Animal Behaviour 145 (2018) 29–37

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Should I stay or should I go? Perching damselfly use simple colour and size cues to trigger flight

Roland Schröder [1], Christina N. Linkem [2], Julio A. Rivera [3], Marguerite A. Butler*

Department of Biology, University of Hawaii, Honolulu, HI, U.S.A.

ARTICLE INFO

Article history:
Received 9 May 2018
Initial acceptance 8 June 2018
Final acceptance 11 July 2018

MS. number: A18-00312R

Keywords:
colour cue
object discrimination
pursuit behaviour
size estimation
territorial defence

How do flying insects correctly respond to visual stimuli in complex natural environments? The spectacular coloration of some orders suggests that colour cues are important. Size may contain useful information as well, but insects are limited in resolving fine spatial detail due to the structure of their compound eyes. Although there have been many studies of experimentally altered body colour and pattern, we know surprisingly little about simple, isolated cues that insects use to take off after objects. Specifically, whether it is colour, size or some combination that triggers pursuit. We presented artificial bead stimuli of varying colours and sizes to perching males of the Hawaiian orangeblack damselfly *Megalagrion xanthomelas* in their natural forested stream habitat. Damselflies were most vigorously responsive to conspecific colours – attacking red (males) and tracking brown (females). Other colours with lesser biological relevance inspired lower response rates. Interestingly, size strongly modulated responses, whereby attack responses towards the smallest bead sizes transitioned to tracking or avoidance at the larger bead sizes. Although small beads are inherently more difficult to see, they triggered responses from greatest distances, even beyond the calculated stereopsis range of 17.5 cm. Damselflies had an object detection threshold of 0.34° within a high-resolution frontally directed acute zone with an interommatidial angle of 0.82°. We found evidence that size and colour serve as discrimination filters to efficiently identify objects of interest while ignoring visual noise. Simple schemes for object discrimination may help to explain how insects can differentiate among prey and conspecifics, and why multiple species can exist in the same community despite being similar in colour. We discuss implications for visual capabilities, and how visual systems that can readily discriminate size and colour can contribute to extraordinary phenotypic diversity.

Flying insects orient themselves almost entirely based on visual cues (Briscoe & Chittka, 2001; D'Adamo & Lozada, 2003; Dafni, Lehrer, & Kevan, 1997; Egelhaaf & Kern, 2002; Kelber, Balkenius, & Warrant, 2003; Lehrer, 1994). For example, bees use a wavelength-cutoff rule for celestial navigation, interpreting short wavelengths from an extended source to signal the sky, whereas wavelengths greater than about 400 nm from a point source indicate the sun (Menzel, 1979). Many insects, including beetles, bumblebees, dragonflies, honey bees and hawkmoths are known to use size information for object discrimination (reviewed in Dafni et al., 1997; Duong, Gomez, & Sherratt, 2017; Kinoshita, Shimada, & Arikawa, 1999; Menzel, 1979). Both size and colour provide information content over a range of lighting conditions, and therefore are potentially useful cues for the receiver (Briscoe & Chittka, 2001; Kelber, Vorobyev, & Osorio, 2003; Kinoshita & Arikawa, 2000; Menzel, 1979). It would therefore be reasonable for visual systems to evolve to detect these cues and direct appropriate responses to them (reviewed in Endler, 1992; Lind, Henze, Kelber, & Osorio, 2017), allowing the study of behaviour to be used to understand visual system capabilities of insects.

Predators of insects are known to use size to guide their selection of prey. From among a range of insect sizes, insectivorous birds have shown preferences for intermediate (Moreby, Aebischer, & Southway, 2006) or large sizes (Remmel & Tammaru, 2009). Dragonflies use size (Duong et al., 2017; Olberg, Worthington, Fox, Bessette, & Loosemore, 2005; Olberg, Worthington, & Venator, 2000), or angular size or velocity (Lin & Leonardo, 2017) to decide whether to take off after artificial prey. Prey that are too small may not be worth the energetic cost of pursuit, whereas those

* Correspondence: M. A. Butler, Department of Biology, University of Hawaii, 2538 McCarthy Mall, Honolulu, HI 96822, U.S.A.
E-mail address: mbutler@hawaii.edu (M. A. Butler).
[1] *E-mail address*: schroeder.rol@gmail.com (R. Schröder).
[2] *E-mail address*: cnlinkem@hawaii.edu (C. N. Linkem).
[3] *E-mail address*: julio.rivera85@gmail.com (J. A. Rivera).

https://doi.org/10.1016/j.anbehav.2018.08.015
0003-3472/

that are too big may be dangerous, with similar assessments made against conspecific adversaries in territorial interactions. Insect prey on the smaller end of the size range are preferred by many species, including grasshopper mice (Langley, 1989), preying mantis (Iwasaki, 1990) and dragonflies (Olberg et al., 2005; Pritchard, 1964; Rashed, Beatty, Forbes, & Sherratt, 2005).

Colour cues are important in the social behaviour of many insects. Colour is associated with territorial status in many odonate species, particularly damselflies (Corbet, 1980; Cordoba-Aguilar, 2009; Cordoba-Aguilar, Lesher-Trevino, & Anderson, 2007; Fitzstephens & Getty, 2000; Grether, 1996; Schultz, Anderson, & Symes, 2008) and in species and gender recognition (Miller & Fincke, 1999; Xu, Cerreta, Schultz, & Fincke, 2014). In some species, polymorphism in female coloration allows females to mimic males (Fincke, 2004; Hammers & Van Gossum, 2008; Sherratt, 2001; Van Gossum et al., 2011; Van Gossum, Stoks, & De Bruyn, 2001), although males may learn to recognize females based on additional cues. Schultz et al. (2008) found that activity periods are adjusted to optimize visual contrast, with sympatric species choosing to be most active when lighting conditions promote optimal contrast between body colour and the background. Wing coloration is known to indicate territorial status in males of the calopterygid damselfly *Calopteryx maculata* and the rubyspot damselfly, *Hetaerina americana* (Cordoba-Aguilar et al., 2007; Grether, 1996), and is used by choosy females to assess attractiveness of males (Siva-Jothy, 1999). Furthermore, experimental studies altering the colours of male and female body parts have concluded that males use abdomen and thorax colour as important cues for sexual recognition (Gorb, 1998; Miller & Fincke, 1999; Xu et al., 2014).

However, most studies examining the visual cues used for object detection have primarily tested stimulus size alone (e.g. Olberg et al., 2000, 2005) or whole body parts (Gorb, 1998; Miller & Fincke, 1999; Xu et al., 2014). One of the few studies to isolate both size and colour pattern (using solid versus an aposematic striped colour pattern, e.g. black and yellow stripes mimicking wasps) was conducted in dragonflies and found no effect of colour pattern (Duong et al., 2017). Therefore, whether simple, isolated colour and size cues are sufficient to trigger behavioural responses, and how insects rank colour, size or some combination in the decision to take off in pursuit of an object remains an open question.

We used artificial beads to study the use of colour and size in visually guided behaviour in the Hawaiian damselfly *Megalagrion xanthomelas* (Selys-Longchamps) in the field. *Megalagrion xanthomelas* is an endemic forest-dwelling species (adult length 33–37 mm) that inhabits small streams and pools on Oahu. It is part of an adaptive radiation of damselflies that varies spectacularly in colour and microhabitat use and possesses visual adaptations for their microhabitat type (Henry, Rivera, Linkem, Scales, & Butler, 2018; Polhemus & Asquith, 1996; Scales & Butler, 2016). *Megalagrion xanthomelas* is sexually dimorphic, with red males and brown females, and is a territorial sit-and-wait predator. It is believed to prey upon dipterans (Corbet, 1980; Polhemus & Asquith, 1996; this study), which vary from very melanistic to beige and live in forest understory dominated by greens and browns. Males perch on stream rocks or low-lying vegetation, readily taking off in flight to chase male intruders (Polhemus & Asquith, 1996). We hypothesized that they use colour and size to modulate behavioural responses appropriate to their ecological context. We presented beads of varying colour and sizes to perched males in the field and noted the type of behavioural response to each. We considered responses to brown and red beads to reflect responses to conspecifics, responses to black and white beads to reflect responses to prey (e.g. Olberg et al., 2000) and responses to yellow and green beads to reflect responses to environmental background. We tested whether response frequencies and take-off distances were explained by detectability, and we investigated what we can infer about the visual capabilities of *M. xanthomelas*. We also explored whether colour or size information dominates in the decision to pursue objects and whether there are simple rules governing these decisions.

METHODS

Field Studies

We videotaped behavioural responses of male damselflies in the field along a stream at the Tripler Army Medical Center (TAMC) located on leeward Oahu (Honolulu, HI, U.S.A.). The 100 m reach of stream is an unnamed tributary to Moanalua Stream at an elevation of 79 m. Generally no more than 1 m across and 10 cm deep, the small stream flows slowly through a forest that provides canopy with occasional gaps (Preston, Englund, & McShane, 2007). Damselflies variously perch on branches, vines and other streamside vegetation as well as boulders and stream litter within the stream and in close proximity to the stream margin (<1 m), and view a generally dim, cluttered habitat (Fig. 1a and b). Further description of this location can be found elsewhere (Englund, 2001; Polhemus, 1996; Preston et al., 2007).

This study focused on male *M. xanthomelas*, as females are rarely encountered out of tandem with males (typical of all *Megalagrion* species). Our study was conducted from July to October 2007 during 0930–1400 hours local time, which captures the main activity period of this species (Polhemus & Asquith, 1996), on sunny days with blue sky and little cloud cover (less than ~30%). In Hawaii, damselflies are active year-round (Henry et al., 2018; Polhemus & Asquith, 1996; Preston et al., 2007), with no obvious seasonal difference in prey activity (R. Schröder, personal observation).

Behavioural Categories

We conducted preliminary focal observations of natural damselfly behaviour and identified four possible behavioural responses that occurred towards conspecifics, potential prey or other objects: (1) attacking (taking off in flight directed towards the object and touching it); (2) tracking (taking off towards the object and inspecting but not touching it, then breaking off pursuit); (3) avoiding (taking off away from the object, increasing distance to the object); and (4) no response (no take-off when object was within 5 cm of the front of the focal subject's head). These categories correspond to those observed by Frantsevich and Mokrushov (1984).

Stimulus Presentation

Perching *M. xanthomelas* will readily respond to artificial bead stimuli. Bead stimuli in various colours (red, brown, black, white, yellow, green; see Fig. 1 for reflectance spectra) and sizes (2.5, 3, 4 and 5 mm diameter) were presented to perching male *M. xanthomelas* in the field ($N = 744$ total bead presentations). This size range spanned the head size of this species (~4 mm; see Results). The beads were spherical plastic beads ('Pearls', K Crafts, U.S. Bussan Co., U.S.A.) painted with acrylic craft paint. Each bead was tied with 30-pound (0.55 mm diameter) clear fishing line of approximately 30 cm in length on the end of a telescoping fishing rod.

On any given experimental day, three to four arbitrarily chosen colour–size stimulus combinations were tested out of 24 possible combinations. The population size is over 200 individuals based on a mark–recapture study (Preston et al., 2007). Individuals were not marked, but care was taken to avoid testing the same individual