The visual ecology of directed aerial descent in first-instar nymphs of the stick insect *Extatosoma tiaratum*

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Abstract

Many wingless insects perform directed aerial descent (DAD) to return to vegetational structures after falling. Given the complex visual environment and spatial structures of tree canopies, those visual signals used as directional cues are not fully understood. Here, we address the role of visual contrast for DAD in newly hatched nymphs of the stick insect *Extatosoma tiaratum* under controlled laboratory conditions. Landing preferences of gliding *E. tiaratum* in various visual environments were studied. We used a single vertical stripe defined by variable contrast edges to test the use of contrast consistency and sharpness. We also used aggregate patterns to examine the effects of target size and the effectiveness of luminance contrast and chromatic contrast. *E. tiaratum* nymphs were attracted to single stripes with well-defined edges, and particularly favored narrow dark targets. The directionality and accuracy of landing were dependent on target size. Lastly, luminance contrasts were more effective in attracting landings than were chromatic contrasts. Visual contrasts are therefore used as spatial references for landing behavior in DAD. These behaviors may enable nymphs to quickly locate dark or shaded sides of vertically oriented vegetational structures in natural habitats.

Keywords canopy, gliding, invertebrate, vision
List of symbols and abbreviations

B/W pattern of black stripe with white background
W/B pattern of white stripe with black background
C_B background contrast, the average of pairwise contrast formed between a stripe and 2 adjacent stripes
C_P pairwise contrast between 2 adjacent surfaces
CI confidence interval
CS contrast sharpness setup
CT contrast edge continuity setup
DAD directed aerial descent
f_L frequency of landing
f_L,stripe frequency of landing on stripe
f_c spatial frequency
GLMM generalized linear mixed model
N sample size
L reflectance
r_L count of landing
R fraction of white surface with respect to total area
s sharpness coefficient
P patchiness, number of black or white stripes
W stripe width
Δφ angular period
θ circular direction in von Mises distribution
κ concentration parameter in von Mises distribution
1 INTRODUCTION

Many wingless arthropods perform directed aerial descent (DAD) to return to vegetational structures after intentional jumps or accidental falls. The ability of DAD is an important adaptation in many arboreal taxa, e.g. it is often associated with aerial escape or dispersal in canopy space. Its repeated evolution in different lineages suggests common selective force for the ability to travel laterally during descent (Dudley et al., 2007; Yanoviak et al., 2010, 2011; Dudley and Yanoviak, 2011). In an evolutionary perspective, DAD may represent an early stage of animal flight (Dudley, 2007). Nevertheless, despite an extensive literature on the significance of visual information for flying insects (see Dudley, 2000), very little is known about the visual ecology of DAD. *Cephalotes* ants target vertically oriented columns with a high reflectance and which resemble bright tree trunks relative to a darker background of foliage (Yanoviak and Dudley, 2006). Given the wide ecological diversity characterizing arboreal taxa that are capable of DAD, many other visual signals of the canopy environment may be used as directional cues.

The visual ecology of DAD is presumably characterized by the changing visual field perceived during descent. First, variable lighting and complex spatial structures of tree canopies together create abundant yet noisy visual environments. In addition to a vertical light gradient (Endler, 1993), temporal changes of sun position and atmospheric conditions can interact with vegetation, e.g. with trunks, branches, lianas and epiphytic plants, producing heterogeneous light microclimates (Chazdon and Fetcher, 1984). The interactions between vegetational surfaces and ambient lighting (e.g. reflection and transmission of incident light) further enhance heterogeneity of the visual landscape. Second, a descending animal perceives a dynamically changing and upward moving visual field. Extracting characteristic signals unique to the desired targets is necessary for successful landing. Furthermore, landing may require robust tactics for locating targets in various environmental conditions, as the visual appearance of tree trunks can vary with a viewer’s perspective, sun angle and weather condition.

One potentially informative signal for DAD is visual contrast, which is the difference in luminance or color perceived by the animal. Contrasts can be useful for discriminating objects from the background. Differences in luminance or coloration due to patterns or shadows on a surface may also be perceived as contrast signals. Insects can utilize contrast to recognize shape and patterns (Prokopy and Owens, 1983) and to discriminate spatial configuration (Lehrer et al., 1990; Kern et al., 1997; Pratt et al., 2001). The contrast edges formed between vertically oriented vegetative structures and background, e.g. the foliage or sky, may provide stationary signals for descending animals and thus can be used as spatial references for maneuvering and as indicators of landing targets. Nevertheless, the reliability of this feature is highly dependent on the spatial configuration of the canopy, as edges can be disrupted by shadow, sunflecks and inconsistent backgrounds. Furthermore, any tactic utilizing contrast edges as visual cues also needs to accommodate variation due to the observer’s perspective. For example, tree trunks under angled incident light may appear as bright surfaces with patchwork patterns on the sunny side but as a shaded surface on the darker side (e.g. Fig. 1A).

Here, we studied the role of visual contrasts during DAD by first-instar nymphs of the stick insect *Extatosoma tiaratum*, Macleay 1826. *E. tiaratum* is an arboreal species native to coastal rainforests of eastern Australia. Female *E. tiaratum* drop eggs to the forest floor, and new hatchlings emerge from the understory during the rainy season (Brock, 2001). Different from many nocturnal species, newly hatched *E.
*tiaratum* are diurnally active and mimic *Leptomyrmex* ants during the first 3–5 days after hatching (Calberg, 1981, 1983, 1984). They also exhibit strongly negative gravitaxis and positive phototaxis, which result in ascent and dispersal towards tree canopies by climbing upwards through vegetation. When exposed to threats and perturbations, they may perform intentional drops followed by directed aerial descent, with glide indices averaging 0.24 over the entire descent (Y. Zeng, unpublished). Compared to vertical parachuting, DAD reduces net height loss in climbs characterized by repeated drops, which reduces the energetic expenditure for ascending; it may also serve as an antipredatory behavior. Although we lack detailed measurements about the visual environments for the native habitat of *E. tiaratum*, they are presumably characterized by complex three-dimensional geometries and high heterogeneity in the visual landscape (Fig. 1B). The tropical rainforest floor receives 0.5%–2.4% of the light at the top of the canopy (Pearcy, 1983). Since newly hatched *E. tiaratum* are presumably active between the forest floor and canopy, they are likely subjected to a wide range of environmental luminance, e.g. ~600 Lux to $2.3 \times 10^4$ Lux (Bjorkman and Ludlow, 1972; Pearcy, 1983; Lee, 1987).

In this study, contrast stimuli were created using vertically oriented stripes to test the landing responses of newly hatched *E. tiaratum* following DAD (Table 1). The goals of this study were: 1) to test the effectiveness of visual contrast and the importance of contrast quality, including consistency and strength, for successful landing; 2) to examine how landing behavior is affected by the size of targets and the heterogeneity of visual environments consisting of dark and light surfaces; and 3) to address the differences in effectiveness between luminance contrast and chromatic contrast for attracting landing. Studying such visual responses, while of immediate physiological relevance to this one taxon, can also illustrate the potential diversity of mechanisms underlying DAD in the numerous arboreal arthropods for which this behavior has been described.
## Table 1. Summary of experimental trials, experimental variables, and results.

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2 RESULTS

2.1 Single-stripe patterns

Nymphal E. tiaratum were attracted by single-stripe patterns. With both B/W and W/B patterns (Fig. 3A), the majority of landings was on the target screen (B/W: 85%, N=41 drops; W/B: 95%, N=37 drops), and at a rate significantly greater than that expected by the null (B/W: $G_{adj}=45.07$, $P<0.001$; W/B: $G_{adj}=60.01$, $P<0.001$). Also, landing frequencies on the stripe ($f_{L,\text{stripe}}$) were significantly greater than the null expectation (B/W: $f_{L,\text{stripe}}=91.4%$; W/B: $f_{L,\text{stripe}}=48.6%$; Fig. 3D; supplementary material Table S1).

The consistency of contrast edges was found to be essential for successful targeting. Neither CT1 (contrast edges defined by alternating black and white surfaces) nor CT3 (W/B pattern with discontinuous edges) attracted landings. Directionally biased landing was only found in the trial using pattern CT2 (B/W pattern with discontinuous edges), for which $f_{L,\text{stripe}}$ exceeded 50% with no significant differences among individuals (Fig. 3D; supplementary material Table S2).

Nymphal E. tiaratum also showed a preference for sharper contrast edges over blurred ones. In contrast sharpness trials (CS series, Fig. 3C), all landing points (N=255) were on the target half of the arena (i.e. with an angular range from -90° to 90°, see Fig. 2E). In both CS1 and CS2 trials, the majority of landings (>50%) were concentrated near the stripe with sharper edges. This result was significantly different from the null expectation, yet results from the control experiments showed no such bias (Fig. 3E). There was no significant differences among individuals tested in the same trials (supplementary material Table S3).

2.2 Size effect of stripe patterns

When exposed to multiple-stripe patterns in Arena 2, most drops (264 of 324, 81%) landed on the arena wall. The trial-specific mean frequency from missing the arena wall ranged between 2% and 7% among trials (supplementary material Fig. S2). Landing points were concentrated near contrast edges in trials with low stripe density (e.g. P2 series). The concentration of landing points was negatively correlated with the spatial density of stripes (Fig. 4). Surface luminance-biased landing was only found in two trials, both of which were characterized by two narrow stripes against a wide background (P2-R10 and P2-R90; Fig. 4A,C; supplementary material Table S4), and the majority of landing occurred on the stripes regardless of its surface luminance. Overall, stripes of intermediate width (i.e. 14 cm) were the most favored, and stripes of large width (i.e. 127 cm) were the least favored (Fig. 5). When both black and white surfaces were of intermediate width (17 cm in trial P8-R50), the majority of landings also occurred on black stripes, although a repeated G-test suggested non-significant deviation from the null expectation (supplementary material Table S4).

2.3 Patterns of parallel stripes in gray shades and colors

In gray shade trials, nymphal E. tiaratum showed a significant preference for darker targets. The majority of drops (136 of 140, 97%) landed on the target screen, which was significantly different from the null expectation (supplementary material Table S5). The landing count ($r_t$) was significantly influenced by the presence of the gray shade ($\chi^2=16.4$, $P<0.001$, repeated Poisson mixed-effects models), but not by the position of stripes ($\chi^2=3.5$, $P=0.32$). Black and dark gray stripes were the most attractive (Fig. 6A), i.e. $f_{L}>30\%$ for the black stripe in five trials and for the dark gray stripe in four trials (supplementary material
Fig. S3). We also found a negative correlation between $r_L$ and surface luminance of the stripes (slope of -1.03±0.39 [estimate±s.e., $P<0.01$, Poisson GLMM], but found no correlation between $r_L$ and the horizontal distance between release point and the midpoint of each stripe ($P=0.39$).

In color trials, the majority of drops (690 of 729; i.e. 95%) landed on the target screen, a result which was significantly different from the null expectation (supplementary material Table S5). We found $r_L$ to be significantly influenced by both stripe color (Poisson mixed-effect models: $\chi^2=87.7$, $P<0.001$) and stripe position ($\chi^2=166.6$, $P<0.001$). Comparing among individual stripes, the mean values of $f_L$ for black and white stripes were significantly greater than those of blue and green, and values of $f_L$ for red and yellow stripes were at an intermediate level (Fig. 6B; supplementary material Fig. S4). Contrary to the results from gray shade trials, $r_L$ was negatively correlated with the distance between release point and stripe (slope of -0.09±0.01 [estimate±s.e., $P<0.001$, Poisson GLMM]). Comparing thirteen pairs of chromatic contrast used in experiments, there was significant variation in $r_L$ ($\chi^2=357.03$, $P<0.001$, Poisson GLMM). The mean landing frequencies associated with different chromatic contrasts were not significantly different, although the most attractive contrasts consisted of black, white, yellow and red (Fig. 7).

Landing preferences were significantly influenced by contrasts formed between stripes in gray shade trials. Values of $r_L$ for gray shade trials show that preference for the darker stripe is positively correlated with the magnitude of pairwise contrast (i.e. $|C_P|$), indicating landing preferences for darker stripes associated with stronger contrast edges (sub-hypothesis 1; Fig. 6C). Also, values of $r_L$ were negatively correlated with background contrast $C_B$, suggesting landing preferences for darker stripes intermediate to two lighter ones (sub-hypothesis 2; Fig. 6D; Table 2).

<table>
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<tr>
<th>correlation</th>
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<td>$r_L$ (darker) vs. $</td>
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<td>$r_L$ (lighter) vs. $</td>
<td>C_P</td>
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<td>$r_L$ vs. $C_B$</td>
<td>-0.052 (0.090)</td>
<td>-0.730 (0.170) ***</td>
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<td>$r_L$ vs. $</td>
<td>C_B</td>
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Table 2. Results from Poisson GLMM analyses for correlation of landing ($r_L$) correlates with either pairwise contrast ($|C_P|$) or background contrast ($C_B$) in gray shade trials. $r_L$ (darker) and $r_L$ (brighter) are counts of landing on the relatively darker and lighter surfaces within each stripe pair, respectively. Estimates from a Poisson regression are shown, with s.e. given in parentheses (*, $P <0.05$; **, $P <0.01$; ***, $P <0.001$). See Fig. 6.
3 DISCUSSION

Our results demonstrate that vertically oriented contrast edges are used as directional cues during DAD by nymphal *E. tiaratum*. Experimental insects targeted contrast edges and preferred those with greater consistency and strength, particularly favoring narrow stripes with low luminance. Both directionality and accuracy of landing were found to be influenced by the size of potential targets. When exposed to parallel stripe patterns in various gray shades and colors, insects were more attracted by luminance contrasts than by chromatic contrasts (Table 1). Using visual contrasts during DAD likely helps nymphal *E. tiaratum* quickly identify preferred landing targets, e.g. the shaded side of vegetational structures, under a variety of lighting conditions.

3.1 The utility of visual contrast

Trials using single-stripe patterns demonstrated that first-instar *E. tiaratum* were attracted to narrow stripes defined by two contrast edges. The landing frequency on the stripe in B/W trials was higher than that in W/B trials, suggesting preference for dark targets rather than simply gliding towards contrast edges. This preference for narrow dark stripes was shown when the black stripes had no consistent edges (i.e. trial CT2; Fig. 3A,B) and when there were multiple black stripes (i.e. in P2-R50 and P8-R50; Fig. 4C,E). Insects also exhibited preference for contrast edges with greater consistency and strength. The quality of contrast is likely derived from the spatial consistency and strength of visual signals, which represent sharp discontinuities in the angular differential of perceived brightness and color (Land, 1997). Discriminating by contrast strength and consistency may help them select among candidate targets (see below).

In the visual environments formed by black and white stripes, the distribution of landing points was dependent on the size of target stripes. The directionality of landing was reduced in trials with narrower stripes (e.g. stripe width ≤ 4 cm) and higher stripe density (e.g. series P32), which may derive from behavioral avoidance of small objects and the lack of ability to effectively respond through maneuvering. Besides size effects for potential targets, landing choice can be significantly influenced by the proportion of dark and light surfaces in the visual field. When the black surfaces were relatively narrow (i.e. width 14–17 cm in series P8-R50 and P2-R90), they were highly attractive for landing. However, such bias was not observed with the black surfaces in greater sizes (i.e. >70 cm, series P2-R10 and P2-R50). These results suggest that first-instar *E. tiaratum* target both contrast edges and narrow dark surfaces.

In trials with parallel stripes in gray shades and colors, we found that contrast in luminance is more effective in attracting landing. In gray shade trials, nymphal *E. tiaratum* exhibited an overall preference for dark stripes; their landing preferences also significantly correlate with the contrasts between stripes (|C_C| and C_B, Fig. 6C,D). Insects did not favor any particular color, but clearly avoided blue and green. They also tended to land on the closest stripe, suggesting a lack of directional preference when exposed to color stripes. The most attractive chromatic contrasts were formed between black and a lighter color (i.e. white or yellow), suggesting that the contrasts due to luminance differences remained attractive with the presence of chromatic signals. Overall, chromatic contrasts were found to be less predictable in influencing landing direction than luminance contrasts. Orthopteroid insects have photoreceptors for UV, blue and green (Briscoe and Chittka, 2001), and thus actual chromatic contrast perception is more complex than estimated in this study. Future measurements of the spectral sensitivity of first-instar *E. tiaratum* would enable better
3.2 Adaptive significance and future directions

Using contrasts as cues to select landing targets is potentially useful in natural habitats. First, selection by signal consistency may help to identify vertically oriented components of vegetational structures, including tree trunks and some branches. As DAD is dominated by translation in descent (~2 m/s), a temporal lag between visual perception and corresponding maneuvers is unavoidable. Identifying objects that are stationary can thus help to predict landing location and enhance landing success. Second, dark stripes favored in experiments resemble either dark or shaded vegetational structures in natural habitats. Targeting the shaded side may also enhance the success of landing. For example, in a falling insect’s perspective the shaded side of a tree trunk may appear to be more stationary and to present less variation than the sunny side, which may present more obvious bark patterns and less consistent contrast edges due to sunflecks (see Fig. 1A). Also, landing on shaded areas may act to hide newly hatched *E. tiaratum*, especially if DAD was initiated for escape. Lastly, nymphal *E. tiaratum* avoided both blue and green surfaces, which behavior is similar to that of *Cephalotes* ants (Yanoviak and Dudley, 2006). This comparison suggests that both taxa may avoid foliage, probably because it is a complex source of spatial reference for falling animals. Due to vegetational and structural diversity within tree canopies (e.g. variable branching structures, presence of lianas and epiphytes), the visual appearance of vegetation can be more variable and inconsistent along the vertical direction than that of tree trunks, thus providing a noisier signal. Also, this behavior may be associated with the avoidance of more complex 3D structures, especially if recovering the height loss is a prior task in both nymphal *E. tiaratum* and *Cephalotes* ants.

Use of characteristic signals for object identification and navigation is commonly seen in invertebrates. For example, honeybees can extract pattern features for use as spatial references (Srinivasan et al., 1993), and hymenopteran insects can use contrasts for edge and pattern detection (Lehrer et al., 1990; Kern et al., 1997; Pratt et al., 2001). Quantitative documentation of the visual environment of relevant habitat, e.g. determining the reflective properties of different vegetational surfaces, along with temporal and spatial variation in the light environment, would further elucidate the effectiveness of using visual contrasts as directional cues. Our experiments were also spatially constrained. Insects were released in haphazard initial orientations, which likely resulted in variation in their initial trajectory (e.g. aerial righting typically occurs if the insects fall upside-down). The high landing frequencies on targets suggest, however, that our experimental setup provided sufficient space for maneuvering in the majority of cases. Future studies might investigate the role of visual signals in control of DAD by correlating maneuvering kinematics with aspects of the visual field. Integrating DAD trajectories with virtual canopy environments could also simulate the dynamic visual fields perceived during descent.

Although the targeting tactics found in this study may be specific to newly hatched *E. tiaratum*, the need to quickly identify landing targets in a complex visual environment is common among different taxa. Habitat-specific lighting and spatial geometry within vegetational structures may play important roles in the ecology of aerial behavior. The preference for dark and shaded surfaces in *E. tiaratum* is opposite to that in *Cephalotes* ants (Yanoviak and Dudley, 2006), which may be due to differences in habitat, e.g. in canopy type and active height, and in behavioral tactics associated with landing, e.g. whether to hide or remain exposed after landing. Given the diverse types of forest canopies (Erwin, 1988) and independent evolution
of DAD in many different taxa (Dudley and Yanoviak, 2011), other mechanisms of visual guidance likely remain to be documented. For instance, preliminary experiments in other nymphal and adult stick insects have shown DAD landing unassociated with visual contrast (e.g. Phyllium spp.; Y. Zeng, unpublished). Furthermore, the ability to distinguish standing vegetative structures from their surroundings may have originated concurrently with plant arborescence (Dudley et al., 2007). Identifying landing targets and control of body orientation by changing appendicular postures can be driven by selection favoring higher landing success and greater accuracy, both of which will depend on size and visual appearance of structures within in the visual environment.

4 MATERIALS AND METHODS

_E. tiaratum_ eggs from our lab colony were incubated under constant temperature (25°C) and humidity (50–70%). Newly hatched nymphs were collected within the first 24 hours and were transferred to clear plastic cups (355–470 ml) with lids. Constant humidity was maintained in the plastic cups by regular water spraying, and all cups were kept in an environmentally controlled room at constant temperature (25–27°C) and 12-hour light-dark cycles. Each experimental trial was conducted using a new batch of past-hatch insects up to one days old (see below). The insects were transported and kept in the same type of cups with constant humidity maintained by wet paper towels during experiments. Statistical tests were conducted using custom-written R scripts (R Core Team, 2014).

4.1 Experimental setups and protocols

Environmental temperature ranged from 26–30°C in experimental spaces. Environmental luminance was measured with a light meter (Extech 401025, Waltham, MA, USA). A standard spatial setup was applied for two experimental arenas, each equipped with a release point located 40 cm horizontally from the target screen and 3.5 m above ground, to ensure sufficient space for maneuvering. Insects were released through a funnel, the inner wall of which was coated with Teflon (no. 2871, BioQuip Products, Gardena, CA, USA), with the nozzle (diameter 3 cm) pointing vertically downwards. The environmental luminance was between the luminance of canopy and understory in tropical rainforests (e.g. ~600 Lux to 2.4×10⁴ Lux; Bjorkman and Ludlow, 1972; Pearcy, 1983; Lee, 1987).

Arena 1 was built in the central green house at the Plant Lab, University of Washington. Visual targets were attached on a suspended screen (height 3.5 m, width 1.5 m). The glass ceiling of the room allowed sunlight to enter, and under cloudy circumstances additional lighting was used to maintain sufficient environmental luminance (2500 Lux to 1×10⁴ Lux). In experiments, insects were dropped into the funnel by triggering the flip of a Teflon-coated vial attached to the end of a hand-held pole (Fig. 2A). The horizontal distance between the release point and the furthest edges of the screen was about 1.55 m. The experimenter (height 1.7 m) stood at least 1.5 m away from the screen. The maneuvering choice of the insects was thus not impeded during the first 1.8 m of descent. The feasibility of this protocol was further confirmed by high landing success rates using the setup described below (e.g. _f_\textsubscript{L,strip}<99% with B/W setup).

Arena 2 was a cylindrical enclosure (height 3.5 m, inner diameter 80 cm) built in the Animal Flight Laboratory, University of California, Berkeley. Eight floodlight bulbs (General Electric PAR38-29529,
Fairfield, CT, USA) were installed directly above the space for sufficient luminance (1500 Lux to 4500 Lux, from enclosure floor to enclosure top). One experimenter stood on a ladder outside of the arena and dropped insects at haphazard initial orientations through the release funnel mounted at the top center of the enclosure (Fig. 2D). Successful landings on targets were confirmed visually from above. The landings were recorded by a high-definition video camera at 50 fps (SONY XR160, Japan) mounted directly above the arena. Landing points were then extracted based on a single landmark at the longitudinal midpoint of the insect body using commercial software (ProAnalyst, Xcitex, Inc., Cambridge, MA, USA), which were then converted into a polar coordinate system (Fig. 2E) using custom-written scripts in MatLab (R2012a, MathWorks, Natick, MA, USA). To assess the effect of digitizing error, a single landing point was digitized five times by the same observer, and the means of the associated standard deviations were <0.20%. The circular directionality of landing positions was analyzed using the ‘CircStats’ package (Lund and Agostinelli, 2012), which calculates the mean direction \( \theta \) and concentration \( \kappa \) based on von Mises maximum likelihood estimates, and which generates bootstrap confidence intervals for each of \( \theta \) and \( \kappa \).

4.2 Quantifying visual contrast

We use ‘pattern’ to represent any given set of visual stimuli used in experimental trials. Visual patterns installed in experimental arenas were made with felt and paper. Design details are specified in particular sections below. The reflectance properties of all materials were measured using a spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA) over the range of 300–650 nm using a deuterium-halogen light source (AIS Model MIMI DTA, Analytical Instrument Systems, Flemington, NJ, USA; supplementary material Fig. S1) calibrated with a diffuse white standard (Labsphere, Inc., NH, USA). In this study, pairwise contrast \( (C_P) \) is based on the differences in reflectance between 2 adjacent surfaces normalized by their total reflectance:

\[
C_p = \frac{L_1 - L_2}{L_1 + L_2}
\]

where \( L_1 \) and \( L_2 \) are the average reflectance of two surfaces. Given that the receptor spectral sensitivity of our experimental insects is unknown, perceived luminance was estimated by averaging the reflectance over 300–550 nm, which covers the visible spectrum of most invertebrates, including orthopteroid insects (Osorio, 1986; Briscoe and Chittka, 2001).

4.3 Single-stripe patterns

Patterns of a single stripe against uniform background were used to test the use of contrasts for DAD landing and to test the effects of signal consistency and strength. All trials were designed to have similar numbers of landings recorded across individuals. The count of landing \( (r_L) \) was used in regression analyses, and the frequency of landing \( (f_L) \) in specific trials was calculated based on individual averages.

First, the effects of contrast were tested in two modes: a black stripe against a white background (B/W) and the opposite (W/B) (Fig. 3A). Both patterns consisted of one stripe (width 15 cm) with a background, and were installed in Arena 1. With each setup, 5–6 trials were conducted for each of six individuals. Landing location was recorded after each drop. In Arena 1, each stripe surface can be projected on a 2D circumference centered at the location of release point; assuming random drift, the landing frequency
associated with each stripe should equal to the angular fraction of its projection relative to a total of 360° (Fig. 2B). Based on this random drift assumption, the null landing frequency is 6.0% for a 15 cm wide stripe positioned 40 cm away from the release point, and is 18.9% for the background screen. The observed count of landings was compared with the null expectation using a repeated G-test of goodness-of-fit with William’s correction, which addresses the overall deviation from expected proportions and whether there are significant differences among repeated experiments (McDonald, 2015).

Second, the role of contrast signal continuity was examined using modifications of B/W and W/B patterns, whereby alternation of black and white surfaces (height 5 cm) in the vertical direction was applied to either or both of the stripe and background surfaces, resulting in three patterns with disrupted contrast edges (CT1, CT2 and CT3; Fig. 3B). CT1 provided two consistent contrast edges defined by alternating black and white surfaces, whereas CT2 and CT3 provided a consistent stripe with discontinuous contrast edges. For each setup, 5–7 drops were conducted for each of six individuals. The calculation of null frequencies followed the protocol described above, and the null frequencies of landing in and out of the stripe region were 6.0% and 94.0%, respectively. Statistical tests were the same as given above.

Third, the effect of contrast signal strength was tested using stripe patterns with different edge sharpnesses in a series of binary choice tests. As single-stripe patterns were found to be attractive, paired single-stripe patterns may thus impose a binary choice for insects. The luminance profile $y$ of a single stripe in the transverse direction was determined by both black surfaces ($y=0$) and white surfaces ($y=1$). The luminance profile of a vertical stripe (centered at $x=0$) was thus defined as a function of horizontal location ($x$):

$$y = \begin{cases} 
(\text{erf}((x + \delta)s) - 1)/2 & x < 0 \\
(\text{erf}((x + \delta)s) + 1)/2 & x \geq 0 
\end{cases}
$$

where $\text{erf}(x) = 2\pi^{-0.5} \int_0^x e^{-t^2} dt$ is an error function, $\delta$ is one half of the stripe width (15 cm), and $s$ is a sharpness coefficient. Stripes of different edge sharpnesses can be produced by changing $s$, e.g. the greatest sharpness occurs when $s=0$ (Fig. 3C), whereas the overall luminance of the stripe region and background combined remains constant. Luminance profiles were generated using custom-written MatLab scripts, and were then printed on paper by commercial printer (Staples Inc., Framingham, MA, USA). The spectral reflectances of the white and black regions of these printed patterns were similar to those of the felt used in experiments (supplementary material Fig. S1). Binary choice patterns were made with two paired stripes of different edge sharpnesses ($s=0$ vs. $s=0.5$), and were installed 90° apart from one other in Arena 2. Two trials were conducted based on two contrast modes modified from patterns B/W and W/B (Fig. 3C). In each trial, 5–6 drops were recorded for each of five individuals. The distributions of landing points were analyzed within a polar coordinate system centered at the arena’s center, with 0° defined by the midline between left and right stripes (Fig. 2E). In addition, three sets of controls were conducted following the same protocols. Each control setup provided two stripes of identical edge sharpness (Control-1, 2 black stripes with sharp edges; Control-2, 2 black stripes with blurred edges; Control-3, 2 white stripes with sharp edges). The null hypothesis predicted non-significant bias of landing on either stripe. Observed counts of landing associated with each stripe were compared to null proportions (1:1) using a repeated G-test with William’s correction.
4.4 Size effect of stripe patterns

Patterns of alternating black and white stripes were installed in Arena 2 to test the effects of target size. Nine different patterns were produced as combinations of three levels of total screen luminance (i.e. the proportion of black relative to white surfaces) and three different stripe densities. For different total screen luminances, the proportion of white and black surfaces (P) was set as 1:9, 1:1, and 9:1, coded as R1, R5 and R9, whereby the embedded numbers represent the fraction of white surface assuming total area to equal a value of 10. For different stripe densities (R), the number of stripes in a given color was set as 2, 8 and 32, coded as P2, P8 and P32, respectively (Fig. 4). The width (W) of black stripes was then determined as $d=\pi(1-R)/P$, where $d$ is the inner diameter of arena (80 cm). The spatial frequency of a given surface can be calculated as $f_c=1/\Delta\phi$, where $\Delta\phi$ denotes the angular period between two edges (Table 3).

With each setup, 6–8 drops for each of 4–6 individuals were recorded. Using a repeated G-test with William’s correction, the counts of landing on black or white were tested against a null hypothesis based on random drift, which predicted that the frequency of landing on a particular color is the same as the area proportion presented by that color. To summarize the angular distribution of landing locations, the angular distance between each landing point and the nearest contrast edge was calculated. The distribution of all landing locations within each trial was then calculated with respect to an interval defined by the midlines of two adjacent surfaces (Fig. 4).

<table>
<thead>
<tr>
<th>trial</th>
<th>N</th>
<th>W (cm)</th>
<th>$\Delta\phi$ (°)</th>
<th>$f_c$ (cyc/rad)</th>
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<tr>
<td>P2-R10</td>
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</tbody>
</table>

Table 3. Pattern design in the size effect experiment, as shown by the variables associated with black surfaces. N, number of stripes; W, stripe width; $\Delta\phi$, angular period; $f_c$, spatial frequency. See Fig. 4.

4.5 Patterns of parallel stripes

Potential use of contrast was tested with patterns of parallel stripes with different gray shades or colors, design of which followed a previous study (Yanoviak and Dudley, 2006). In Arena 1, parallel felt stripes (15 cm width) were suspended as a target, behind which a dark green background screen was placed 1.5 m distant. In gray shade trials, four felt stripes representing a gray shade gradient (black, dark gray, gray and white) were used. The juxtaposition of stripes formed a screen 60 cm in width (Fig. 2A,B). Under each of seven trials of possible stripe combinations (supplementary material Table S6), landing choice was recorded.
for the same group of 4–5 individuals, each of which was dropped 4–5 times, and 16–20 successful landings was obtained. In color trials, fourteen different combinations of stripes in six colors (red, black, white, yellow, green, and blue) were used (supplementary material Table S7). Blue and green stripes were not used together for logistical reasons, because increasing the number of candidate colors by one would double the total number of trials to an unfeasible number of 28. For each trial, the same groups of 5–8 individuals were each dropped 7–11 times, and totals of 45–63 landings were recorded. For both gray shade and color trials, each landing was scored as to the color of the corresponding stripe, or as a ‘miss’ if the insect failed to land on the target screen. The null hypothesis was made based on random drift, which predicts the landing frequency on the target screen to be 34.4% based on the same assumptions as in single-stripe trials. Repeated G-tests with William’s correction were used to analyze the counts of landing on screen against the null expectation.

With parallel stripes, insects may choose to land on a favored color, or to land on the nearest stripe if all colors are equally effective. These possibilities were addressed by testing how either or both the juxtaposition and coloration of stripes predicted landing locations. Poisson mixed-effect models for landing count were conducted using the ‘lme4’ package (Bates et al., 2012), in which stripe position and coloration were set as fixed factors, and individual and trial number were set as random factors. P-values were calculated through likelihood ratio tests of the full model using the effect in question, set against the model without the effect in question. Similarly, a Poisson generalized linear mixed model (GLMM) was used to analyze the relationship between the count of landing and the horizontal distance between the midpoint of each stripe and release point, using individual identity and trial numbers as random factors.

Furthermore, correlations between landing preferences and visual contrasts were analyzed at two size scales for gray shade trials, as specified by the following sub-hypotheses. Sub-hypothesis 1 was that landing frequency is positively correlated with the strength of contrast between two adjacent stripes. Therefore, we expected among all pairwise combinations across all trials that those with stronger pairwise contrast ($C_{P}$, Eqn 1; Fig. 2C) would attract more landings. Based on results from previous trials (e.g. P8-R50, Fig. 4E), a preference for darker stripes was expected. Poisson GLMM was used to analyze the correlation between the magnitude of pairwise contrast ($|C_{B}|$) and the corresponding count of landing ($r_L$) on each of darker and lighter stripes among all stripe pairs, for which individuals and trial numbers were set as random factors. Sub-hypothesis 2 was that landing preference is correlated with the mean contrast between a stripe and its two adjacent surfaces, predicting that stripes that form stronger contrast relative to two adjacent stripes tended to attract more landings. The background contrast was calculated as the average of pairwise contrasts

\[
C_B = \frac{C_{PL} + C_{PR}}{2}
\]

(3),

where $C_{PL}$ and $C_{PR}$ are pairwise contrasts of the stripe of interest against adjacent stripes to the left and to the right, respectively (Fig. 2C). Contrast against the dark green background was used for marginal stripes. Poisson GLMM was used to analyze the correlation between $C_B$ (relative value) or $|C_{B}|$ (magnitude) and counts of landing associated with individual stripes across all trials, with individual identity and trial numbers as random factors.
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7 AUTHOR CONTRIBUTIONS

Y.Z. devised the experiments. Y.Z., Y.L. and A.A. performed the experiments and collected the data. Y.Z., R.D. and Y.L. analyzed the data. All authors contributed to writing the manuscript.
References


Fig. 1. Complex visual environment and spatial structures at tree canopies. (A) Examples of the variation of visual appearance of tree trunks under sunny sky, which may appear as shaded surfaces (a), bright surfaces (b), or be covered by sunflecks (c). Photo taken at UC Berkeley campus. (B) Natural habitat of *E. tiaratum*, lowland Mesophyll forest of Polly Creek, Garradunga, Queensland, Australia (Photo courtesy of Jack W. Hasenpusch). Inset, a newly hatched first-instar *E. tiaratum*. 
Fig. 2. The spatial setups of two experimental arenas. (A) The configuration of Arena 1, demonstrated with setup of parallel color stripes. (B) Arena 1 in top view. Letters denote five stripe positions. (C) Schematic demonstration of the calculations of pairwise contrast $C_p$ and background contrast $C_b$. (D) Arena 2 in semisectional view. Two vertical stripes represent landing targets. (E) Arena 2 in top view. Red bars denote the positions of landing targets.
Fig. 3. Single stripes with well-defined edges attract *E. tiaratum* landing. (A) Pattern designs for single-stripe experiments in Arena 1. (B) Pattern designs for contrast edge continuity experiments in Arena 2. (C) Pattern designs contrast sharpness experiments in Arena 2. All patterns are shown as sample sections trimmed from the original (height 3.5 m). (D) Landing frequencies on different single stripe patterns, corresponding with (A) and (B). Dash line denotes null frequency 6.0%. Asterisk symbols indicate landing frequencies that are significantly greater than null predictions (*P <0.0001*; also see Tables S1, S2). (E) Comparison of the angular distribution of landing points in one run with pattern CS1 and control (also see Table S3). Sample sizes (the number of landings and individuals, respectively) in parentheses.
**Fig. 4. E. tiastratum nymphs preferred to land on black stripes of median sizes.** Each subplot is a summary of the design and results for one trial of parallel stripe experiment: (Top) A schematic diagram of the visual environment in top view, as installed in Arena 2. (Middle) The distribution of landing points summarized within a representative interval defined by the midlines of two adjacent surfaces, as indicated by the gray sector in schematic diagram. Sample sizes (the number of landings and individuals, respectively) in parentheses. Red dot denotes mean angular position. Longer error bars denote 25th and 75th percentiles, and shorter error bars denote 10th and 90th percentiles. (Bottom) Landing frequencies on black and white surfaces. Red dash lines indicates null frequencies. Asterisk symbol denotes trials with frequencies significantly different from the null expectations (also see Table S4).
Fig. 5. *E. tiaratum* nymphs landed on median sized stripes and avoided wide stripes. Landing preference on black and white surfaces plotted against log-transformed stripe width. Landing preference is represented by the differential between observed landing frequency and the null frequency ($\Delta f = f_{\text{observed}} - f_{\text{null}}$). Each subplot summarizes results from three trials with the same proportions of black and white surfaces in parallel stripe experiments. Dots representing data of the same trials are connected by dash lines. Two shades highlight the range of stripe size on which significant landing preferences were observed. Error bars denote s.e.m.
Fig. 6. *E. tiaratum* nymphs were more attracted by luminance contrasts than by chromatic contrasts in parallel stripe experiments. (A)-(B) Landing frequencies in gray shade and color trials, respectively. Values are mean±s.e.m. Same letters indicate the lack of difference between means. Bk, black; Dk, dark gray; Gy, gray; Wh, white; Rd, red; Yl, yellow; Bu, blue; Gr, green. (C) The correlations between the count of landing and the magnitude of pairwise contrast (|C_p|) in gray shade trials. Significant correlation is associated with darker stripes (also see Table 1). (D) The negative correlation between the count of landing and background contrast in gray shade trials (also see Table 1). Values are mean±s.e.m.
Fig. 7. *E. tiaratum* nymphs were not attracted by any particular chromatic contrast. Landing frequencies associated with pairwise chromatic contrasts in parallel color stripe trials. Dots demonstrate the color pairs. Values are mean±s.e.m. Horizontal bars indicate the lack of difference between means.