visits also did not significantly differ between flower colours (Table 3) but did differ between species, with *M. domestica* foraging for  $4.95 \pm 5.10$  min, over one minute longer than both *C. stygia* ( $3.37 \pm 4.18$  min) and *E. tenax* ( $3.65 \pm 4.91$  min) on average (Figure 2; Table 3).

#### 3.3 | Colour Features

We found clear evidence for decision-making being guided by the chromatic features of artificial flowers in all species (Table 4 for full numerical results). *Eristalis tenax* strongly favoured more saturated (or 'vivid') colours, which echoes their demonstrated preference for the yellow flower, which is the most strongly saturated in the choice set (Figure 1E; Table 1). *Calliphora stygia* showed no overall preference for any one colour (as above), but nonetheless favoured more saturated options, as well as those which chiefly stimulated their R7p–R8p (i.e., short- vs. long-wavelength) opponency channel, as indicated by more negative hue angles (Table 1). *Musca domestica* showed the same effects, albeit with different relative strengths. They strongly favoured

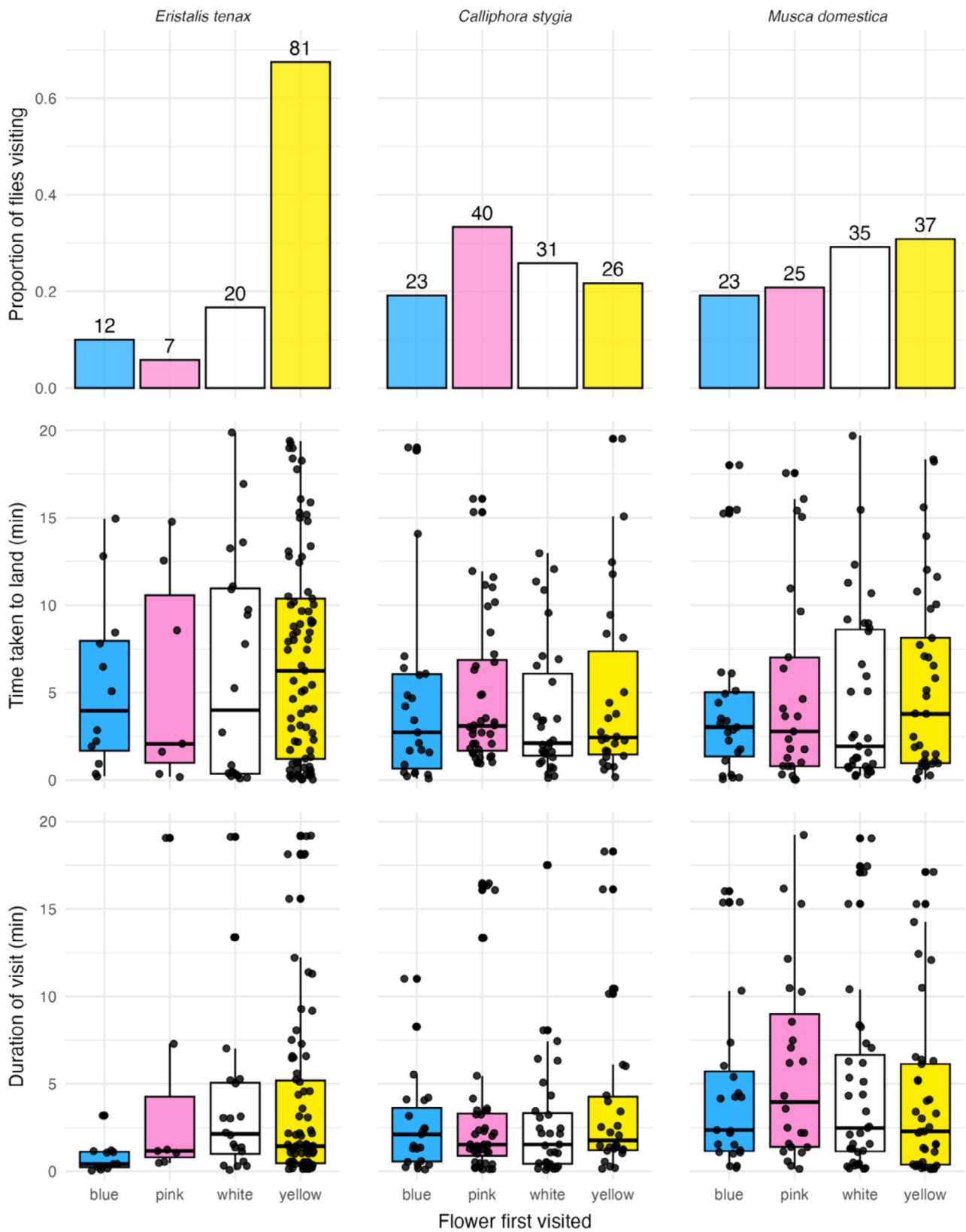
more saturated colours, with only a weakly negative influence of flower hue. We found no evidence that flower luminance influenced the choices of any species.

## 4 | Discussion

Insect foraging is a driving force in the evolution of colourful floral displays, with colour being a vital trait influencing flower-visitation (Chittka and Menzel 1992; Ellis et al. 2021; Garcia et al. 2022; Lunau 1996; Matouskova et al. 2023). Work to date has overwhelmingly centred on decision making in honeybees, with comparatively little known of the visual preferences of alternative pollinators, such as flies, in a foraging context (Lunau 2014). Here we tested the innate colour preferences of three species of fly by offering individuals a choice between blue, pink, yellow and white artificial flowers in simultaneous choice assays. *Eristalis tenax* exhibited a pronounced overall preference for yellow, while *Calliphora stygia* and *Musca domestica* showed no such gross colour preferences. Decisions in all three species, however, were guided by the fly-subjective chromatic features of flowers, namely saturation and hue, but not their luminance. Together, our results reveal differences in the innate sensory and foraging ecology of flower-visiting flies, with implications for their role as potential alternative pollinators.

The pronounced innate attraction towards yellow displayed by *E. tenax* is consistent with past study (An et al. 2018; Hannah et al. 2019; Kelber 2001; Lunau et al. 2018; Matouskova et al. 2023) and confirms that the use of artificial flowers in a controlled assay design is a reliable method for testing colour preferences in flies more broadly (Chapman et al. 2023). The innate yellow preference in *E. tenax* has been hypothesised by Lunau and Wacht (1994) to be linked to the yellow pollen typical of the flowers *E. tenax* forages on in natural environments. While innate, this preference for yellow persists with experience in nature, with De Buck (1990) finding that *E. tenax* foraged on 150 plant species of which 73% had yellow flowers.

By contrast, we found no such overall preferences in *M. domestica* and *C. stygia*. We did, however, identify cue-specific influences on their first choices, with both responding to the saturation and hue of flowers, albeit to different extents (Table 3). *Eristalis tenax* also responded readily to saturation (Table 3). This suggests the choices of these fly species are not strongly guided by categorical preferences but rather the continuous colour attributes of flowers. Though well documented among insects including honeybees (Hempel de Ibarra et al. 2014), it is a curious finding in these flies for two reasons. One is that the colour vision of many flies, including blowflies specifically, has been hypothesised as 'categorical'. The study of Troje (1993) with *Lucilia* sp. has been particularly influential in suggesting the existence of four broad categories of colour, with flies being unable to discriminate between stimuli in each category. Our results argue against this view, given that individuals' choices are well predicted by continuous measures of our floral signals, which otherwise all sit within a single colour 'category' in Troje's hypothesised model (the bottom left quadrant of colour space; Figure 1F). This finding is, however, consistent with recent dedicated tests (Hannah et al. 2019), which also showed no support for the assumption of broad colour categorisation.



**FIGURE 2** | The proportion of first choices (top row), time taken to land (middle row) and duration of visits (bottom row) in simultaneous choice assays of innate colour preferences across three species of pollinating fly. Raw counts are displayed above bar graphs, and raw data are overlaid on boxplots, which otherwise denote medians and interquartile ranges ( $n=120$  per species). The middle and bottom panels correspond to the GLMM analyses reported in Tables 3 and S1.

**TABLE 2** | Summary Chi-square results testing for overall colour preferences among three species of fly, and across both sexes, in simultaneous-choice assays with four artificial flowers (blue, pink, white, yellow).

Response metrics	$\chi^2$	df	n	p
Colour visited				
Difference within <i>E. tenax</i>	118.470	3	120	<b>&lt;0.001</b>
Difference within <i>C. stygia</i>	5.533	3	120	0.137
Difference within <i>M. domestica</i>	4.933	3	120	0.177
Colour visited by sex				
Difference within <i>E. tenax</i>	0.341	3	120	0.952
Difference within <i>C. stygia</i>	6.230	3	120	0.101
Difference within <i>M. domestica</i>	1.183	3	120	0.757

Note: Statistical significance is denoted by bolding, and  $n=120$  per species, balanced across sexes. These tests indicate whether flies deviated from random choice, with significant results reflecting a measurable preference for some colours over others within the assay context.

**TABLE 3** | Summary type II Wald  $\chi^2$  test results from Gamma GLMs testing for behavioural differences among three fly species and/or four artificial flower colours in their latency to land and duration spent on a flower.

Variable	df	Chi Sq	p
Latency: time taken to land			
Fly species	2	4.81	0.090
Colour visited	3	1.70	0.638
Fly species*Colour visited	6	0.62	0.996
Duration: time spent on a flower			
Fly species	2	10.98	<b>&lt;0.004</b>
Colour visited	3	4.03	0.258
Fly species*Colour visited	6	11.41	0.077

Note: Statistical significance ( $\alpha < 0.05$ ) is denoted in bold, and  $n=120$  individuals per species.

The second reason is that flies are often thought to attend foremost to the brightness of colour patterns; this view is supported by the overrepresentation of white and pale (low-saturation) floral signals in many fly-dominated habitats (Arnold et al. 2009; Pickering and Stock 2003). We identified no such preference for the subjectively brightest colour in our set (white; Figure 2), and to the contrary found that it was the chromatic features of flowers (saturation and hue) which exclusively predicted visitation, with no discernible influence of luminance (Table 3). Related studies have yielded mixed result on the subject, with blue (Geden 2006; Kafle et al. 2019; Khan and Yogi 2017),

**TABLE 4** | The results of binomial GLMMs examining how fly-subjective colour features guide the choice of artificial flowers in simultaneous-choice assays with three species of pollinating fly ( $n=120$  per species).

Model	Est.	SE	z	p	R <sup>2</sup>
<i>Eristalis tenax</i>					
Intercept	−8.30	2.70	−3.07	<b>&lt;0.01</b>	0.66
Hue	−0.03	0.56	−0.59	0.95	
Saturation	14.15	2.76	5.121	<b>&lt;0.001</b>	
Luminance	0.526	0.89	0.591	0.55	
<i>Calliphora stygia</i>					
Intercept	−0.70	1.07	−4.38	<b>&lt;0.001</b>	0.44
Hue	−0.89	0.25	−3.57	<b>&lt;0.001</b>	
Saturation	2.51	1.26	1.99	<b>0.046</b>	
Luminance	0.32	0.68	0.47	0.640	
<i>Musca domestica</i>					
Intercept	−5.98	1.54	−3.90	<b>&lt;0.001</b>	0.53
Hue	−0.67	0.34	−1.98	<b>0.049</b>	
Saturation	5.74	1.62	3.54	<b>&lt;0.001</b>	
Luminance	1.22	0.68	1.79	0.07	

Note: Statistical significance ( $\alpha < 0.05$ ) is denoted in bold.

white (Diclaro et al. 2012; Dieng et al. 2021) and yellow (Burg and Axtell 1984) proving attractive to *M. domestica*, and yellow (Brodie et al. 2015; Fukushi 1989), blue (Fukushi 1989), red (Chen et al. 2015) and green (Fraga and d'Almeida 2005) preferred by related blowflies (*Chrysomya megacephala* and *Lucilia sericata*). An enduring challenge in uniting these results is the variation in available colours within a set or community, their exact visual properties, and the nature of the preference under consideration. Nonetheless, these findings, taken with our own results, support an emerging view that colour-based cues may play a richer role in floral foraging in many flies than has historically been appreciated.

It should be noted, however, that while our results do suggest a stronger role for hue and saturation over luminance in shaping fly choices, the restricted number and spacing of colour stimuli limits the strength of this inference. Future study incorporating a broader and more continuous range of stimuli, particularly isoluminant sets or systematic manipulations of individual colour dimensions, will be valuable for more precisely teasing apart these effects.

The gross difference in innate preferences between *E. tenax* on the one hand and *M. domestica* and *C. stygia* on the other (Figure 2) highlights both shared and divergent aspects of the innate sensory ecology of flower-visiting flies. In particular, the tendency for *M. domestica* and *C. stygia* to forage on a variety of non-floral resources including carrion, faeces, rotting fruit and vegetables, as well as nectar and pollen, suggests they may be less responsive to visual floral traits than *E. tenax* which obtains nearly all its nutrients from flowers (Gilbert 1981; Willmer 2011).

To that end Hall (1995) argued that visual cues may play only a secondary role in attracting blowflies, and conceivably houseflies, with odour being the primary attractant. In our assays, then, the odour of equivalent honey rewards across all choices may have served to temper the expression of overall colour preferences in *M. domestica* and *C. stygia*, if they weigh odour cues more heavily than *E. tenax* during foraging.

Insect-pollinator foraging decisions have profound implications for plant communities, including in agricultural settings. Understanding why insect pollinators choose one flower over another is therefore a question of practical significance (Latty et al. 2020). Field-based studies indicate the identified yellow preference of *E. tenax* persists in natural settings (De Buck 1990; Lunau and Maier 1995), which suggests yellow-flowered crops such as mustard (*Sinapis alba*), canola (*Brassica napus*), yellow capsicum (*Capsicum annuum*) and watermelon (*Citrullus lanatus*) may benefit from preferential visitation by *E. tenax*. Proximity to attractive yellow flowers could also favour non-preferred crops via ‘spillover’ effects in mixed planting systems, or through the targeted use of wildflower strips (Ghazoul 2006; Johnson et al. 2003; Pereira-Peixoto et al. 2014). For *M. domestica* and *C. stygia* their interest in specific colour features over gross differences supports a view of them as more generalised and flexible foragers. It also invites further research into the combined influence of suites of floral traits (such as scent, shape, reward quality, or floral guides) in guiding multi-attribute decision making (Latty et al. 2020). These stand as exciting lines of future inquiry, as we continue to address the urgent challenge of cultivating resilience in wild and managed pollination systems.

## Author Contributions

**Eliza G. Crossley:** conceptualization, investigation, writing – original draft, data curation, methodology, writing – review and editing, formal analysis, visualization. **Caitlyn Y. Forster:** methodology, supervision, writing – review and editing, conceptualization. **Tanya Latty:** resources, supervision, methodology, conceptualization, funding acquisition. **Thomas E. White:** conceptualization, funding acquisition, formal analysis, supervision, resources, project administration, writing – review and editing, methodology, visualization, data curation.

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## Ethics Statement

No ethics permits were required for this study, though to the best of our knowledge, there was no experimentally induced injury or lasting stress among study animals. All flies, once used in a single assay, were returned to a cage where they were fed *ad libitum* for the natural duration of their lives.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

All underlying data and code are available via GitHub ([https://github.com/SEElab-18/ms\\_innate\\_fly/](https://github.com/SEElab-18/ms_innate_fly/)) and is archived via Zenodo (<https://dx.doi.org/10.5281/zenodo.17254716>).

## References

- An, L., A. Neimann, E. Eberling, H. Algora, S. Brings, and K. Lunau. 2018. “The Yellow Specialist: Dronefly *Eristalis tenax* Prefers Different Yellow Colours for Landing and Proboscis Extension.” *Journal of Experimental Biology* 221: jeb184788.
- Arnold, S. E. J., V. Savolainen, and L. Chittka. 2009. “Flower Colours Along an Alpine Altitude Gradient, Seen Through the Eyes of Fly and Bee Pollinators.” *Arthropod-Plant Interactions* 3, no. 1: 27–43.
- Biesmeijer, J. C., M. Giurfa, D. Koedam, S. G. Potts, D. M. Joel, and A. Dafni. 2005. “Convergent Evolution: Floral Guides, Stingless Bee Nest Entrances, and Insectivorous Pitchers.” *Die Naturwissenschaften* 92, no. 9: 444–450.
- Brodie, B., M. Smith, J. Lawrence, and G. Gries. 2015. “Effects of Floral Scent, Color and Pollen on Foraging Decisions and Oocyte Development of Common Green Bottle Flies.” *PLoS One* 10: e0145055.
- Burg, J. G., and R. C. Axtell. 1984. “Monitoring House Fly, *Musca domestica* (Diptera: Muscidae), populations in Caged-Layer Poultry Houses Using a Baited Jug-Trap 1.” *Environmental Entomology* 13, no. 4: 1083–1090.
- Chapman, K. M., F. J. Richardson, C. Y. Forster, et al. 2023. “Artificial Flowers as a Tool for Investigating Multimodal Flower Choice in Wild Insects.” *Ecology and Evolution* 13, no. 11: e10687.
- Chen, G., X.-K. Ma, A. Jürgens, et al. 2015. “Mimicking Livor Mortis: A Well-Known but Unsubstantiated Color Profile in Saproxyophily.” *Journal of Chemical Ecology* 41, no. 9: 808–815.
- Chittka, L., and R. Menzel. 1992. “Evolutionary Adaptation of Flower Colours and the Insect Pollinators’ Colour Vision.” *Journal of Comparative Physiology A* 171, no. 2: 171–181.
- Clement, S., B. Hellier, L. Elbertson, R. Staska, and M. Evans. 2007. “Flies (Diptera: Muscidae: Calliphoridae) are Efficient Pollinators of *Allium ampeloprasum* L. (Alliaceae) in Field Cages.” *Journal of Economic Entomology* 100: 131–135.
- Cook, D., M. Tufail, S. Voss, et al. 2023. “Blow Flies (Diptera: Calliphoridae) Ability to Pollinate Hass Avocado Trees Within Paired Tree Enclosures.” *Journal of Applied Entomology* 147: 577–591.
- Cook, D., S. Voss, J. Finch, R. Rader, J. Cook, and C. Spurr. 2020. “The Role of Flies as Pollinators of Horticultural Crops: An Australian Case Study With Worldwide Relevance.” *Insects* 11: 341.
- Croft, L., P. Matheson, C. Flemming, N. J. Butterworth, and A. McGaughan. 2024. “Population Structure and Interspecific Hybridisation of Two Invasive Blowflies (Diptera: Calliphoridae) Following Replicated Incursions Into New Zealand.” *Ecology and Evolution* 14, no. 1: e10832.
- Damayo, J. E., R. C. McKee, G. Buchmann, et al. 2023. “Virus Replication in the Honey Bee Parasite, *Varroa destructor*.” *Journal of Virology* 97, no. 12: e0114923.
- De Buck, N. 1990. *Flower Visitation and Pollination Ecology of Hoverflies (Diptera, Syrphidae) in Particular for Belgium*. Vol. 60. Royal Belgian Institute of Natural Sciences.
- de Camargo, M. G. G., K. Lunau, M. A. Batalha, S. Brings, V. L. G. de Brito, and L. P. C. Morellato. 2019. “How Flower Colour Signals Allure Bees and Hummingbirds: A Community-Level Test of the Bee Avoidance Hypothesis.” *New Phytologist* 222, no. 2: 1112–1122.
- Deheyn, D. D. 2017. “How Flowers Get the Blues to Lure Bees.” *Nature* 550, no. 7677: 467–468.
- Diclaro, J., L. Cohnstaedt, R. Pereira, S. Allan, and P. Koehler. 2012. “Behavioral and Physiological Response of *Musca domestica* to Colored Visual Targets.” *Journal of Medical Entomology* 49: 94–100.
- Dieng, H., T. Satho, N. Radzi, et al. 2021. “Flower Mimics Roll out Multicolored Carpets to Lure and Kill the House Fly.” *Insects* 12: 1097.



- Dyer, A. G., S. Boyd-Gerny, M. Shrestha, et al. 2016. "Innate Colour Preferences of the Australian Native Stingless Bee *Tetragonula carbonaria* Sm." *Journal of Comparative Physiology* 202, no. 9–10: 603–613.
- Ellis, A. G., B. Anderson, and J. E. Kemp. 2021. "Geographic Mosaics of Fly Pollinators With Divergent Color Preferences Drive Landscape-Scale Structuring of Flower Color in Daisy Communities." *Frontiers in Plant Science* 12: 617761.
- Faegri, K., and L. v. d. Pijl. 1979. *The Principles of Pollination Ecology*. 3rd rev ed. Pergamon Press.
- Fraga, M., and M. d'Almeida. 2005. "Preliminary Remarks on Attractiveness to Different Colours in Calliphoridae (Diptera), Niterói, RJ, Brazil." *Entomologia y Vectores* 12, no. 1: 155–158.
- Frisch, K. v., and O. v. Frisch. 1975. *Animal architecture*. Hutchinson.
- Fukushi, T. 1989. "Learning and Discrimination of Coloured Papers in the Walking Blowfly, *Lucilia Cuprina*." *Journal of Comparative Physiology A* 166, no. 1: 57–64.
- Garcia, J., L. Hannah, M. Shrestha, M. Burd, and A. Dyer. 2022. "Fly Pollination Drives Convergence of Flower Coloration." *New Phytologist* 233: 52–61.
- Geden, C. 2006. "Visual Targets for Capture and Management of House Flies, *Musca domestica* L." *Journal of Vector Ecology* 31: 152–157.
- Ghazoul, J. 2006. "Floral Diversity and the Facilitation of Pollination." *Journal of Ecology* 94: 295–304.
- Gilbert, F. 1981. "Foraging Ecology of Hover-Flies - Morphology of the Mouthparts in Relation to Feeding on Nectar and Pollen in Some Common Urban Species." *Ecological Entomology* 6: 245–262.
- Giurfa, M., J. Nunez, L. Chittka, and R. Menzel. 1995. "Color Preferences of Flower-Naive Honeybees." *Journal of Comparative Physiology A* 177, no. 3: 247–259.
- Goyret, J., M. Pfaff, R. A. Raguso, and A. Kelber. 2008. "Why Do *Manduca sexta* Feed From White Flowers? Innate and Learnt Colour Preferences in a Hawkmoth." *Die Naturwissenschaften* 95, no. 6: 569–576.
- Gruson, H., T. White, and R. Maia. 2019. "Lightr: Import Spectral Data and Metadata in R." *Journal of Open Source Software* 4, no. 43: 1857.
- Gumbert, A. 2000. "Color Choices by Bumble Bees (*Bombus terrestris*): Innate Preferences and Generalization After Learning." *Behavioral Ecology and Sociobiology* 48, no. 1: 36–43.
- Hall, M. J. R. 1995. "Trapping the Flies That Cause Myiasis: Their Responses to Host-Stimuli." *Annals of Tropical Medicine and Parasitology* 89, no. 4: 333–357.
- Hannah, L., A. Dyer, J. Garcia, A. Dorin, and M. Burd. 2019. "Psychophysics of the Hoverfly: Categorical or Continuous Color Discrimination?" *Current Zoology* 65: 483–492.
- Hansen, K., S. Wacht, H. Seebauer, and M. Schnuch. 1998. "New Aspects of Chemoreception in Flies Olfaction and Taste Xii." *Annals of the New York Academy of Sciences* 855: 143–147.
- Hardie, R. C. 1986. "The Photoreceptor Array of the Dipteran Retina." *Trends in Neurosciences* 9, no. 9: 419–423.
- Haslett, J. 1989. "Interpreting Patterns of Resource Utilization: Randomness and Selectivity in Pollen Feeding by Adult Hoverflies." *Oecologia* 78: 433–442.
- Hempel de Ibarra, N., M. Vorobyev, and R. Menzel. 2014. "Mechanisms, Functions and Ecology of Colour Vision in the Honeybee." *Journal of Comparative Physiology* 200, no. 6: 411–433.
- Howlett, B. G., and M. Gee. 2019. "The Potential Management of the Drone Fly (*Eristalis tenax*) as a Crop Pollinator in New Zealand." *New Zealand Plant Protection* 72: 221–230.
- Inouye, D. W., B. M. H. Larson, A. Ssymank, and P. G. Kevan. 2015. "Flies and Flowers III: Ecology of Foraging and Pollination." *Journal of Pollination Ecology* 16: 115–133.
- Johnson, S. D., C. I. Peter, L. A. Nilsson, and J. Ågren. 2003. "Pollination Success in a Deceptive Orchid Is Enhanced by co-Occurring Rewarding Magnet Plants." *Ecology* 84, no. 11: 2919–2927.
- Kafle, L., J. Gonzales, and A. Neupane. 2019. "Colored Bait Preferences of House Fly (*Musca domestica* L.) (Diptera: Muscidae)." *Entomological Research* 49: 451–455.
- Kandori, I., S. Fukada, T. Kurosaki, T. Yokoi, and D. R. Papaj. 2021. "Comparison of Color-Learning Rates Among Eight Species of Three Insect Orders (Hymenoptera, Diptera, and Lepidoptera)." *Ecological Research* 36, no. 5: 830–841. <https://doi.org/10.1111/1440-1703.12249>.
- Karasiński, J. 2018. *The Economic Valuation of Australian Managed and Wild Honey Bee Pollinators in 2014–2015*. Curtin University.
- Kelber, A. 2001. "Receptor Based Models for Spontaneous Colour Choices in Flies and Butterflies." *Entomologia Experimentalis et Applicata* 99, no. 2: 231–244.
- Khan, H., S. Shad, and W. Akram. 2013. "Combination of Phagostimulant and Visual Lure as an Effective Tool in Designing House Fly Toxic Baits: A Laboratory Evaluation." *PLoS One* 8: e77225.
- Khan, M. S., and M. K. Yogi. 2017. "Insect Crop Pollinators." In *Industrial Entomology*, 397–412. Springer Singapore Pte. Limited.
- Kuenzinger, W., A. Kelber, J. Weesner, J. Travis, R. A. Raguso, and J. Goyret. 2019. "Innate Colour Preferences of a Hawkmoth Depend on Visual Context." *Biology Letters* 15, no. 3: 20180886.
- Lang, M. D., G. R. Allen, and B. J. Horton. 2006. "Blowfly Succession From Possum (*Trichosurus vulpecula*) Carrion in a Sheep-Farming Zone." *Medical and Veterinary Entomology* 20, no. 4: 445–452.
- Larson, B., P. Kevan, and D. Inouye. 2001. "Flies and Flowers: Taxonomic Diversity of Anthophiles and Pollinators." *Canadian Entomologist* 133: 439–465.
- Latty, T., and V. Dakos. 2019. "The Risk of Threshold Responses, Tipping Points, and Cascading Failures in Pollination Systems." *Biodiversity and Conservation* 28, no. 13: 3389–3406.
- Latty, T., J. S. Trueblood, and G. Machovsky-Capuska. 2020. "How Do Insects Choose Flowers? A Review of Multi-Attribute Flower Choice and Decoy Effects in Flower-Visiting Insects." *Journal of Animal Ecology* 89, no. 12: 2750–2762.
- Levot, G., and G. Casburn. 2016. *Sheep Blowflies*. Primefact.
- Lunau, K. 1996. "Signalling Functions of Floral Colour Patterns for Insect Flower Visitors." *Zoologischer Anzeiger* 235: 11–30.
- Lunau, K. 2014. "Visual Ecology of Flies With Particular Reference to Colour Vision and Colour Preferences." *Journal of Comparative Physiology A* 200: 497–512.
- Lunau, K., and E. J. Maier. 1995. "Innate Colour Preferences of Flower Visitors." *Journal of Comparative Physiology A* 177, no. 1: 1–19.
- Lunau, K., L. N. An, M. Donda, M. Hohmann, L. Sermon, and V. Stegmanns. 2018. "Limitations of Learning in the Proboscis Reflex of the Flower Visiting Syrphid Fly *Eristalis tenax*." *PLoS One* 13, no. 3: e0194167.
- Lunau, K., and S. Wacht. 1994. "Optical Releasers of the Innate Proboscis Extension in the Hoverfly *Eristalis-Tenax* L (Syrphidae, Diptera)." *Journal of Comparative Physiology A* 174, no. 5: 575–579.
- Maia, R., H. Gruson, J. A. Endler, T. E. White, and R. B. O'Hara. 2019. "pavo 2: New Tools for the Spectral and Spatial Analysis of Colour in R." *Methods in Ecology and Evolution* 10, no. 7: 1097–1107.
- Maia, R., and T. E. White. 2018. "Comparing Colors Using Visual Models." *Behavioral Ecology* 29, no. 3: 649–659.

- Matouskova, E., J. Stenc, and Z. Janovsky. 2023. "Innate Preferences of *Eristalis tenax* L. (Syrphidae) for Flower Colour, Size and Symmetry Are More Intricate Than the Simple Additive Model." *Biological Journal of the Linnean Society* 140, no. 1: 110–119.
- Mello, R. S., M. M. C. Queiroz, A. F. Nunes-Freitas, and V. M. Aguiar-Coelho. 2009. "Calliphorid Fly (Diptera, Calliphoridae) Attraction to Different Colored Traps in the Tingua Biological Reserve, Rio de Janeiro, Brazil." *Iheringia Série Zoologia* 99, no. 4: 426–430.
- Mu, J., Y. Yang, Y. Luo, R. Su, and K. J. Niklas. 2017. "Pollinator Preference and Pollen Viability Mediated by Flower Color Synergistically Determine Seed Set in an Alpine Annual Herb." *Ecology and Evolution* 7, no. 9: 2947–2955.
- Muñoz-Galicia, D., C. Castillo-Guevara, and C. Lara. 2021. "Innate and Learnt Color Preferences in the Common Green-Eyed White Butterfly (*Leptophobia aripa*): Experimental Evidence." *PeerJ* 9: e12567.
- Nuzhnova, O. K., and N. V. Vasilevskaya. 2013. "Effect of Color Preferences on the Foraging Behavior of the Green-Veined White Butterfly (*Pieris napi* L.)." *Contemporary Problems of Ecology* 6, no. 1: 45–50.
- Pereira-Peixoto, M. H., G. Pufal, C. F. Martins, and A.-M. Klein. 2014. "Spillover of Trap-Nesting Bees and Wasps in an Urban–Rural Interface." *Journal of Insect Conservation* 18, no. 5: 815–826.
- Phaboutdy, E., and M. Ward. 2024. "Investigation of Landscape Risk Factors for the Recent Spread of Varroa Mite (*Varroa destructor*) in European Honeybee (*Apis mellifera*) Colonies in New South Wales, Australia." *Geospatial Health* 19, no. 1: 1282. <https://doi.org/10.4081/gh.2024.1282>.
- Pickering, C. M., and M. Stock. 2003. "Insect Colour Preference Compared to Flower Colours in the Australian Alps." *Nordic Journal of Botany* 23, no. 2: 217–223.
- Prokopy, R. J., A. P. Economopoulos, and M. W. McFadden. 1975. "Attraction of Wild and Laboratory-Cultured *Dacus oleae* Flies to Small Rec Tangles of Different Hues, Shades, and Tints." *Entomologia Experimentalis et Applicata* 18: 141–152.
- R Core Team. 2013. "R Language Definition." *R Foundation for Statistical Computing* 3, no. 1: 116.
- R Core Team. 2018. "R: A Language and Environment for Statistical Computing." R Foundation for Statistical Computing.
- Rader, R., S. A. Cunningham, B. G. Howlett, and D. W. Inouye. 2020. "Non-Bee Insects as Visitors and Pollinators of Crops: Biology, Ecology, and Management." *Annual Review of Entomology* 65, no. 1: 391–407.
- Reverté, S., J. Retana, J. Gómez, and J. Bosch. 2016. "Pollinators Show Flower Colour Preferences but Flowers With Similar Colours Do Not Attract Similar Pollinators." *Annals of Botany* 118: 249–257.
- Rodríguez-Gironés, M., and L. Santamaría. 2004. "Why Are So Many Bird Flowers Red?" *PLoS Biology* 2: 1515–1519.
- Stavert, J. R., D. E. Pattemore, I. Bartomeus, A. C. Gaskett, and J. R. Beggs. 2018. "Exotic Flies Maintain Pollination Services as Native Pollinators Decline With Agricultural Expansion." *Journal of Applied Ecology* 55, no. 4: 1737–1746.
- Streinzer, M., J. Neumayer, and J. Spaethe. 2021. "Flower Color as Predictor for Nectar Reward Quantity in an Alpine Flower Community." *Frontiers in Ecology and Evolution* 9: 721241.
- Tiusanen, M., P. D. N. Hebert, N. M. Schmidt, and T. Roslin. 2016. "One Fly to Rule Them All—Muscid Flies Are the Key Pollinators in the Arctic." *Proceedings of the Royal Society. B, Biological Sciences* 283, no. 1839: 20161271.
- Troje, N. 1993. "Spectral Categories in the Learning Behaviour of Blowflies." *Zeitschrift Für Naturforschung. C, A Journal of Biosciences* 48, no. 1: 96–104.
- van der Kooi, C. J., D. G. Stavenga, K. Arikawa, G. Beluš, and A. Kelber. 2021. "Evolution of Insect Color Vision: From Spectral Sensitivity to Visual Ecology." *Annual Review of Entomology* 66, no. 1: 435–461.
- Willmer, P. 2011. "Pollination by Flies." In *Pollination and Floral Ecology*, 304–321. Princeton University Press.
- Wilson, R. L., M. P. Widrechner, and K. R. Reitsma. 1991. "Pollination Methods for Maintaining Carrot Germplasm Collections." *FAO/IBPGR Plant Genetic Resources Newsletter* 85, no. 1: 1–3.
- Woodcock, T. S., B. M. H. Larson, P. G. Kevan, D. W. Inouye, and K. Lunau. 2014. "Flies and Flowers II: Floral Attractants and Rewards." *Journal of Pollination Ecology* 12: 63–94.
- Yurtsever, S., O. Zuhali, and G. Necmettin. 2010. "What Colour of Flowers Do Lepidoptera Prefer for Foraging?" *Biologia* 65, no. 6: 1049–1056.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The experimental arena for simultaneous-choice assays. Flies entered the arena from a small opening in the top centre of the cage, within which they could move freely. Artificial flowers were placed in fixed holes 14 cm apart, with wells containing diluted honey rewards in the centre of each flower. **Figure S2:** The proportion of first choices for artificial flowers, separated by males (M) and female (F), in three species of pollinating fly. The raw counts are displayed above each column ( $n = 120$  per species), and columns are coloured to match the colour of the artificial flower in the experiments. **Table S1:** Parameter estimates from Gamma GLMMs testing for differences among three fly species and/or four artificial flower colours in latency to first landing and duration of first visit. Reference categories are *C. stygia* for species and blue for colour. Models include fly identity as a random intercept. **Table S2:** Likelihood-ratio test (LRT) results for fixed effects in binomial GLMMs testing fly colour choice for each species. *p*-values are from Chi-squared tests comparing the full model to reduced models omitting each term in turn.