Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

UV-absorbing white attracts more non-syrphid flower-visiting flies than UV-reflecting white

Tsuguhiro T Osugi

sora2017ddr@gmail.com

University of Toyama Faculty of Science Graduate School of Science and Engineering: Toyama Daigaku Rigakubu Daigakuin Rikogaku Kyoikubu Rigaku Ryoiki https://orcid.org/0009-0003-0898-0701

Shohei G Tsujimoto

Meijo University Faculty of Agriculture Graduate School of Agriculture: Meijo Daigaku Nogakubu Daigakuin Nogaku Kenkyuka

Atsushi Ushimaru

Kobe University Graduate School of Human Development and Environment Faculty of Human Development: Kobe Daigaku Daigakuin Ningen Hattatsu Kankyogaku Kenkyuka Hattatsu Kagakubu

Hiroshi S Ishii

University of Toyama Faculty of Science Graduate School of Science and Engineering: Toyama Daigaku Rigakubu Daigakuin Rikogaku Kyoikubu Rigaku Ryoiki

Research Article

Keywords: Diptera (Brachycera), ultraviolet reflectance and absorption, visual cues, pollinator behavior, flower–visitor interactions

Posted Date: June 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9700862/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Ultraviolet (UV) reflectance and absorption are widespread floral traits known to influence pollinator behavior, yet their effects remain poorly understood for many pollinator taxa beyond bees. In particular, the responses of non-syrphid Brachycera, a diverse and abundant group of flower-visiting flies, have received little empirical attention. Here, we provide the first empirical evidence that flower-visiting non-syrphid flies are more attracted to UV-absorbing than UV-reflecting white visual cues. We conducted field experiments in a Japanese alpine ecosystem using colored pan traps and sticky traps that differed in UV reflectance (UV-absorbing vs. UV-reflecting) and human-visible color (white and yellow). UV-absorbing white traps consistently captured significantly more non-syrphid Brachycera than UV-reflecting white traps across years and trap types, whereas no significant difference was detected between UV-absorbing and UV-reflecting yellow traps. These results indicate that the influence of UV on attractiveness to these flies cannot be explained solely by UV absorption or reflection per se, but likely depends on interactions with the non-UV spectral properties. In contrast, non-eusocial bees showed no significant differences in responses to UV-absorbing versus UV-reflecting visual cues, highlighting taxon-specific variation in sensitivity to UV-related signals. Together, our findings reveal previously unrecognized UV-mediated responses in flower-visiting flies and underscore the importance of considering pollinator identity when evaluating the ecological and evolutionary roles of floral UV traits.

Introduction

Many pollinators possess the ability to perceive ultraviolet light in the wavelength range of 320–400 nm (hereafter referred to as UV). Behavioral studies have demonstrated that UV sensitivity is an integral component of the color vision systems of diverse pollinator taxa, including bees (Peitsch et al. 1992), butterflies (Arikawa et al. 1987), flies (Troje 1993), and birds (Kreithen and Eisner 1978). Correspondingly, UV reflectance and UV patterns are widespread among angiosperm flowers (Horovitz and Cohen 1972; Guldberg and Atsatt 1975). Taken together, these findings suggest that UV vision helps pollinators locate flowers (Klomberg et al. 2019), discriminate among individual flowers that differ in UV coloration within plant communities (Johnson and Andersson 2002), identify appropriate landing sites (Jones and Buchmann 1974), and detect floral resources within a given flower (Jones and Buchmann 1974; Lunau et al. 2005).

To date, research on floral UV signals has focused primarily on how UV patterns and UV reflectance influence pollinator attraction and guidance to rewards. For example, UV bullseye patterns—composed of UV-absorbing petal bases and UV-reflective petal apices—function as nectar guides, particularly for bees (Horth et al. 2014; Papiorek et al. 2016), and occasionally for other pollinator groups such as bee flies (Johnson and Dafni 1998), syrphid flies (Dinkel and Lunau 2001), butterflies (Borges et al. 2003), hawk moths (Hirota et al. 2019), and birds (Papiorek et al. 2016). In addition, the attractive effects of floral UV patterns and UV reflectance itself have been documented predominantly in bees (Horth et al. 2014; Papiorek et al. 2016; Klomberg et al. 2019), with only limited evidence from syrphid flies (An et al. 2018; Neiman et al. 2018), butterflies and hawk moths (Hirota et al. 2019), and birds (Lunau et al. 2011;

Papiorek et al. 2016). Evidence from studies of neotropical orchid bees further suggests that the attractive effects of UV reflectance can depend on visible color context: bees preferred UV-reflecting red and UV-absorbing white stimuli over red and white stimuli with the opposite UV properties (Lunau et al. 2011). Nevertheless, empirical work on the effects of UV reflectance and UV patterns has remained strongly biased toward bees, with relatively limited studies examining other taxa. Consequently, how other pollinator groups, including non-syrphid flies, respond to differences in UV properties remains poorly understood.

Non-syrphid flies, although often overlooked, play a substantial role as pollinators (Inouye et al. 2015). Evidence from agricultural landscapes indicates that the pollination services provided by non-syrphid flies are comparable to those of syrphid flies (Orford et al. 2015). In Arctic and alpine ecosystems, non-syrphid flies are frequently the most abundant flower visitors (Kevan 1972; Elberling and Olesen 1999; Lázaro et al. 2008; Ishii et al. 2019), and they often function as the most important pollinators (Tiusanen et al. 2016). Thus, evaluating how non-syrphid flies respond to floral traits including floral UV signals is essential for achieving a comprehensive understanding of plant-pollinator interactions.

Our long-term goal is to clarify how floral UV reflectance and absorption influence the visitation behavior of diverse pollinator groups for better understanding how floral color diversity is maintained. As an important step toward this goal, this study examines the attractive function of UV reflectance in non-syrphid flies, a pollinator group that has largely been overlooked. To achieve this, we placed colored pan traps and sticky traps in a Japanese alpine ecosystem, where non-syrphid flies dominate the diurnal pollinator community. Pan and sticky traps are widely used as passive sampling methods to collect flower visitors with minimal collector bias (Leon and Thorp 2001; Vrdoljak and Samways 2012; Gill and O'neal 2015), although they may introduce taxon-specific sampling biases (Disney et al. 1982; Kirk 1984; Dafni et al. 1990).

Because dipteran pollinators at our study site primarily visit white or yellow flowers (Ishii et al. 2019), we tested four trap colors: UV-reflecting white, UV-absorbing white, UV-reflecting yellow, and UV-absorbing yellow. We addressed two questions: (1) Does UV-absorbing white attract more non-syrphid flies than UV-reflecting white? and (2) Does UV-reflecting yellow attract more non-syrphid flies than UV-absorbing yellow, as has occasionally been reported for bee pollinators (e.g., Johnson and Andersson 2002; Klomberg et al. 2019)? These questions are motivated in part by the broader observation that flowers that appear white to humans are almost always UV-absorbing, whereas some red, blue, or yellow flowers reflect UV light (Chittka et al. 1994; Kevan et al. 1996). This pattern also holds in the Japanese alpine flora at our study site (Ishii et al. 2019, 2025). Kevan et al. (1996) proposed that UV-reflecting white flowers may provide little chromatic contrast against vegetation and other backgrounds, potentially reducing detectability and thereby contributing to the rarity of UV-reflecting white flowers. Consistent with this idea, orchid bees have been reported to show reduced preference for UV-reflecting white, which was interpreted as avoidance of bee-achromatic (low-contrast) stimuli (Lunau et al. 2011). Nevertheless, whether dipteran pollinators similarly avoid UV-reflecting white or instead show a contrasting preference remains poorly understood. This gap is notable given the prevalence of fly visitation and pollination in

many white-flowered species across diverse plant communities, including alpine systems (Ishii et al. 2019, 2025). By addressing this gap, we evaluate how UV properties interact with visible color to shape attraction in a major but understudied pollinator group.

Materials and methods

Study site

The study was conducted on the Murodo Plateau (36°34'35"–36°36'35" N, 137°34'43"–137°36'59" E, 2400–2450 m a.s.l.), which is located within the Tateyama Mountain Range in Chubu Sangaku National Park, central Honshu, Japan. The study site, situated above the timberline, consisted of alpine meadow vegetation. At this site, Diptera (primarily from the suborder Brachycera) are the most abundant flower visitors, followed by Hymenoptera (mainly bees) (Ishii et al. 2019, 2025).

Pan and sticky trap experiments

Two types of traps were used in our experiments: pan traps and sticky traps. Using both trap types allowed us to assess whether the observed color preferences were robust to trap design and unlikely to be an artifact of trap-specific capture biases. Both trap types employed four test colors: UV-absorbing white (W-), UV-reflecting white (W+), UV-absorbing yellow (Y-), UV-reflecting yellow (Y+). The materials used to produce these colors are detailed in Online resource 1.

Pan traps consisted of a large plastic Petri dish (8.5 cm in diameter), circular (8.5 cm in diameter) and rectangular (6 × 8 cm) papers colored in one of the four test colors, a green circular paper (2.5 cm in diameter), and a small Petri dish (2.5 cm in diameter) filled with a 3% sodium dodecyl sulfate (SDS) solution (Fig. 1). The traps were elevated above the ground using iron nails (3.8 cm in length) as legs. Unlike in previous studies (e.g., Toler et al. 2005; Wilson et al. 2008; Gonzalez et al. 2020), the colored papers were not submerged in the solution, because aqueous solutions strongly absorb ultraviolet light. In the 2021 experiments, the colored papers were used without lamination. In the 2022 experiments, in contrast, the colored papers were laminated with a 50-μm-thick plastic film (50-μm Pouch Laminating Film, GMP Co., Ltd.) to minimize potential effects of surface texture and odor associated with the paint material and paper.

Sticky traps, used in the 2023 experiments, consisted of plastic Petri dishes (8.5 cm in diameter), circular papers (8.5 cm in diameter) colored in one of the four test colors, and iron nails (10 cm in length) as legs. The colored papers were laminated, and an adhesive aerosol (Fly Adhesive Aerosol, Nisso Green Co., Ltd.) was evenly sprayed onto the surface.

The spectral reflectance of the test colors (Fig. 2) was measured using a charge-coupled device (CCD) spectrometer (BRC112, B&W Tek, Newark, DE, USA), a Y-shaped fiber-optic cable (FRP-400-0.22-1.5-UV, B&W Tek), a white standard (freshly pressed pellet of dry $BaSO_4$), and a deuterium–tungsten light source

(BDS130, B&W Tek). Reflectance was measured at 1-nm intervals from 300 to 700 nm, following the methodology described by Chittka and Kevan (2005). The measured reflectance spectra were translated into loci in two-dimensional color spaces representing fly and beevision (Fig. 3).

For the fly visual system, we used the model proposed by Troje (1993), with mathematical details following Makino and Yokoyama (2015) and Ohashi et al. (2015). This model represents chromatic information based on the relative excitation of two p-type and two y-type photoreceptors. Color perception is determined by which receptor within each pair shows greater excitation, resulting in four chromatic categories: fly-UV (p+/y+), fly-blue (p−/y+), fly-yellow (p−/y−), and fly-purple (p+/y−). Under this framework, stimuli within the same category are considered chromatically indistinguishable to flies (Troje 1993).

For the bee visual system, we employed the bee color hexagon model (Chittka 1992), a chromaticity diagram based on the relative excitation of three hymenopteran photoreceptors (UV, blue, and green). In this model, loci can be expressed in polar or Cartesian coordinates, where the angular position corresponds to perceived hue and the Euclidean distance between loci represents perceived color difference (Chittka 1992; Dyer and Chittka 2004a; Dyer and Chittka 2004b; Dyer 2006).

Although lamination and the application of adhesive aerosol altered the spectral reflectance of the colored papers to some extent (Fig. 2), the resulting stimuli were plotted in closely similar positions within both the fly and bee vision model (Fig. 3). In the fly visual system, the four test colors were plotted in different chromatic categories (Fig. 3b), suggesting that they are likely perceived as distinct colors by flies. In the bee visual system, W − was plotted within the achromatic locus (within 0.1 hexagon units from the center: Chittka et al. 1994; Lunau et al. 2011), whereas the other three colors were plotted in different directions away from the center (Fig. 3a), indicating that they are likely perceived as different hues by bees. Moreover, for each experimental year, the distances between any two test colors exceeded 0.17 hexagon units, which falls within the range where bees can reliably discriminate colors even under absolute conditioning (threshold > 0.11 units; Dyer 2006).

The experiments were conducted under sunny or slightly cloudy conditions during daytime hours (8:00–14:00) from June to September in both 2021 and 2022, and June to August in 2023, approximately once every two weeks. In total, we placed 68 sets of pan traps in 2021 and 40 sets in 2022 (ranging from 3 to 12 sets per experimental day), and 23 sets of sticky traps in 2023 (ranging from 6 to 11 sets per day). Each set of pan and sticky traps consisted of four traps of different colors (W-, W+, Y-, and Y+). Within each set, traps were positioned at the vertices of a 1-m square, and the color assigned to each position was randomized. For pan traps, we oriented the traps to prevent the shadows cast by the rectangular paper from falling onto the traps. Each set was placed at least 5 m apart from the others. The pan and sticky traps were collected four hours after installation. Insects captured in the pan traps were preserved in 70% ethanol, and the colored papers from sticky traps were stored in a dry place for later identification.

Net sampling

To confirm which insects captured by pan traps were flower visitors, we conducted additional sampling of flower visitors using insect nets. Sampling was conducted by randomly patrolling the study sites under sunny or slightly cloudy conditions during daytime hours (9:00–15:00), approximately once every two weeks from June to August in 2023. During patrols, we did not target particular plant species; instead, we opportunistically collected any insects observed visiting flowers. An insect was classified as a flower visitor when physical contact between the insect and a flower was observed. We also recorded the plant species on which each visitor was observed. In total, 20 h were dedicated to net sampling. Insects captured during this process were preserved in 70% ethanol for later identification.

Insect identification

Insects captured using pan traps, sticky traps, and insect nets were initially classified into seven functional groups: syrphid flies (Diptera: Brachycera), non-syrphid Brachycera (Diptera), Nematocera (Diptera), bumble bees (Hymenoptera), non-eusocial bees (Hymenoptera), wasps (Hymenoptera), and others. sawflies (Hymenoptera: Symphyta) and ants (Hymenoptera: Formicidae) were categorized as “others” because of their distinct ecological roles compared with bees and wasps, as well as their small numbers of captured individuals. All other insect orders, including Lepidoptera, Coleoptera, Hemiptera, and Orthoptera, were also classified as “others” owing to low capture numbers. No non-insect animals were captured in our study.

Given that Brachycera (including both syrphid and non-syrphid flies) and bees (including bumble bees and non-eusocial bees) represent the primary and secondary flower-visiting insect groups at our study sites, specimens from these groups captured using pan traps and insect nets were further identified to the family level, and in some cases to the genus or species level. In contrast, family level identification was not feasible for specimens collected with sticky traps, because the adhesive frequently damaged morphological characters required for reliable identification.

To determine which insects captured by pan traps were flower visitors, and to identify flower-visiting taxa that were not captured by the traps, all insects collected using pan traps in 2022, as well as those captured using insect nets, were further classified into morphospecies or species levels (hereafter referred to as “species”). This classification was conducted under a stereomicroscope (CartonDSZT44FT, Carton Optical Industries, Ltd.). For the identification of Brachycera and bee species, we referred to Tanaka (2000) and Tadauchi and Murao (2014), respectively.

Analysis

To evaluate the influence of trap color on the attractiveness to different functional groups of flower visitors, we analyzed the relationship between trap color and the number of insects captured, separately for each functional group and each year. Syrphid flies and bumble bees were excluded from the analysis

because only small numbers of individuals were captured in pan traps. The analyses were conducted using generalized linear mixed models (GLMMs), in which trap color was included as the fixed effect, the number of individuals captured per trap was used as the response variable, and sampling date was included as a random effect. All GLMMs were fitted using the lme4 package in R version 4.3.2 (R Core Team 2023). The models assumed a negative binomial error distribution with a log link function to account for frequent overdispersion in the count data. To avoid zero inflation, trap sets in which the focal functional group was not captured in any of the four traps were excluded from the analysis.

To assess how trap color influenced attraction of different families within non-syrphid Brachycera and non-eusocial bees, we further analyzed the relationship between trap color and the number of captured individuals for each family within each group, using the same analytical framework described above. For this analysis, we pooled data from the pan trap experiments conducted in 2021 and 2022, because these datasets exhibited similar patterns.

To determine which functional groups captured by the traps are likely to represent flower-visiting insects, and which flower-visiting groups are less effectively sampled by pan traps, we calculated an overlap between pan traps and insect nets for each functional group as follows:

$$OI_j = \frac{2\sum_i \min\left(a_{i,j}, b_{i,j}\right)}{\sum_i a_{i,j} + \sum_i b_{i,j}}$$

where OI_j represents the overlap index for functional group *j*, and $a_{i,j}$ and $b_{i,j}$represent the proportions of the focal species (i.e., species *i* of functional group *j*) in the total number of insects captured by pan traps and insect nets, respectively. An OI_j value close to 1 indicates that the species in the functional group *j* were captured in similar proportions by pan traps and insect nets, whereas a value close to 0 indicates that species within the functional group *j* were captured predominantly by either pan traps or insect nets, but not both.

Results

Pan and sticky trap experiments

In total, 2,979 insects were captured in 2021 and 1,071 insects in 2022 using 68 and 40 sets of pan traps, respectively. These insects were classified into 99 species. Additionally, 1,760 insects were captured in 2023 using 23 sets of sticky traps. Among the seven functional groups, non-syrphid Brachycera was the most frequently captured group across all years. The relative abundances of the remaining functional groups varied among years, with groups listed in descending order: Nematocera, bees, wasps, and others in 2021; bees, Nematocera, others, and wasps in 2022; and Nematocera, others, bees, and wasps in 2023. Syrphid flies and bumble bees were rarely captured by either pan traps or sticky traps, with only 12 syrphid flies and one bumble bee captured by these traps over the three-year study period. The non-syrphid Brachycera captured by pan traps comprised 40 species from 21 families, and the non-eusocial

bees captured comprised eight species from three families. Among the non-eusocial bees, *Lasioglossum nupricola* (Halictidae) was the dominant species, accounting for 69.1% (N = 149) in 2021 and 90.8% (N = 207) in 2022.

Among the four trap colors, W- traps consistently captured significantly more non-syrphid Brachycera than W+ traps across all years (Fig. 4a-c). In contrast, no significant differences were detected between Y- and Y+ traps in any year. In the pan trap experiments conducted in 2021 and 2022, W- traps generally captured more non-syrphid Brachycera than both Y- and Y+ traps, although the differences were not always statistically significant. However, this trend was not observed in the sticky trap experiments: Y- and Y+ traps captured a similar number of non-syrphid Brachycera as W- traps. There were no significant differences in the number of non-eusocial bees captured between W- and W+ traps, or between Y- and Y+ traps, in any year (Fig. 4d-f). However, yellow pan traps (both Y- and Y+) captured significantly more non-eusocial bees than white pan traps (both W- and W+) in both 2021 and 2022, whereas this pattern was not significant in the sticky trap experiments in 2023. There were no significant differences in the number of Nematocera captured among the four different colored pan traps in both 2021 and 2022 (Fig. 4g-h). However, Y+ sticky traps captured significantly more Nematocera than W- and W+ sticky traps in 2023 (Fig. 4i). There were no significant differences in the number of wasps (Fig. 4j-l) or others (Fig. 4m-o) captured among the four trap colors in any years.

Within non-syrphid Brachycera, family-level analyses revealed that W- pan traps consistently captured more individuals than W+ traps across all major families (Fig. 5a-e), as well as for the “others” category of non-syrphid Brachycera (Fig. 5f), although this trend was not statistically significant in the Anthomyiidae (Fig. 5e). Overall, W- traps exhibited the highest captures, whereas W+ traps exhibited the lowest captures across most taxonomic groups within non-syrphid Brachycera. Anthomyiidae represented the only exception, with Y+ traps showing the lowest captures.

For the two main families of non-eusocial bees (Halictidae and Andrenidae), no significant differences in the number of captured individuals were detected between Y- and Y+ traps, or between W- and W+ traps (Fig. 6a-b). However, yellow pan traps (both Y- and Y+) captured significantly more individuals of both the bee families than white pan traps (both W- and W+). Other non-eusocial bee families were not analyzed owing to small sample size (n = 9).

Comparison of insect compositions captured by pan traps and insect nets

In total, 407 flower-visiting insects were captured using insect nets. Among the seven functional groups, non-syrphid Brachycera (196 individuals) were the most frequently captured group, followed by non-eusocial bees (80 individuals), syrphid flies (65), and bumble bees (39). These insects were classified into 82 species, 37 of which overlapped with those captured by pan traps.

Among the species recorded using insect nets, 25 of 39 non-syrphid Brachycera species (64.1%), 5 of 8 non-eusocial bee species (62.5%), 1 of 3 Nematocera species (33.3%), 1 of 5 wasp species (20.0%), 1 of 13 “other” insect species (7.6%), 1 of 3 bumble bee species (33.3%), and 3 of 11 syrphid fly species (27.2%) were also captured by pan traps (Fig. 7).

The overlap index values for non-syrphid Brachycera (0.434) and non-eusocial bees (0.773) were substantially higher than those for other functional groups, which ranged from 0.034 to 0.051. These results indicate that a large proportion of non-syrphid Brachycera and non-eusocial bees captured by pan traps were confirmed as flower visitors, and that flower-visiting species within these two groups were effectively sampled by pan traps. In contrast, the data suggest that many Nematocera, wasps, and insects classified as “others” captured by the pan traps were unlikely to be flower visitors, whereas flower-visiting bumble bees and syrphid flies were less effectively sampled by pan traps.

Discussion

Effect of UV-absorption and reflection on the attractiveness to non-syrphid flies

To the best of our knowledge, this study provides the first empirical evidence that ultraviolet (UV) properties influence the attractiveness of visual cues to flower-visiting non-syrphid flies. Specifically, we found that UV-absorbing white was more attractive to non-syrphid Brachycera than UV-reflecting white (Fig. 4). This pattern was consistent across trap types (pan traps and sticky traps) and regardless of whether the colored papers were laminated, indicating that differences in material properties or scent associated with the papers had little influence on the observed response.

By contrast, no difference in attractiveness to non-syrphid flies was detected between UV-absorbing and UV-reflecting yellow. Together, these results suggest that UV effects on attractiveness are not determined solely by UV absorption or reflection per se, but rather by interactions between UV signals and non-UV spectral cues. Importantly, the greater attractiveness of UV-absorbing white compared with UV-reflecting white was consistently observed across multiple families within non-syrphid Brachycera group (Fig. 5), indicating that this response is not restricted to a particular lineage within the group. Moreover, most of the non-syrphid Brachycera species captured by traps were also recorded visiting flowers using insect nets, and vice versa (Fig. 7), confirming that the majority of species responding to trap colors are indeed flower-visiting insects. These findings therefore suggest that this UV-mediated response may be widespread among flower-visiting non-syrphid Brachycera.

Chittka et al. (1994) and Kevan et al. (1996) reported that most flowers that appear white to human absorb, rather than reflect, UV, a pattern also documented at our study site (Ishii et al. 2019). Using a bee-vision framework, they proposed that UV-reflecting white flowers, which may appear “truly white” to UV-sensitive insects (cf. Kevan et al. 1996), are rare because they provide little chromatic contrast against vegetation and other backgrounds from the insect's perspective, making them less likely to be

detected. Consistent with this idea, Lunau et al. (2011) showed that, for orchid bees, UV-reflecting white was less attractive than UV-absorbing white. Accordingly, the greater attractiveness of UV-absorbing white to non-syrphid Brachycera in our study may also reflect the lower visual salience of UV-reflecting white to these flies. However, the bee-based framework of chromatic and achromatic channels should not be directly applied to flies. Fly color vision is often described as categorical, in which stimuli are grouped into a limited number of color categories (Troje 1993). Under this framework, UV-related effects on attraction may arise not from bee-style chromatic contrast per se, but from whether UV absorption versus reflection shifts a stimulus across fly-specific category boundaries or changes how reliably it is classified against natural backgrounds.

Alternatively, or additionally, non-syrphid flies may have evolved or learned to associate UV-absorbing white with floral rewards, thereby optimizing foraging efficiency in environments where UV-reflecting white frequently occurs in non-floral elements, such as snow (Wuttke et al. 2006) and mineral/soil surfaces (Adams and Filice 1967; Bogrekci and Lee 2006). Disentangling the relative contributions of evolutionary adaptation and individual learning will require further experimental work. More broadly, future studies integrating behavioral experiments with comparative and ecological approaches will be essential for elucidating how flower coloration and pollinator sensory biases jointly shape plant–fly interactions.

Effect of UV-absorption and reflection on attractiveness to non-eusocial bees

Previous studies have suggested that UV reflection from flowers can enhance their attractiveness to non-eusocial bees (Johnson and Andersson 2002; Welsford and Johnson 2011; Klomberg et al. 2019; but see Horth et al. 2014). In contrast, in our trap-based assays, we found no difference in attractiveness between UV-absorbing and UV-reflecting traps for the two main families of non-eusocial bees at our study site (Halictidae and Andrenidae), regardless of human-perceived trap color (Fig. 6). This discrepancy between our findings and previous studies may be attributable to differences in bee species composition among study systems. Alternatively, it may reflect differences in experimental approaches. In many previous studies, the effects of UV reflection on bee attraction were examined using natural flowers in which UV reflectance was experimentally suppressed in a subset of individuals within a population. Such manipulated flowers likely represented a minority phenotype, whereas the majority of flowers retained their natural UV-reflecting properties. Under these conditions, bees may have preferentially visited the more common floral phenotype (i.e., the wild type), rather than exhibiting an intrinsic preference for UV reflection per se. Thus, whether UV reflection itself—independent of its role in floral UV patterning—functions as a general attractant for non-eusocial bees remains unclear. In contrast, within flower UV patterns, which operate at a finer spatial scale by guiding bees toward floral resources and appropriate landing sites, have been more consistently demonstrated to influence bee behavior (Jones and Buchmann 1974; Klomberg et al. 2019; Johnson and Andersson 2002). However, because our assays used uniformly colored traps rather than patterned floral displays, our results do not adress the role of UV patterns.

Nematocera, wasps, and other insects

Although less abundant than non-syrphid Brachycera and non-eusocial bees, insects belonging to Nematocera, wasps, and the “other” category were also captured by the traps. However, a large proportion of species within these groups were not recorded as flower visitors, as most were not captured during net sampling on flowers (Fig. 7). This pattern suggests that many individuals from these groups captured by the traps may not have been actively visiting flowers. Furthermore, with the exception of Nematocera in the 2023 sticky trap experiment, no significant differences were detected in the attractiveness of the four test colors for these groups (Fig. 4g–o), suggesting that their capture was not systematically influenced by trap color. Instead, their presence in the traps is more likely attributable to incidental or random encounters. The clear contrast in color-dependent capture patterns between flower-visiting insects (non-syrphid Brachycera and non-eusocial bees) and groups that rarely visited flowers (Nematocera, wasps, and other insects) supports the interpretation that the selective capture observed in flower-visiting taxa reflects biologically meaningful responses to flower-associated visual cues, rather than methodological artifacts of the trapping approach.

Limitation of this study

One limitation of this study arises from the use of pan and sticky traps. Although these methods allowed us to test how ultraviolet reflectance influences the attractiveness of visual cues to certain insect groups, such as non-syrphid Brachycera and non-eusocial bees, they were less effective for sampling other important pollinator groups, including bumble bees and syrphid flies (Fig. 7). Nevertheless, previous studies have demonstrated that UV reflectance can influence the foraging behavior of bumble bees (Papiorek et al. 2016; Chen et al. 2020) and syrphid flies (An et al. 2018; Neiman et al. 2018). We occasionally observed bumble bees forcefully escaping after contacting the traps, as well as syrphid flies hovering in front of traps without being captured, indicating that these insects may have responded to the visual stimuli but avoided capture. Future studies could overcome this limitation by combining visual trapping approaches with non-invasive observation methods, such as motion-detection cameras or video recordings, which may more effectively record behavioral responses of flower-visiting insects that are adept at avoiding physical traps. Such approaches would allow a more comprehensive assessment of how floral UV reflection and absorption influence visitation patterns across a broader range of pollinator taxa.

Conclusion

This study provides the first empirical evidence that ultraviolet light (UV) influences the attractiveness of visual cues to flower-visiting non-syrphid Brachycera, demonstrating that UV-absorbing white attracts these flies more strongly than UV-reflecting white. This pattern is consistent with the observation that many human-white flowers absorb, rather than reflect, UV. In contrast, no difference in attractiveness to these flies was detected between UV-absorbing and UV-reflecting yellow, indicating that the influence of UV on attractiveness cannot be explained solely by UV absorption or reflection per se, but likely depends

on interactions with non-UV spectral properties. These flies' preference for UV-absorbing white may reflect an evolved or learned association with floral rewards in environments where UV-reflecting white is common in non-floral backgrounds, such as snow and mineral surfaces. Alternatively, UV-related attractions might be shaped by how UV modifies the conspicuousness of a stimulus against natural backgrounds, including vegetation. Nevertheless, further research is required to disentangle the evolutionary and ecological processes underlying the apparent association between the preference of non-syrphid flies for UV-absorbing white and the prevalence of UV-absorbing white flowers.

In this study, non-eusocial bees did not exhibit significant differences in responses to UV-absorbing versus UV-reflecting stimuli, suggesting that the role of UV cues in mediating attractiveness varies among pollinator taxa. Although our trap-based approach was less effective for certain flower-visiting groups, such as bumble bees and syrphid flies, the present results highlight taxon-specific responses to UV-related visual cues among flower visitors. Future studies integrating improved sampling techniques with non-invasive behavioral observations will be essential for advancing our understanding of how floral UV traits shape the diverse and context-dependent interactions between plants and their pollinators.

Declarations

Acknowledgement

We are also grateful to K. Okusawa, A. Tashima, T.W. Ueno and Y.H. Tamura for their assistance with fieldwork.

Funding; This work was supported by JSPS KAKENHI Grant Numbers JP22K06400 to H.S.I. and JP19H03303 to U.A. and H.I.S.

Conflicts of interest/Competing interests ; The authors declare that they have no conflict of interest.

Ethics approval; Not applicable. This study involved field trapping of unregulated invertebrate insects and did not involve human participants, vertebrate animals, or cephalopods. No ethical approval was required under institutional or national guidelines.

Consent to participate; Not applicable.

Consent for publication; Not applicable.

Availability of data and material; Our raw data is available from FigShare: https://figshare.com/s/79db2a35e31d1eff546d.

Code availability; Not applicable. Our analysis conducted was quite standard.

Authors' contributions; TTO: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, validation, visualization, writing, editing. SGT: Conceptualization, methodology, resources, supervision, reviewing. AU: funding acquisition,

methodology, supervision, validation, reviewing. HSI: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing, reviewing, editing.

References

1. Adams JB, Filice AL (1967) Spectral reflectance 0.4 to 2.0 microns of silicate rock powders. J Geophys Res 72:5705–5715
2. An L, Alexander N, Eberling E, Algora H, Brings S, Lunau K (2018) The yellow specialist: dronefly Eristalis tenax prefers different yellow colours for landing and proboscis extension. J Exp Biol 221:jeb184788
3. Arikawa K, Inokuma K, Eguchi E (1987) Pentachromatic visual system in a butterfly. Naturwissenschaften 74:297–298
4. Bogrekci I, Lee WS (2006) Effects of soil moisture content on absorbance spectra of sandy soils in sensing phosphorus concentrations using UV–VIS–NIR spectroscopy. Trans ASABE 49:1175–1180
5. Borges RM, Gowda V, Zacharias M (2003) Butterfly pollination and high-contrast visual signals in a low-density distylous plant. Oecologia 136:571–573
6. Chen Z, Liu CQ, Sun H, Niu Y (2020) The ultraviolet colour component enhances the attractiveness of red flowers of a bee-pollinated plant. J Plant Ecol 13:354–360
7. Chittka L, Kevan PG (2005) Flower colour as advertisement. In: Dafni A, Kevan PG, Husband BC (eds) Practical pollination biology. Enviroquest Ltd, Cambridge (UK), pp 157–196
8. Chittka L, Shmida A, Troje N, Menzel R (1994) Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. Vis Res 34:1489–1508
9. Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J Comp Physiol A 170:533–543
10. Dafni A, Bernhardt P, Shmida A, Ivri Y, Greenbaum S, O'Toole C, Losito L (1990) Red bowl-shaped flowers: convergence for beetle pollination in the Mediterranean region. Isr J Plant Sci 39(1–2):81–92
11. Dinkel T, Lunau K (2001) How drone flies (Eristalis tenax L., Syrphidae, Diptera) use floral guides to locate food sources. J Insect Physiol 47:1111–1118
12. Disney RHL, Erzinclioglu YZ (1982) Collecting methods and the adequacy of attempted fauna surveys, with reference to the Diptera. Field Stud 5:607–621
13. Dyer AG, Chittka L (2004a) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (Bombus terrestris) as a case study. J Comp Physiol A 190:105–114
14. Dyer AG, Chittka L (2004b) Fine colour discrimination requires differential conditioning in bumblebees. Naturwissenschaften 91:224–227

15. Dyer AG (2006) Discrimination of flower colours in natural settings by the bumblebee species Bombus terrestris (Hymenoptera: Apidae). Entomol Gen 28:257–268
16. Elberling H, Olsen JM (1999) The structure of a high latitude plant–flower visitor system: the dominance of flies. Ecography 22:314–323
17. Gill KA, O'Neal ME (2015) Survey of soybean insect pollinators: community identification and sampling method analysis. Environ Entomol 44:488–498
18. González E, Landis DA, Knapp M (2020) Forest cover and proximity decrease herbivory and increase crop yield via enhanced natural enemies in soybean fields. J Appl Ecol 57:1–11
19. Guldberg LD, Attat PR (1975) Frequency of reflection and absorption of ultraviolet light in flowering plants. Am Midl Nat 93(1):35–43
20. Hirota SK, Miki N, Yasumoto AA, Yahara T (2019) UV bullseye contrast of Hemerocallis flowers attracts hawkmoths but not swallowtail butterflies. Ecol Evol 9:52–64
21. Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70
22. Horovitz A, Cohen Y (1972) Ultraviolet reflectance characteristics of flowers of crucifers. Am J Bot 59:706–713
23. Horth L, Campbell L, Bray R (2014) Wild bees preferentially visit Rudbeckia flower heads with exaggerated ultraviolet absorbing floral guides. Biol Open 3:221–230
24. Inouye DW, Larson BMH, Ssymank A, Kevan PG (2015) Flies and flowers III: ecology of foraging and pollination. J Pollinat Ecol 16:115–133
25. Ishii HS, Kubota MX, Tsujimoto SG, Kudo G (2019) Association between community assemblage of flower colours and pollinator fauna: a comparison between Japanese and New Zealand alpine plant communities. Ann Bot 123:533–541
26. Ishii HS, Tsujimoto SG, Kadoya MB, Watanabe Y, Kohyama TI, Hiraiwa MK, Ushimaru A, Kudo G (2025) Linkage between community assemblage of floral traits and pollinator fauna: a comparison among six geographical regions. Plant Biol 27:538–548
27. Johnson SD, Andersson S (2002) A simple field method for manipulating ultraviolet reflectance of flowers. Can J Bot 80:1325–1328
28. Johnson SD, Dafni A (1998) Response of bee-flies to the shape and pattern of model flowers: implications for floral evolution in a Mediterranean herb. Funct Ecol 12:289–297
29. Jones EG, Buchmann SL (1974) UV floral patterns as orientation cues. Anim Behav 22:481–485
30. Kevan P, Giurfa M, Chittka L (1996) Why are there so many and so few white flowers? Trends Plant Sci 1:252–257
31. Kevan P (1972) Floral colors in the high Arctic with reference to insect–flower relations and pollination. Can J Bot 50:2289–2316
32. Kirk WDJ (1984) Ecologically selective coloured traps. Ecol Entomol 9:35–41
33. Klomberg Y, Kouede RD, Bartoš M, Mertens JEJ, Tropek R, Fokam EB, Janeček Š (2019) The role of ultraviolet reflectance and pattern in the pollination system of Hypoxis camerooniana

(Hypoxidaceae). AoB Plants 11(5):plz057
34. Kreithen ML, Eisner T (1978) Ultraviolet light detection by the homing pigeon. Nature 272:347–348
35. Leong JM, Thorp RW (1999) Colour-coded sampling: the pan trap colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. Ecol Entomol 24:329–335
36. Lunau K, Hofmann N, Valentin S (2005) Response of the hoverfly species Eristalis tenax towards floral dot guides with colour transition from red to yellow (Diptera: Syrphidae). Entomol Gen 27:249–256
37. Lunau K, Papiorek S, Eltz T, Sazima M (2011) Avoidance of achromatic colours by bees provides a private niche for hummingbirds. J Exp Biol 214:1607–1612
38. Lázaro A, Hegland SJ, Totland O (2008) The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. Oecologia 157:249–257
39. Makino TT, Yokoyama J (2015) Nonrandom composition of flower colors in a plant community: mutually different co-flowering natives and disturbance by aliens. PLoS ONE 10(12):e0143443
40. Neimann A, An L, Lunau K (2018) The yellow specialist: colour preferences and colour learning of the hoverfly Eristalis tenax (Diptera: Syrphidae). Entomol Heute 30:27–44
41. Ohashi K, Makino TT, Arikawa K (2015) Floral colour change in the eyes of pollinators: testing possible constraints and correlated evolution. Funct Ecol 29:1144–1155
42. Orford KA, Vaughan IP, Memmott J (2015) The forgotten flies: the importance of non-syrphid Diptera as pollinators. Proc. R. Soc. B Biol. Sci. 282:20142934
43. Papiorek S, Junker RR, Alves-dos-Santos I, Melo GAR, Amaral-Neto LP, Sazima M, Wolowski M, Freitas L, Lunau K (2016) Bees, birds and yellow flowers: pollinator-dependent convergent evolution of UV patterns. Plant Biol 18:46–55
44. Peitsch D, Fietz A, Hertel H, de Souza J, Ventura DFX, Menzel R (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. J Comp Physiol A 170:23–40
45. R Core Team (2023) R: a language and environment for statistical computing. Vienna (AT): R Foundation for Statistical Computing. Available from: https://www.R-project.org/
46. Tadauchi O, Murao R (eds) (2014) An illustrated guide to Japanese bees, 1st edn. Bun-ichi Sogo Shuppan
47. Tanaka K (2000) A guide to identification of house and household insect pests. 2nd ed. Key to families of the order Diptera. House Househ. Insect Pests. 22(2):95–141
48. Tiusanen M, Hebert PDN, Schmidt NM, Roslin T (2016) One fly to rule them all—muscid flies are the key pollinators in the Arctic. Proc. R. Soc. B Biol. Sci. 283:20161271
49. Toler TR, Evans EW, Tepedino VJ (2005) Pan-trapping for bees (Hymenoptera: Apiformes) in Utah's west desert: the importance of color diversity. Pan-Pac Entomol 81:103–113
50. Troje N (1993) Spectral categories in the learning behaviour of blowflies. Z Naturforsch C 48:96–104

51. Vrdoljak SM, Samways MJ (2012) Optimising coloured pan traps to survey flower-visiting insects. J Insect Conserv 16:345–354
52. Welsford MR, Johnson SD (2012) Solitary and social bees as pollinators of Wahlenbergia (Campanulaceae): single-visit effectiveness, overnight sheltering and responses to flower colour. Arthropod-Plant Interact 6:1–14
53. Wilson JS, Griswold T, Messinger O (2008) Sampling bee communities (Hymenoptera: Apiformes) in a desert landscape: are pan traps sufficient? J Kans Entomol Soc 81:288–300
54. Wuttke S, Seckmeyer G, König-Langlo G (2006) Measurements of spectral snow albedo at Neumayer, Antarctica. Ann Geophys 24:7–21

Figures

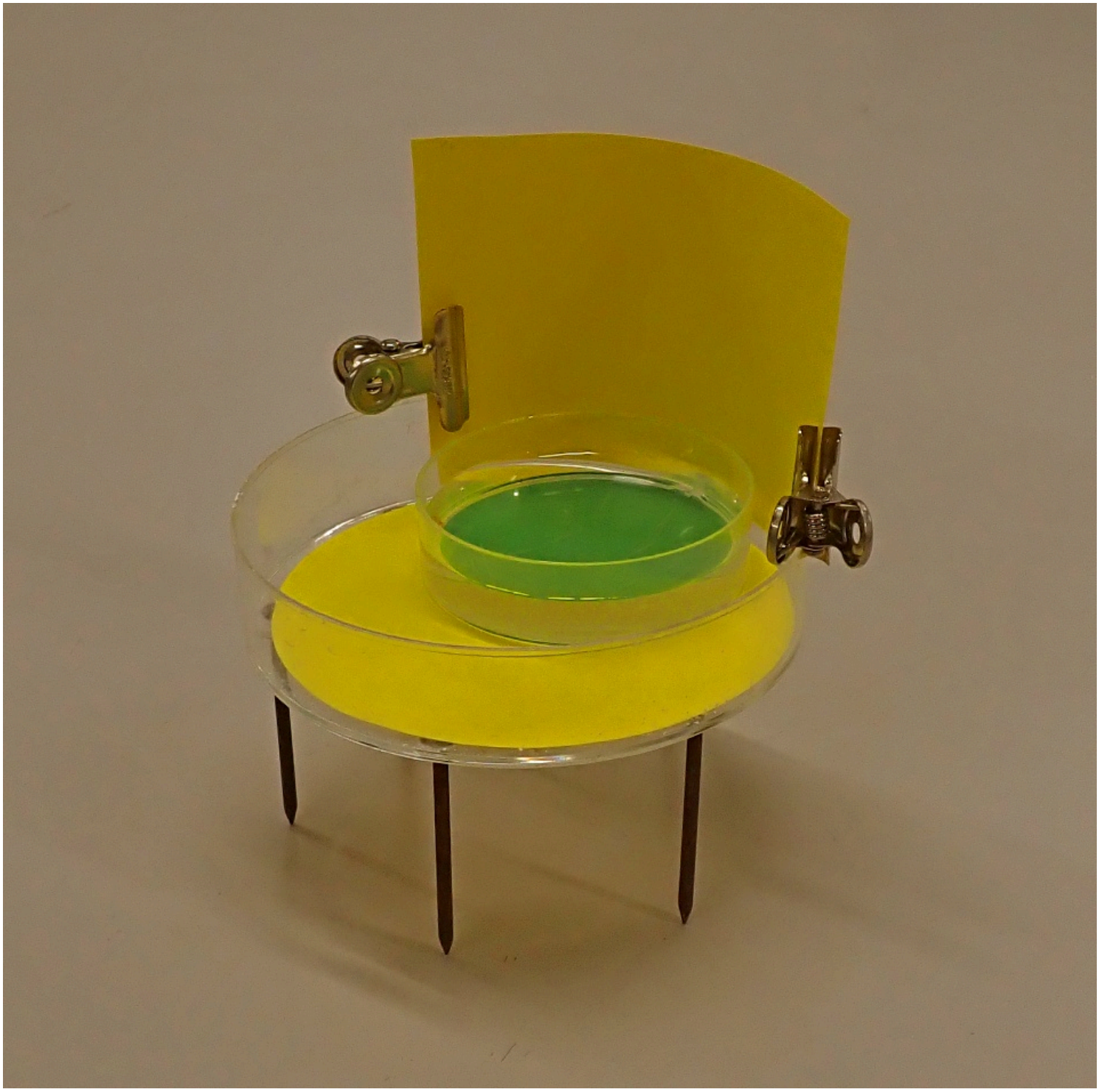


Figure 1

Photograph of a pan trap used in the experiments.

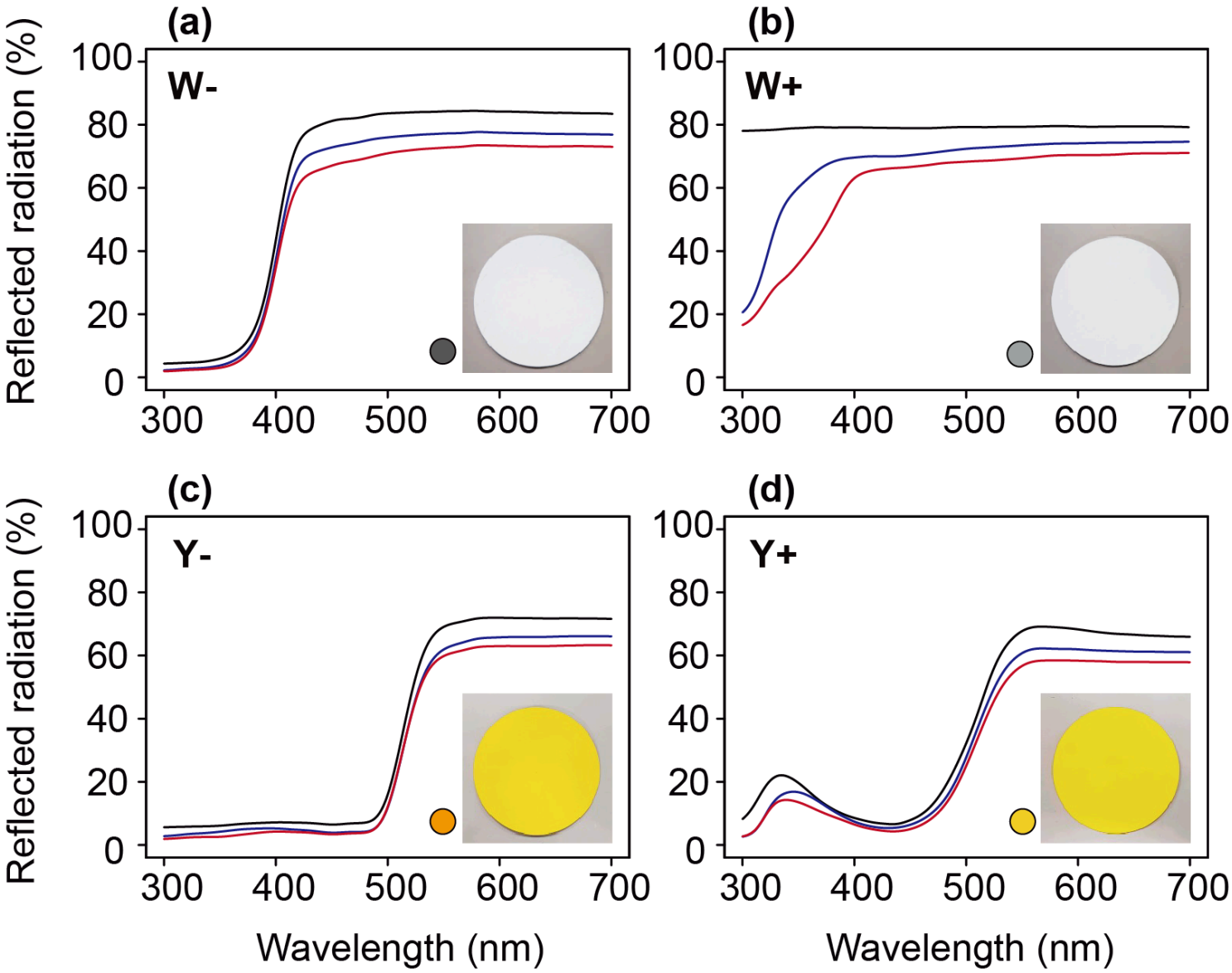


Figure 2

Spectral reflectance of the colored papers used in the experiments. Panels show reflectance spectra for (a) UV-absorbing white (W−), (b) UV-reflecting white (W+), (c) UV-absorbing yellow (Y−), and (d) UV-reflecting yellow (Y+). Black lines indicate the reflectance spectra of the colored papers used in the 2021 experiments, blue lines show spectra after lamination (used in the 2022 experiments), and red lines show spectra after lamination and application of adhesive aerosol (used in the 2023 experiments). Insets in the lower right of each panel show photographs of the corresponding colored papers. Colored circles adjacent to the photographs indicate the color codes used to represent each treatment in Fig. 3.

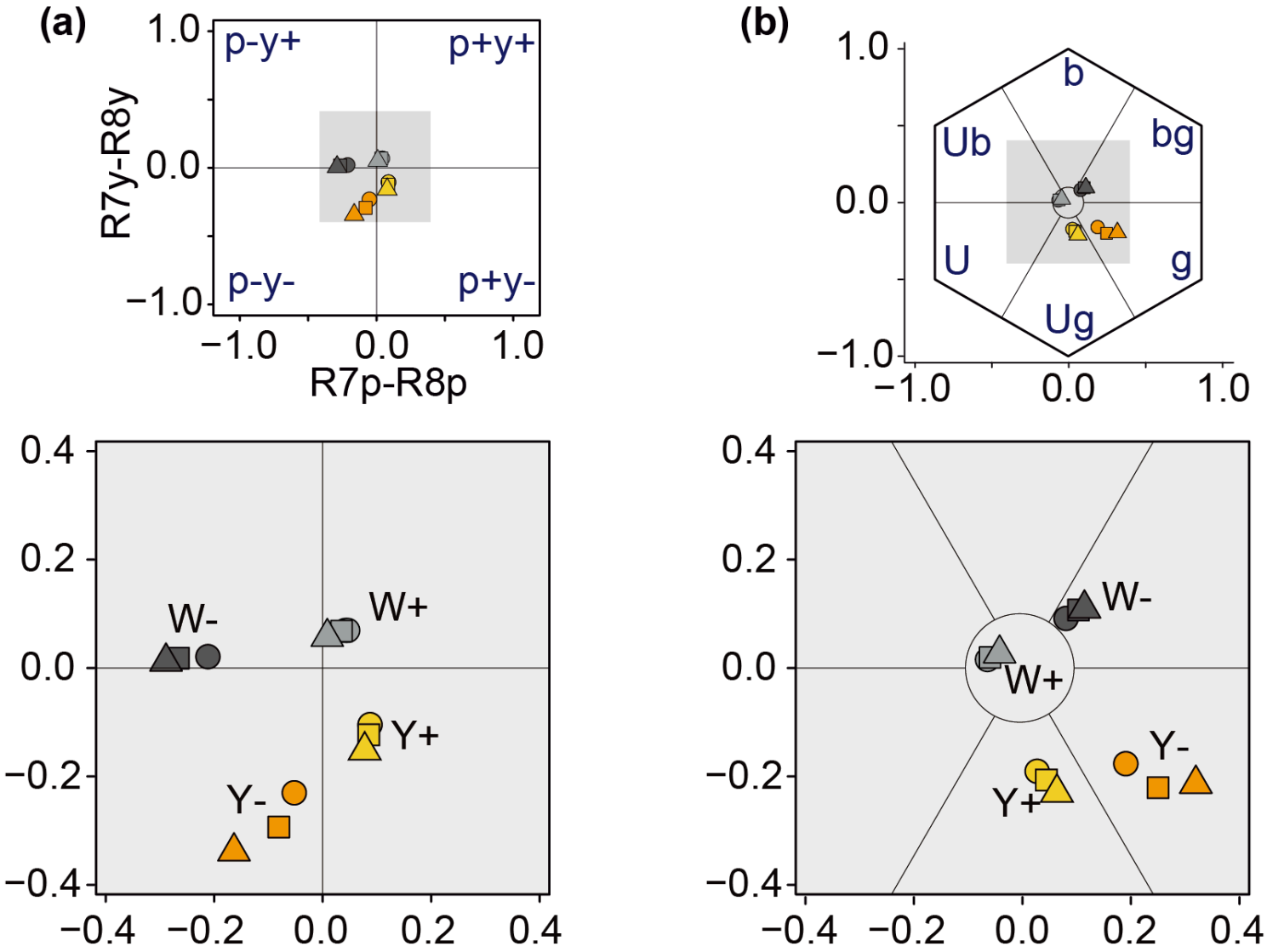


Figure 3

Color loci of the experimental stimuli plotted in models of fly and bee color vision. (a) Color loci of the stimuli within the fly vision model (Troje 1993). (b) Color loci of the stimuli within the bee color hexagon (Chittka 1992). In each panel, the upper plot shows the full color space, and the lower plot shows a magnified view of the region containing the stimuli. In the upper plots, the shaded area indicates the region that is enlarged in the lower plots. Circles indicate colored papers used in the 2021 experiments, squares indicate colored papers after lamination (used in the 2022 experiments), and triangles indicate colored papers after lamination and application of adhesive aerosol (used in the 2023 experiments). Colors correspond to the four test stimuli: UV-absorbing white (W−), UV-reflecting white (W+), UV-absorbing yellow (Y−), and UV-reflecting yellow (Y+). In the bee color hexagon, the central circle represents the achromatic locus, within which stimuli separated by less than 0.1 hexagon units (Chittka et al.1994; Lunau et al. 2011).

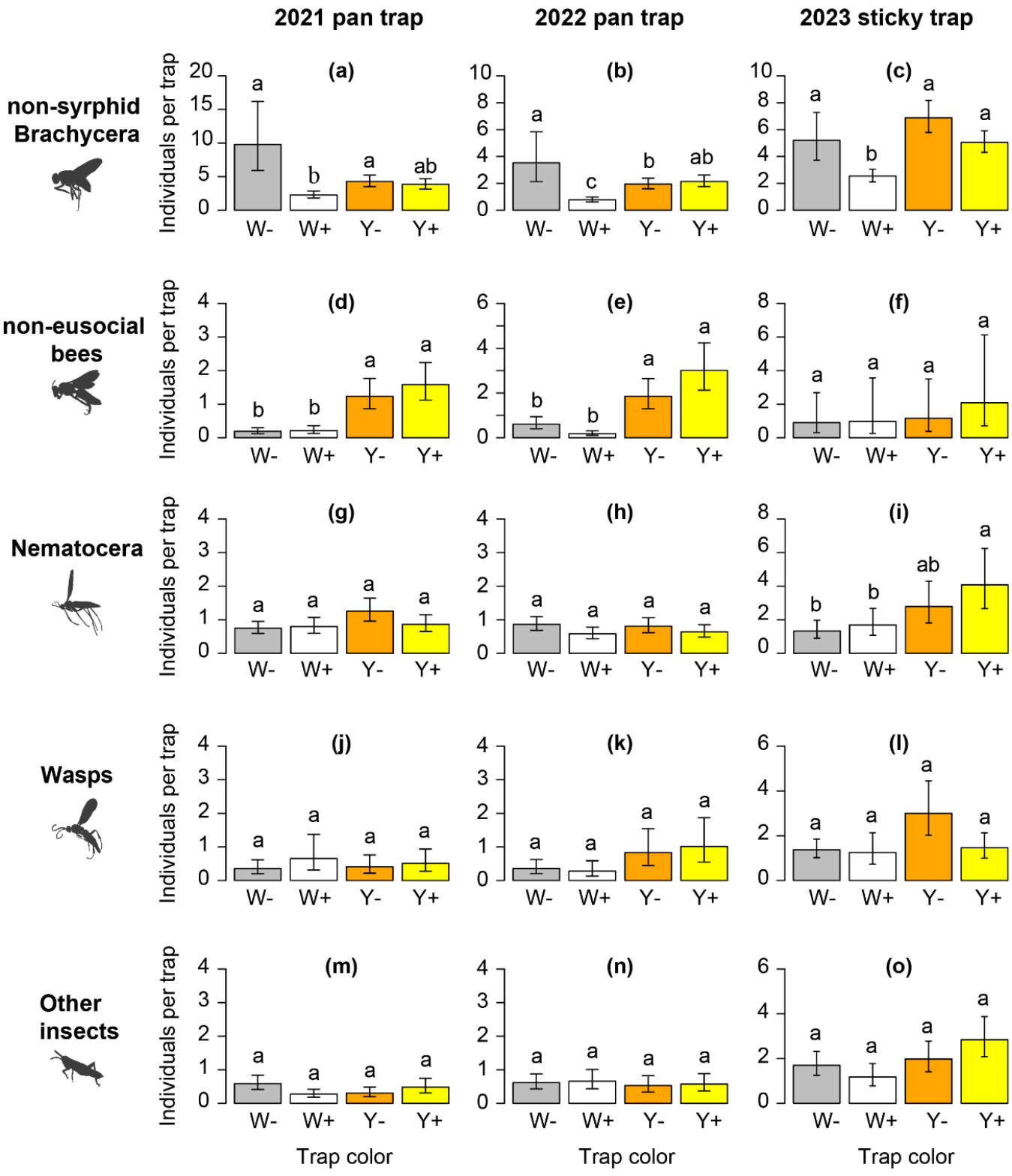


Figure 4

Relationships between trap background color and the estimated number of individuals captured per trap for each functional group across years. Panels (a–c) show non-syrphid Brachycera, (d–f) non-eusocial bees, (g–i) Nematocera, (j–l) wasps, and (m–o) other insects. Estimated values represent the number of individuals captured per trap, derived from generalized linear mixed models (GLMMs). Error bars indicate standard errors. Bars sharing the same letter are not significantly different at $P = 0.05$. Significance

levels were adjusted using the Holm correction (Holm 1979). Note that y-axis scales differ among functional groups.

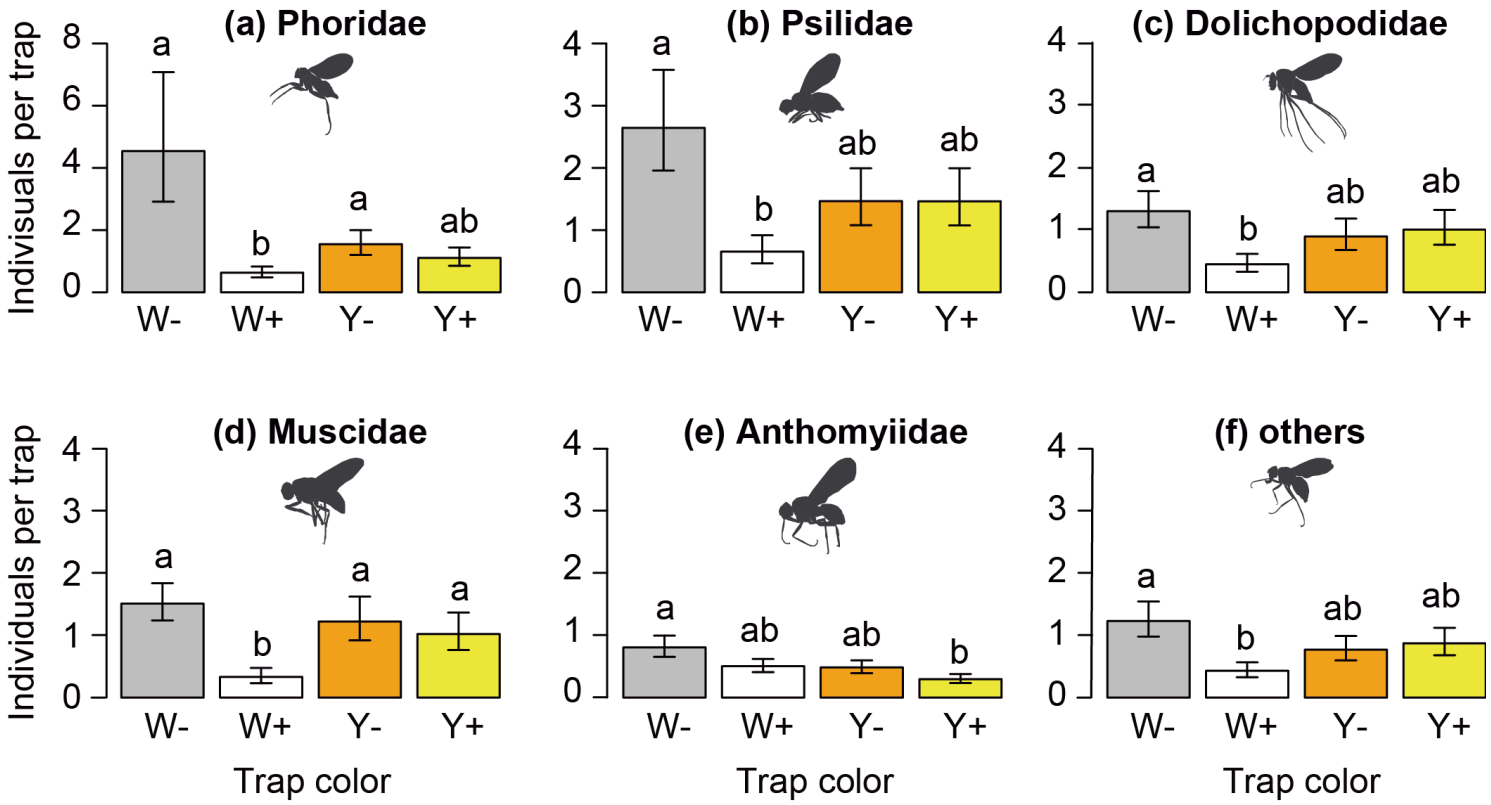


Figure 5

The relationship between trap color and the number of individuals captured for the five major families within non-syrphid Brachycera, as well as for the “others” category of non-syrphid Brachycera. Panels show (a) Phoridae, (b) Psilidae, (c) Dolichopodidae, (d) Muscidae, (e) Anthomyiidae, and (f) other non-syrphid Brachycera. Bars represent the estimated mean number of individuals captured per trap, calculated using generalized linear mixed models (GLMMs). Error bars indicate standard errors. Bars sharing the same letter do not differ significantly at p = 0.05. Significance levels were adjusted using the Holm correction (Holm 1979).

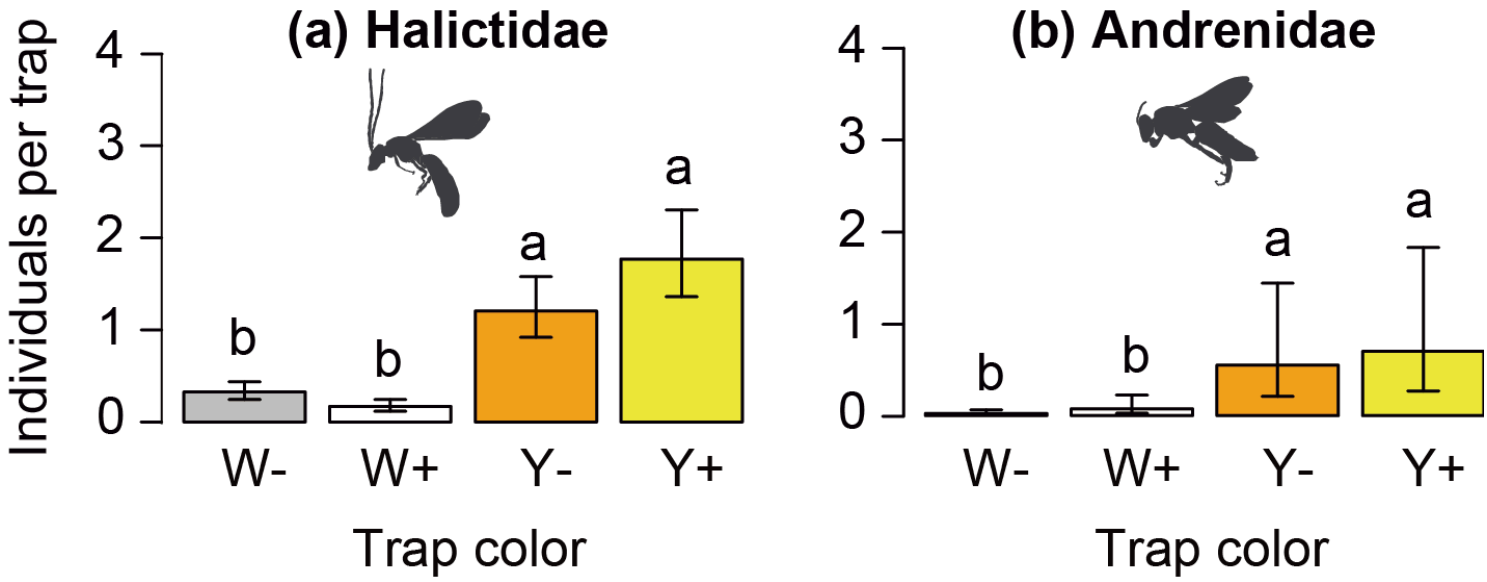


Figure 6

The relationship between trap color and the number of individuals captured for two families of non-eusocial bees: (a) Halictidae and (b) Andrenidae. Bars represent the estimated mean number of individuals captured per trap, calculated using generalized linear mixed models (GLMMs). Error bars indicate standard errors. Bars sharing the same letter do not differ significantly at p = 0.05. Significance levels were adjusted using the Holm correction (Holm 1979).

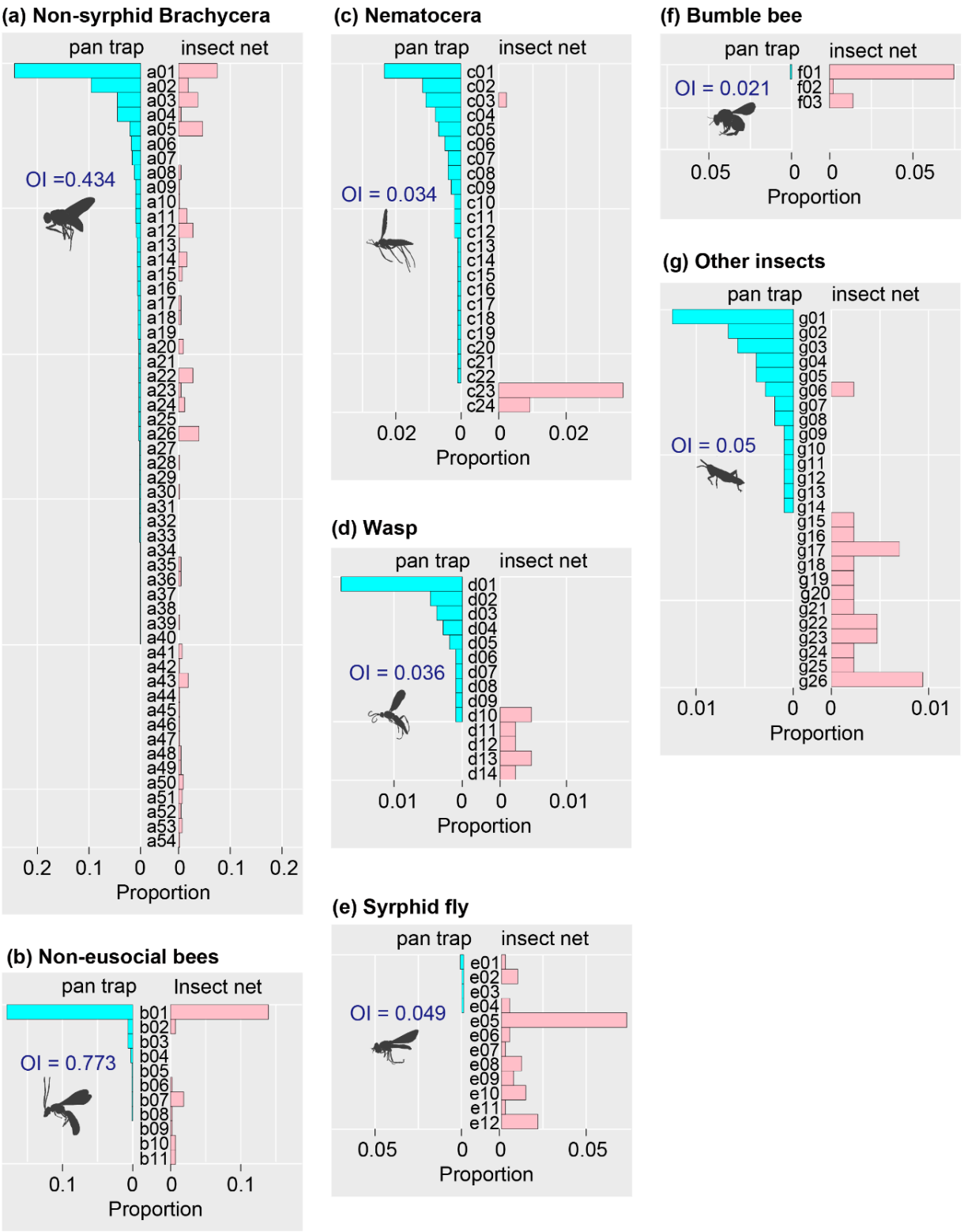


Figure 7

Relative frequency histograms of species captured by pan traps (left side of each panel) and insect nets (right side of each panel), shown separately for each functional group. Relative frequencies were calculated as the proportion of individuals of each species captured by pan traps or insect nets, relative to the total number of individuals captured by each method, respectively. Panels show results for (a) non-syrphid Brachycera, (b) non-eusocial bees, (c) Nematocera, (d) wasps, (e) syrphid flies, (f) bumble

bees, and (g) other insects. Species IDs shown along the central vertical axis of each panel represent individual species or morphospecies recorded in this study. The overlap index (OI) between pan traps and insect nets for each functional group is indicated within each panel (see text for details). Detailed information for each species is provided in Online resource 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial1.pdf
- SupplementaryMaterial2.pdf