

RESEARCH ARTICLE

Canopy parkour: movement ecology of post-hatch dispersal in a gliding nymphal stick insect, *Extatosoma tiaratum*

Yu Zeng^{1,2,*}, Sofia W. Chang¹, Janelle Y. Williams¹, Lynn Y.-Nhi Nguyen¹, Jia Tang¹, Grisanu Naing³, Chandni Kazi¹ and Robert Dudley^{1,4}

ABSTRACT

For flightless arboreal arthropods, moving from the understory into tree canopies is cognitively and energetically challenging because vegetational structures present complex three-dimensional landscapes with substantial gaps. Predation risk and wind-induced perturbations in the canopy may further impede the movement process. In the Australian stick insect *Extatosoma tiaratum*, first-instar nymphs hatch on the forest floor and disperse toward tree canopies in the daytime. Here, we addressed how their tactic responses to environmental cues and movement strategies are adapted to the canopy environment. Newly hatched nymphs ascend with high endurance, travelling >100 m within 60 min. Navigation toward open canopies is underpinned by negative gravitaxis, positive phototaxis and visual responses to vertically oriented contrast patterns. Nymphal *E. tiaratum* also use directed jumping to cross gaps, and respond to tactile stimulation and potential threat with a self-dropping reflex, resulting in aerial descent. Post-hatch dispersal in *E. tiaratum* thus consists of visually mediated displacement both on vegetational structures and in the air; within the latter context, gliding is then an effective mechanism enabling recovery after predator- and perturbation-induced descent. These results further support the importance of a diurnal niche, in addition to the arboreal spatial niche, in the evolution of gliding in wingless arboreal invertebrates.

KEY WORDS: Arboreal, Climbing, Invertebrate, Jumping, Ontogeny, Tactic behavior

INTRODUCTION

Flightless nymphal and adult arthropods compose the majority of arboreal invertebrate fauna (Basset, 2001; Basset et al., 2012); these taxa locomote on vegetational structures (e.g. tree trunks, branches, lianas and leaves) for various behaviors including locating resources, dispersal and homing after dislocation (Cunha and Vieira, 2002; Basset et al., 2003; Yoshida and Hijii, 2005). Nevertheless, legged movements by small invertebrates can be challenged by the three-dimensional landscape and dynamic environment of the canopy space. Irregular habitat formed by different vegetational structures (e.g. foliage, branches and stems) precludes straight displacement, and may also obstruct directed aerial movement within canopy gaps. Complexity and dynamics of


the physical environment can also impede navigational processes and locomotor performance. Diverse vegetational structures form heterogeneous visual environments under sunlight, with sunflecks and shadows projected dynamically onto various surfaces (Evans and Coombe, 1959). The visual environment thus changes with time and with the observer's perspective, potentially compromising the use of long-range visual cues. In addition, vertical movement between the forest floor and canopies can be energetically costly given the relatively long distances (e.g. forest canopy height ranges globally from 8 to 40 m; Simard et al., 2011). For ascent in particular, the efficiency of transport largely depends on the animal's endurance capacity (e.g. the duration of movement at a given speed; Full, 1987; Garland and Else, 1987). Endurance can also be influenced by environmental temperature (e.g. Full and Tullis, 1990), and the daily cycle of temperature in forests (e.g. ~10° C day–night difference in Amazonian rainforest; Shuttleworth, 1985) may constrain the timing and extent of activity. Exposure to predators and parasitoids, many of which are specialized for either searching for or ambushing targets on vegetation (e.g. insects, spiders and birds; Southwood et al., 1982; Russell-Smith and Stork, 1994; Ford et al., 1986; Basset, 2001) may also threaten invertebrates' movement through the canopy space. In addition to biological threats, environmental perturbations (e.g. rain and wind gusts; see McCay, 2003) can interfere with directed movements. Overall, arboreal transport by small invertebrates can be sensorily and energetically challenging, and such demand may be particularly strong in vertical displacement given the concomitant variation of microhabitats and ecological communities (see Erwin, 1988; Shuttleworth, 1985).

Despite such spatiotemporal variation in the forest environment, both gravity and sunlight can serve as useful long-range cues in canopy space. Terrestrial arthropods generally perceive gravity with gravireceptors and integrate it with other signals (e.g. vision) for navigation (Büschges et al., 2001; Brockmann and Robinson, 2007; Robie et al., 2010). Also, spatial light gradients may be used to infer sun direction in the presence of foliar cover (Bjorkman and Ludlow, 1972; Endler, 1993; Wolken, 1995), with the strongest signal and lowest temporal variation occurring at midday (i.e. a peak plateau of solar altitude with <5 deg variation between 11:00 h and 13:00 h). In addition, vegetational structures under sunlight likely provide short-range visual cues for spatial discrimination. For example, insects identify contrast edges in landing response (Kern et al., 1997; Zeng et al., 2015). The use of gravitational and visual cues is potentially more general than chemical cues, which generally depend on a strong source (e.g. odor from flowers) or permanence (e.g. pheromone trails used by ants; Jackson et al., 2007).

Arboreal invertebrates exhibit various locomotor behaviors within canopy space. Jumping is a common and energetically efficient strategy for crossing air gaps (designated 'gaps' hereafter; see Graham and Socha, 2019), as shown in many wingless

¹Department of Integrative Biology, University of California, Berkeley, CA 94720, USA. ²Schmid College of Science and Technology, Chapman University, Orange, CA 92866, USA. ³Department of Earth and Planetary Science, University of California, Berkeley, CA 94720-4767, USA. ⁴Smithsonian Tropical Research Institute, Balboa, Republic of Panama.

*Author for correspondence (dreavoniz@berkeley.edu)

 Y.Z., 0000-0002-2651-227X; J.Y.W., 0000-0002-4078-4159

arthropods (e.g. spiders, nymphal mantids and ants; Parry and Brown, 1959; Burrows et al., 2015; Ye et al., 2020). Accidental loss of foothold (e.g. via perturbation by wind or predators) and defensive self-dropping (e.g. startle response: Haemig, 1997; Humphreys and Ruxton, 2019; Dudley and Yanoviak, 2011), along with failed landings after jumping, can transition to directed aerial descent (hereafter termed ‘gliding’; see Dudley et al., 2007; Socha et al., 2015), which reduces height loss (see Dudley and Yanoviak, 2011; Yanoviak et al., 2011). An arboreal spatial niche may not entirely underpin the evolution of gliding. For tree-dwelling invertebrate taxa, the diurnal niche permits visually mediated aerial maneuvers and landing, which may be essential for the evolution of gliding (Yanoviak et al., 2011; Zeng et al., 2015). Studying the ecology of movement in canopy space, and also initiation mechanisms for aerial descent, can thus help to address the utility of gliding within specific ecological contexts.

The newly hatched nymphs of the Australian stick insect *Extatosoma tiaratum* (Macleay 1826) disperse by ascending from the forest floor, where eggs are deposited, by climbing vegetation into the canopy (Fig. 1A,B). In contrast to the typically nocturnal lifestyle of phasmids, *E. tiaratum* nymphs hatch during the middle of the day (i.e. 11:00 h to 15:00 h) in the rainy season (November to January; Carlberg, 1981, 1983, 1984a,b; Brock and Hasenpusch, 2009; Brock, 2001), and immediately start to rapidly move. The nymphs also possess ant-mimicking coloration and exhibit comparable behaviors (e.g. body shaking during crawling; Fig. 1; Movie 1) while climbing and exploring their surroundings (Carlberg, 1981, 1983; Rentz, 1996; Brock, 2001). Ant mimicry (i.e. myrmecomorphy) presumably facilitates dispersal by deceiving visual predators (e.g. birds and small reptiles). These traits collectively form a disperser’s syndrome (Ronc and Clobert,

2012) that potentially facilitates ascent and the search for suitable post-hatch microhabitats. The hatchlings can also glide to reduce height loss if falling (see Zeng et al., 2015). Such post-hatch dispersal behavior is exhibited only during the first 3–5 days after hatching, following which the nymphs become nocturnally active and lose their ant-mimicking morphology (Fig. 1B) (Brock, 2001, 1999).

Here, we evaluated movement ecology (Nathan et al., 2008) and canopy dispersal in newly hatched *E. tiaratum* (subspecies *E. tiaratum tiaratum* from north Queensland; see Brock, 2001) under controlled laboratory conditions, focusing on their navigational and locomotor mechanisms for traveling through the canopy. We hypothesized that: (1) dispersing nymphs use gravity, light gradients and visual contrast to navigate toward vegetational structures, which are then used for ascent; (2) newly hatched nymphs are capable of considerable vertical ascent; (3) when dispersing, newly hatched nymphs can also initiate aerial descent both volitionally and in response to tactile perturbation, leading to aerial righting and gliding (Zeng et al., 2015, 2017); and (4) jumping is adopted by nymphal stick insects for negotiating gaps, a strategy found in both wingless and winged phasmid species (Burrows and Morris, 2002; Burrows, 2008). Displacement by individual dispersing insects within vegetational structures across gaps derives from both locomotor capacity and sensory responses (Fig. 1C), and can explain both movement ecology of small wingless invertebrates in canopy space and the ecological utility of gliding within the canopy.

MATERIALS AND METHODS

Experimental insect husbandry

Eggs of *E. tiaratum* were incubated on vermiculite substratum at ~70% humidity and 25–27°C air temperature. Newly hatched

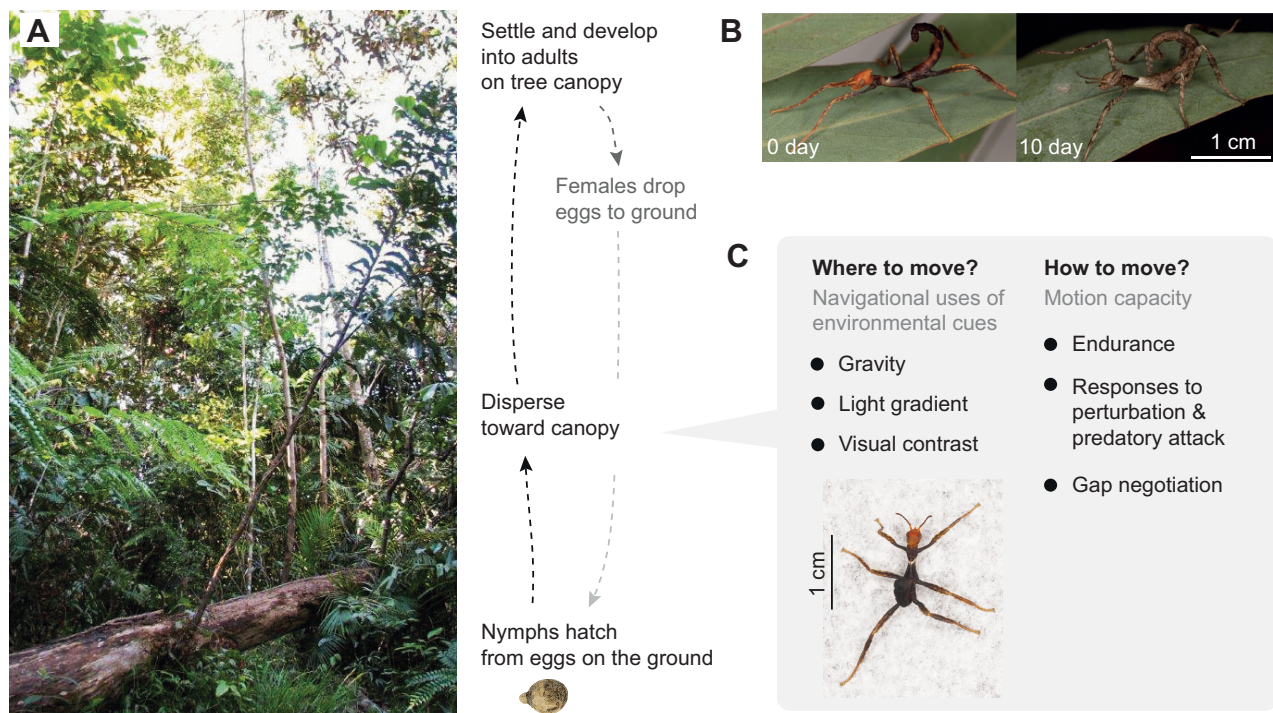


Fig. 1. Post-hatch dispersal in Macleay’s spectre stick insect, *Extatosoma tiaratum*. (A) Left: natural habitat of *E. tiaratum*, illustrating the spatial complexity and vertically varying light environment. Photo was taken in lowland mesophyll forest, Polly Creek, Garradunga, QLD, Australia (courtesy of Jack W. Hasenpusch). Right: schematic summary of *E. tiaratum* life cycle, showing the transient spatial niche of newly hatched nymphs which ascend from the forest floor to tree canopies. (B) Loss of ant-mimicking coloration during the first 3–5 days after hatching (see also Fig. S1). (C) The two main categories of questions in this study, structured following a ‘movement ecology paradigm’ (see Nathan et al., 2008).

nymphs were collected every 12 h and were maintained in clear plastic cups (354 ml) with caps. Nymphs were provided with fresh leaves of Himalayan blackberry (*Rubus armeniacus*) from the day of hatching, and were lightly sprayed with water every 2–3 days. Cups were kept in an environmentally controlled room with a 12 h:12 h light:dark cycle. All experiments were conducted at air temperatures of 25–27°C. The experimenter first collected the insect from the housing cup by letting it climb onto a small wooden rod (diameter ~0.5 cm, length ~20 cm), and then oriented the rod toward the entrance of the experimental setup to allow the insect to enter.

Negative gravitaxis

A T-maze within a uniformly lit chamber was used to evaluate negative gravitaxis. A T-shaped system of wooden rods (diameter, 9 mm), consisting of a horizontal entrance beam and a vertical beam to evaluate binary choice, was placed in the center of the experimental chamber (40×40×40 cm), which was covered with white felt and surrounded by two natural-spectrum bulbs (R30FF100VLX, Verilux Inc., Waitsfield, VT, USA) with voltage control (Fig. 2A; Fig. S2). Luminance at the center of the chamber was measured with a light meter oriented horizontally (Model #401025, Extech, Waltham, MA, USA; spectral range: 400–740 nm). In each trial, the experimenter first released the insect onto the horizontal beam through the entrance window (2×2 cm), and then observed through an observation window (1.5×1.5 cm) for the insect's movement toward the intersection of T-maze and its subsequent directional choice. The insect's directional choice was indicated by its final position when reaching either end of the

vertical beam. A minimum of 3 min resting time was given between trials both within and among individuals. Experiments were conducted for three age groups (0–1, 6–7 and 12–14 days old) under four environmental luminances (0, 10, 600 and 3000 lx, adjusted using the voltage controller). Each combination of age and luminance was tested with 10–26 individuals, using 4–6 trials per individual. A repeated *G*-test (McDonald, 2015) was used to test frequencies of occurrence of negative gravitaxis (f_g) relative to a null frequency of 0.5. A Poisson GLMM was used to test whether counts of ascent responses were significantly correlated with environmental luminance and age, using individuals as random factors.

Phototaxis

A T-maze was used to test phototactic response of insects. The T-maze consisted of two tunnel sections (section 1: diameter 2 cm, length 20 cm; section 2: diameter 4.5 cm, length 10 cm); the interior of both sections was covered with black fabric to reduce light reflection (Fig. 3A). A natural-spectrum bulb (R30FF100VLX, Verilux Inc.) with a voltage controller was placed ~5 cm away from each exit of the T-maze, with a paper screen (5×5 cm) positioned vertically 3 cm distant from each exit. Luminance was measured with a light meter (401025, Extech; spectral range: 400–740 nm) at two exits. Three luminance contrasts (0 lx versus 3 lx, 100 lx versus 500 lx, and 1×10^4 lx versus 2×10^4 lx) were tested, a range which covers the luminance variation between forest understories and canopies (e.g. ~600 lx to 2.4×10^4 lx; Bjorkman and Ludlow, 1972; Percy, 1983; Lee, 1987). Each luminance contrast was tested for insects of three ages (0–1, 5 and 10 days old), each represented by

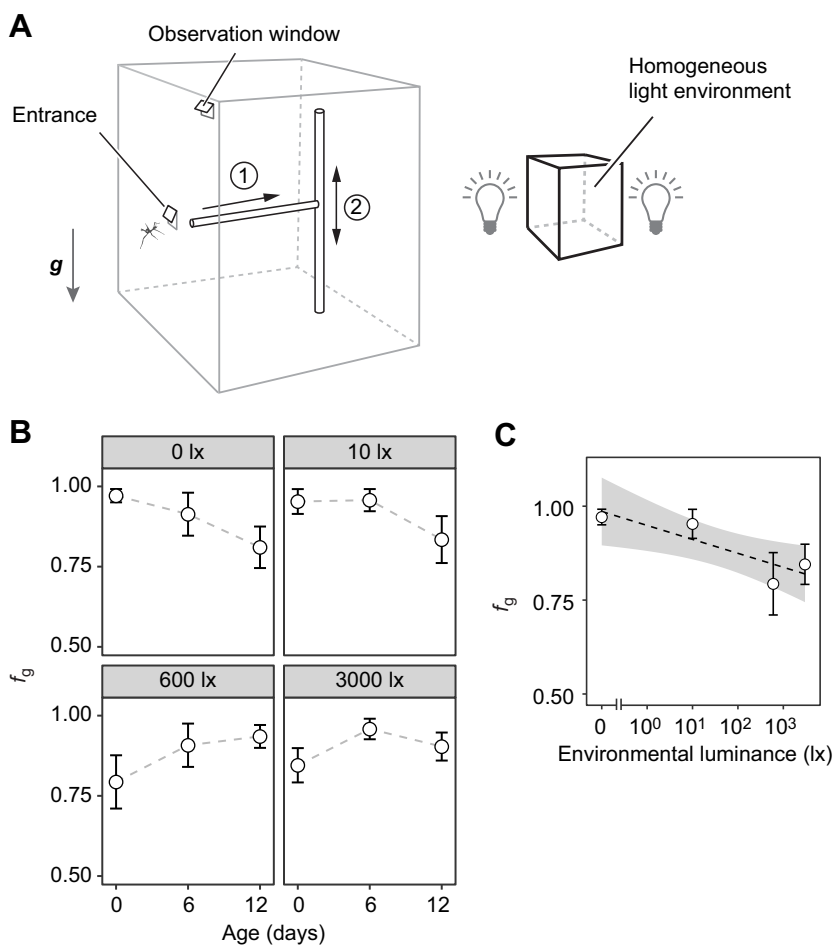


Fig. 2. Nymphal *E. tiaratum* exhibits negative gravitaxis under various environmental luminances. (A) Schematic demonstration of the experimental setup, comprising a vertically oriented T-maze made of two rods installed within a cubic chamber covered with white felt, surrounded by voltage-controlled light bulbs for homogeneous luminance (right). An entrance (2×2 cm) on the chamber wall was opened for the horizontal portion of the T-maze, and an observational window (2×2 cm) was opened at the chamber top. Experimenters first released the insect to the end of the horizontal rod (step 1), and then observed the directional choice of the insect (step 2). (B) Ascent frequency (f_g) versus age under various levels of environmental luminance, showing a general negative gravitaxis throughout the first instar. Ontogenetic decline of ascent frequency was found in total darkness (0 lx). Values represent means±s.e.m. All frequencies are significantly different from the null predictions ($P < 0.001$, repeated *G*-test). See Materials and Methods for sample sizes. (C) In newly hatched (0 day old) nymphs, ascent frequency was inversely correlated with environmental luminance (Poisson regression coefficient = -0.0013 ± 0.0002 , $P < 0.0001$, Poisson GLMM). Values represent means±s.e.m. Trend line represents linear regression model, with shade representing 95% CI. See Table S1 for more details.

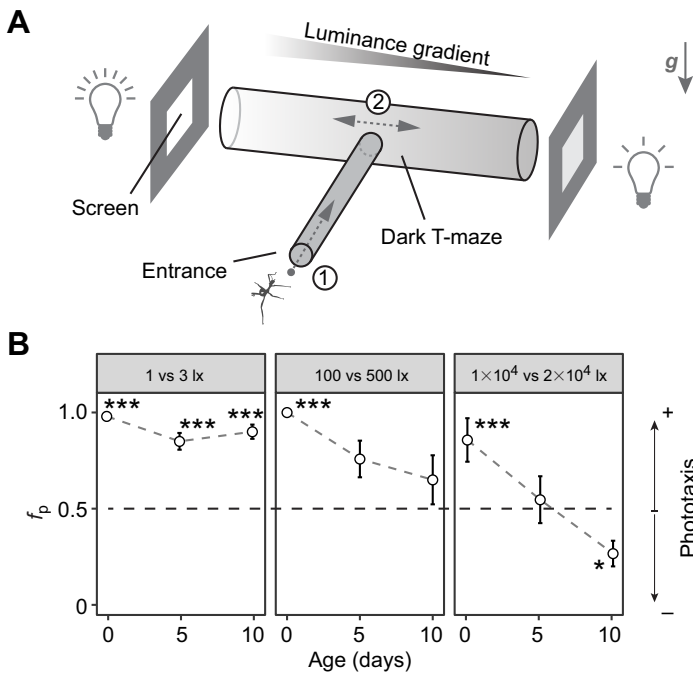


Fig. 3. Nymphal *E. tiaratum* exhibits positive phototaxis. (A) Schematic depiction of the experimental setup in a dark room, consisting of a T-maze made of two dark tunnels with a luminance gradient at the intersection. Experimenters first released the insect at the entrance (step 1), and then recorded its directional choice within the luminance gradient (step 2). (B) Ontogenetic variation of the phototactic response under different luminance gradients. Values represent the frequency of phototactic movements (f_p ; means \pm s.e.m.). Asterisks denote the significance level of results based on repeated *G*-tests: * $P < 0.05$; *** $P < 0.001$. In older nymphs, the frequency of the phototactic response was inversely correlated with average luminance (5 days old, $P < 0.05$; 10 days old, $P < 0.01$; Poisson GLMM). See Materials and Methods for sample sizes. See Table S1 for details.

5–20 individuals (with 5–6 trials per individual). During each trial, the experimenter first released the insect into the entrance, immediately covered the entrance with a lid and then recorded the insect's final directional choice within the light gradient, as indicated by exit from either of the two tunnels. Control experiments were conducted with one 0–1 day old group in complete darkness, for which an infrared video camera (SONY HDR-HC7) was used to observe the insects' directional choice. A repeated *G*-test was used to analyze the significance of directional bias relative to the null frequencies (i.e. 1:1). Poisson generalized linear mixed models (GLMM) were used to test the correlation between counts of phototactic responses and environmental luminance, using individuals as random factors.

Tactic responses to visual contrasts

Directional choices were examined using a cylindrical arena (height 35 cm, diameter 26 cm) decorated internally with vertically oriented contrast patterns. The patterns were made with felt sheets using shades of black, gray and white (average reflectance over 300–550 nm: black, 3%; gray, 38%; white, 47%), as measured with a spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA; see Zeng et al., 2015). A natural spectrum light bulb (R30FF75VLX, Verilux Inc.) was placed on top of the arena and provided ~ 600 lx luminance at the arena floor (as measured with a light meter, Extech 401025) (Fig. 4A; Fig. S3).

Five contrast patterns were used to test directional preferences to contrast strength and stripe size. The patterns formed by black and white surfaces were coded as BW11, BW91 and BW19, where the embedded numbers represent the relative proportions of black (B) and white (W) surfaces. In BW91 and BW19, the narrow stripes feature a spatial frequency of ~ 3.18 cycles rad^{-1} (as viewed from the arena's center). Similarly, two patterns formed by gray surfaces paired with black or white surfaces in equal proportions were coded as BG11 and GW11. The effect of contrast strength on the insect's tactic response was tested using BW11, BG11 and GW11, and the effect of stripe size was tested using BW11, BW19 and BW91. Each contrast pattern was tested using 19–38 individuals, with 5 trials per individual.

In each experimental trial, the insect was released through an entrance (diameter 2 cm) at the center of the arena floor, and was observed from above (Movie 1). Three temporal landmarks were recorded: (1) entrance into the arena; (2) moment of arrival at the arena's wall; and (3) initiation of climbing. The insect's directional preference was represented by the angular distance between the point where the insect first reached the arena wall and the nearest contrast edge. Circular directionality of the insects' directional choice was analyzed using 'CircStats' package in R software (<https://CRAN.R-project.org/package=CircStats>; <http://www.R-project.org/>), which calculates means and confidence intervals of direction θ and concentration κ based on von Mises maximum likelihood estimates. Repeated *G*-tests were used to test directional preference for the darker and the lighter surfaces in each configuration against null proportions, which were derived based on random association with corresponding areal proportions of different surface patterns. In BW19, for example, the null frequency for an insect walking to the black surface is 0.1. Durations between temporal landmarks, and also the average speed of movement on the arena floor, were compared using repeated-measures ANOVA. When examining the insect's preference for narrow surfaces in BW19 and BW91, we tested whether the frequency of moving toward the narrow surface was associated with contrast edges but not surface size. Thus, the frequency of moving to the narrow surface (either black or white) equals the frequency associated with the corresponding shade in BW11. Based on results with pattern BW11 (Table S1), the null frequency is thus 0.57 for BW19 and 0.43 for BW91.

Ontogenetic decline in ascent endurance

We recorded ascent movements of insects moving on a vertically oriented treadmill (width 2 cm, height 28 cm), which was connected to a speed controller and placed in a dark room (Fig. 5A; Movie 1). A light source was placed above the treadmill to phototactically motivate the insects. A reference disc with visual marks was attached to the top shaft of the treadmill to indirectly indicate treadmill speed. A mirror was placed adjacent to the treadmill at 45 deg relative to the plane of the treadmill belt,

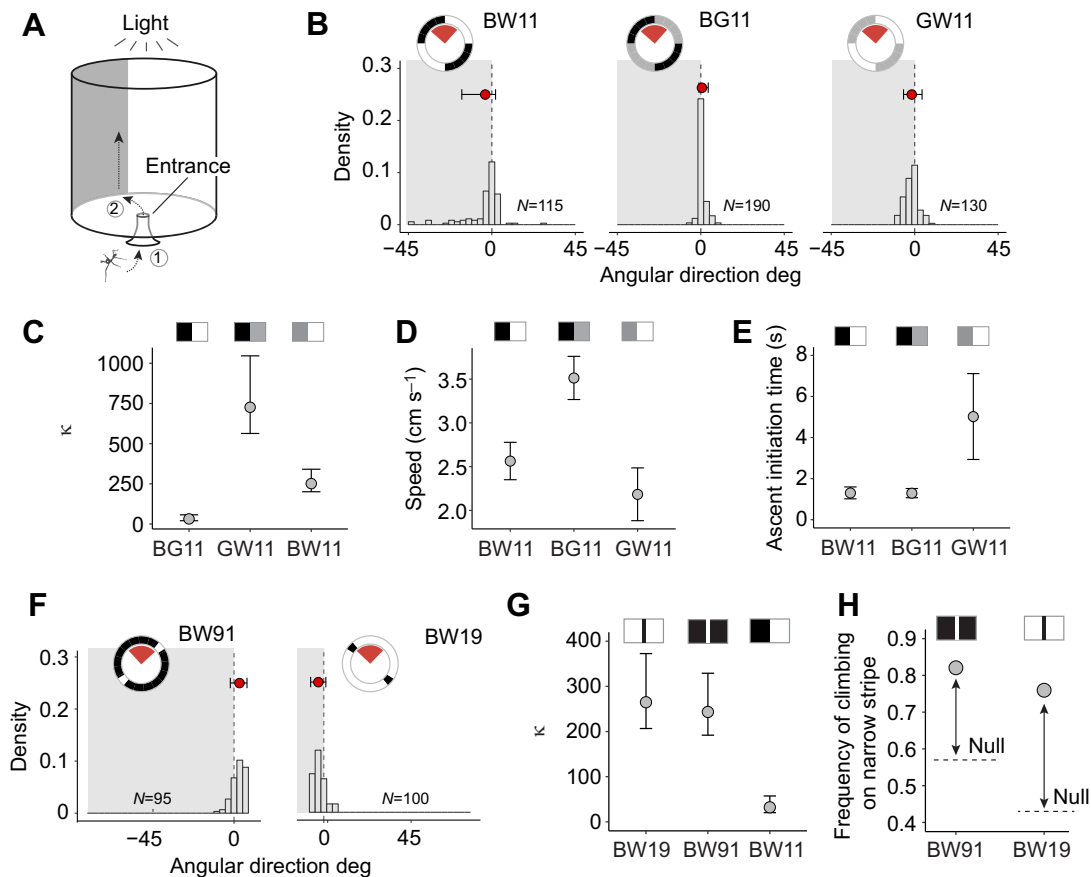


Fig. 4. Newly hatched nymphal *E. tiaratum* are attracted by vertically oriented visual contrasts. (A) Schematic illustration of visual arena, the inner wall of which was lined with different contrast patterns (BW, BG, GW: see Materials and Methods). Experimenters first released the insect into the arena entrance (step 1), and then observed its behavior until it reached the arena wall (step 2). (B,C) Summary of directional choices for visual contrasts formed between surfaces of equal size. Subplots in B summarize directional preferences with respect to the contrast edge, with histograms illustrating the distribution of movement direction. Insets are schematic diagrams of the visual arena from the top view, with the red sectors showing plotted angular ranges. Red dots denote mean angular position, with error bars showing 10th and 90th percentiles. Movement direction exhibited the greatest concentration with a black–gray contrast (BG11), as shown by comparison of the concentration parameter κ (means \pm 95% confidence interval, CI) in C. (D) Comparison of mean speed of movement toward the arena wall with different contrast patterns. The insects moved with fastest speed when exposed to the black–gray contrast. Values are significantly different among trials (repeated-measures ANOVA, $F_{2,27}=5.09$, $P<0.05$). (E) Comparison of ascent initiation time (the period between reaching the arena wall and initiating ascent) with different contrast patterns. Insects exhibited a significant delay when exposed to the gray–white contrast (GW11); values are significantly different between trials ($F_{2,27}=3.43$, $P<0.05$, repeated-measures ANOVA). For D and E, values represent means \pm s.e.m. (F–H) Summary of directional choices for contrasts formed between surfaces of different sizes. Insects exhibited preference for the narrower surface regardless of its brightness, as shown by a higher concentration parameter κ (means \pm 95% CI) relative to contrast formed between equal-sized surfaces (G) and significantly greater frequencies relative to the null (H). For B and F, the number of trials is shown in parentheses. See also Movie 1 and Tables S1–S3. See Materials and Methods for sample sizes.

thus providing an image of the climbing insect in dorsal view. The mirror image and reference disc were filmed using a digital video camera (25 frames s⁻¹; HDR-XR160, Sony Corporation). For each trial, the experimenter first released the insect onto the track and then maintained the insect's position within the live video feed by manually adjusting treadmill speed. Nymphs of six ages were used (2, 4, 48, 96, 120 and 240 h old; see Table S1); each individual was filmed for 60 min. Motion-tracking software (ProAnalyst, Xcitex, Woburn, MA, USA) was used to track the instantaneous position of the insect and the orientation of the reference disc. Custom-written MatLab scripts were then used to calculate ascent speed as a function of time. Treadmill speed was calculated as:

$$U_t = \omega R, \quad (1)$$

where ω is angular speed of the reference disc and R is the radius of treadmill shaft. The ascent speed of the insect was then

calculated as:

$$U = U_t + U_i, \quad (2)$$

where U_i is the insect's vertical speed as derived from the camera's view. Given varying and age-specific climbing speeds and intermittent pauses, we used percent ascent time and total ascent height to characterize the insects' endurance capacity.

Self-dropping behavior

Simulated predatory attacks and induction of accidental loss of foothold (as a proxy for environmental perturbations) were applied to experimental insects ascending on a vertically oriented cardboard sheet (width 15 cm, height 35 cm) placed beneath a light source. Experimental insects were first released near the bottom of the sheet, and were allowed to ascend under volitional phototaxis and negative gravitaxis. To simulate predatory attacks, a compressed cylinder of paper towel (diameter \sim 5 mm) was held against the insect's tibial

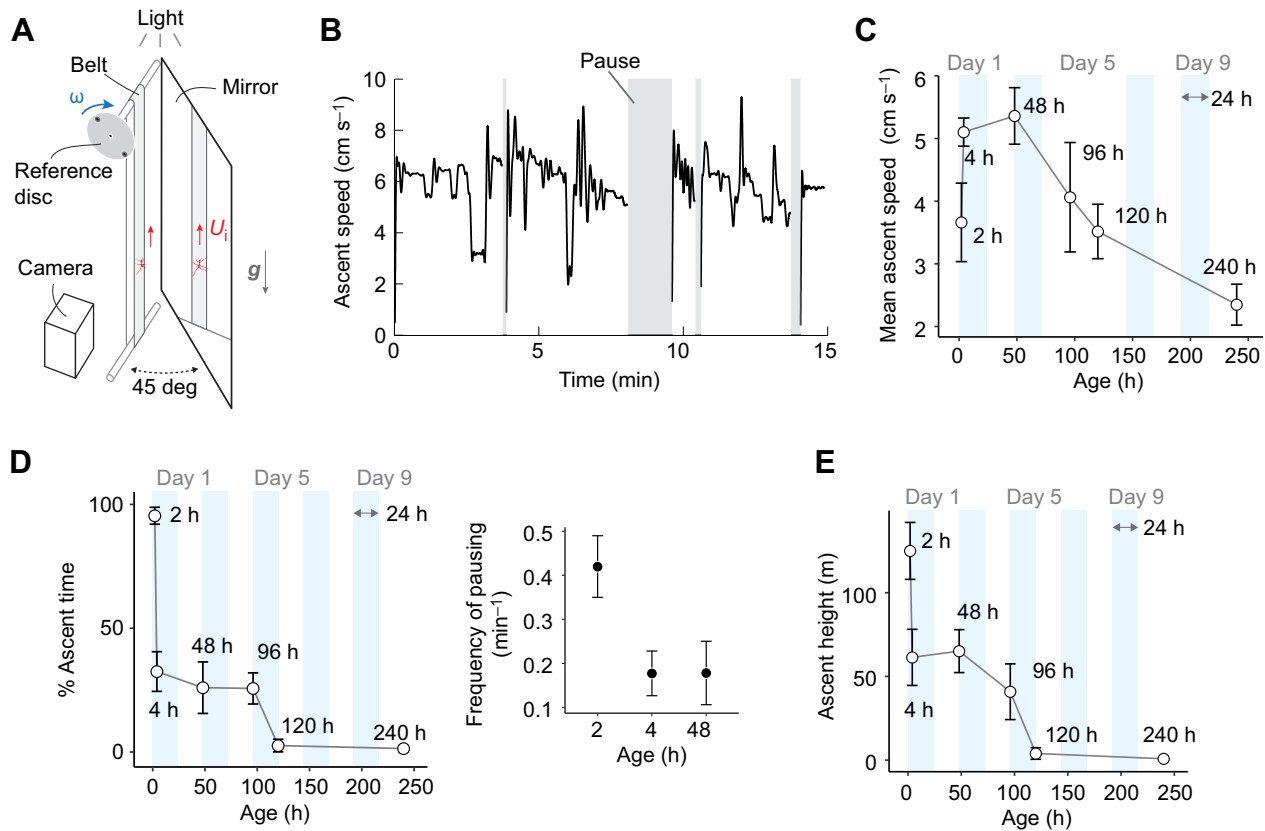


Fig. 5. Ontogeny of ascent behavior in nymphal *E. tiaratum*. (A) Schematic diagram of the experimental setup, illustrating a vertically oriented treadmill with a light source on the top. The mirror image of the ascending insect was maintained in camera view via manual speed control by the experimenter. The ascent speed was calculated based on treadmill belt speed, U_t (as indicated by the angular speed ω of the reference disc), and the insect's speed in the camera's view, U_i (see Movie 1). (B) A representative speed profile, showing active ascent movements with intermittent pauses. (C) Ontogenetic variation in ascent speed. Mean speeds are significantly different between age groups (one-way ANOVA, $F_{5,20}=18.4$, $P<0.001$). (D) The temporal percentage of active ascent relative to the total time, showing a sharp reduction after 2 h. Inset shows a significantly greater frequency of pausing in newly hatched (2 h) relative to older nymphs. (E) Comparison of total ascent height within 60 min between different age groups, showing significant inter-group differences (one-way ANOVA, $F_{5,18}=6.14$, $P<0.01$). For B–E, values represent means \pm s.d. See also Table S1.

and tarsal segments for any given leg, pinning it against the substrate for ~ 20 ms without causing injury (Fig. 6A; Movie 2). The experiment was conducted on nymphs of three age groups (0, 6 and 12 days old), using 10–15 individuals per group. Each individual was tested in five trials; in each trial, each leg pair received two simulated attacks, with a waiting period of 10 s between consecutive attacks. To induce accidental loss of foothold, a Teflon-coated surface (~ 2 cm wide) was introduced onto the cardboard sheet (Fig. 6C). The insects' response to contact with this slippery surface was recorded for nymphs of three age groups (0, 5 and 10 days old), using 7 individuals per group.

Jumping behavior for crossing gaps

We evaluated jumping behavior in nymphs by allowing them to ascend to an elevated platform surrounded by distant visual targets such that jumping would be required to cross the gap to any given target. The platform (2 cm \times 5 cm) was mounted atop a vertically oriented rod and ~ 50 cm above the experimental chamber's floor and >40 cm away from its walls. The floor and walls were covered with white felt, and environmental luminance was ~ 600 lx. Two landing targets were used: (1) a horizontally oriented rod (diameter of 5 mm) wrapped with black felt and positioned 20 cm below the platform, with a white background ~ 30 cm below the platform (Fig. 7A); and (2) a vertically oriented rod, also wrapped with black felt, positioned ~ 8 cm away from the platform (Fig. 7A). For each of

these two configurations, jumping behavior was tested for nymphs of three age groups (0, 6 and 12 days old; horizontal target, 6–9 individuals per age group and 5 trials per individual; vertical target, 5–9 individuals per age group and 2–4 trials per individual). In each trial, the experimenter released the insect onto the middle of the vertical rod and let it ascend to the platform. If the insect stopped moving, the experimenter gently tapped the vertical rod to stimulate the insect upward. The experimenter then observed the insect's response for 4 min after it ascended to the platform. If the insect climbed down the vertical rod, the experimenter would restart the trial by letting the insect walk onto a small rod, and then reversed the rod to allow the insect to walk back to the vertical rod in a head-up orientation. Once on the platform, the insects could descend, rest or jump, with transitions between cursorial and exploratory movements (Fig. 7B; see Results). The ensuing behavior sequences were coded accordingly, whereby repeated descent was treated as a single action. A minimum of 2 min resting period was provided between trials. Control experiments were conducted in five 0 day old individuals following the same protocols, but without any visual landing targets.

RESULTS

Negative gravitaxis

After being released into a vertically oriented T-maze within a visually homogeneous environment (Fig. 2A), nymphs moved to

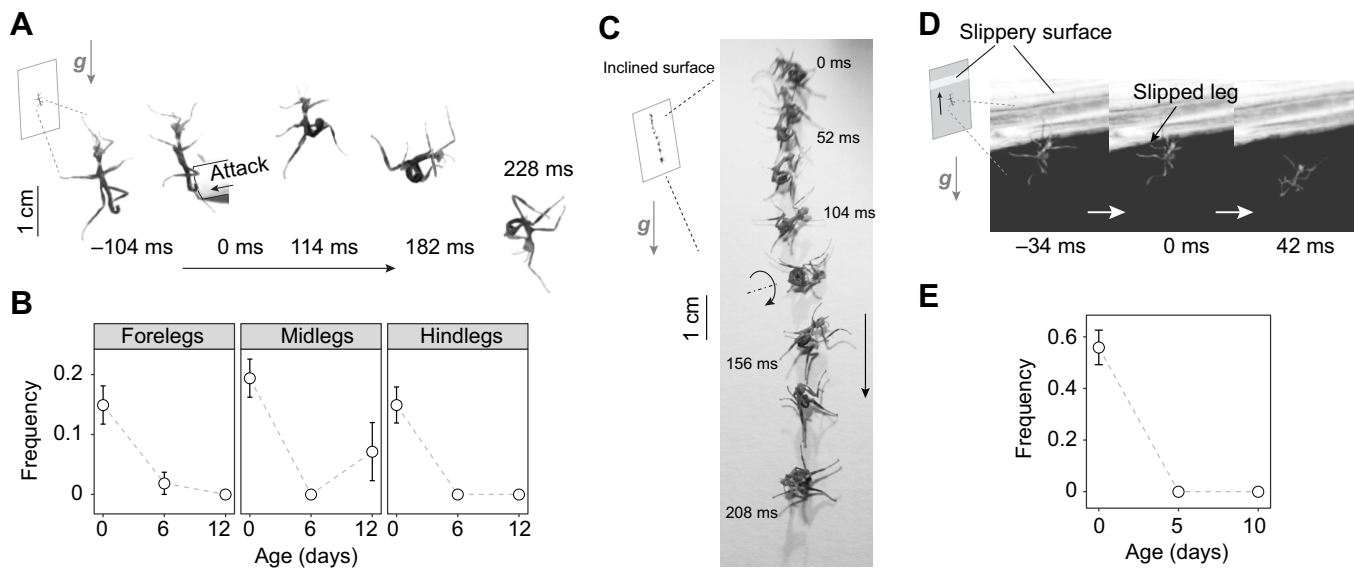


Fig. 6. Newly hatched *E. tiaratum* initiate self-dropping under tactile stimulation. (A) Sequence from a high-speed video of initiation of self-dropping in response to simulated predatory attack. (B) Newly hatched nymphs show a significantly higher frequency of self-dropping after simulated attacks. Values represent means \pm s.e.m. (0 days old, $N=14$ individuals and $N=124$ trials per leg pair; 6 days old, $N=22$ insects and $N=208$ trials per leg pair; 12 days old, $N=10$ insects and $N=100$ per leg pair). See also Movie 2 and Table S1. (C) A sample sequence of rolling immediately after self-dropping along an incline of 70 deg (mean speed ~ 42 cm s $^{-1}$). Note the dorsiflexion of body segments and tucking of legs. See also Movie 3. (D) A sample sequence of self-dropping in response to a sudden loss of foothold when contacting a slippery surface. The inset depicts the experimental setup. (E) Newly hatched nymphs show a significantly higher frequency of self-dropping after a sudden loss of foothold (frequency: 0.56 ± 0.07 ; means \pm s.e.m.; 0 days old, $N=7$ insects and $N=59$ trials; 5 days old, $N=7$ insects and $N=50$ trials; 10 days old, $N=7$ insects and $N=50$ trials). See also Movie 2.

the intersection and ascended along the vertical rod under various environmental luminances (0–3000 lx), exhibiting frequencies of gravitactic response (f_g ranges from 0.81 to 0.97) significantly different from the null ($f_g=0.5$) (Fig. 2B). When there is no light gradient in the vertical direction, gravity alone can thus be used by nymphal *E. tiaratum* as a directional cue. Furthermore, ascent frequency was inversely correlated with environmental luminance in newly hatched (0 day old) nymphs, with the greatest frequency ($f_g=0.97\pm 0.02$; mean \pm s.e.m.) occurring in total darkness and the lowest ($f_g=0.79\pm 0.08$) occurring in 600 lx (Fig. 2C).

Positive phototaxis

After being released into a T-maze of horizontal dark tunnels, nymphs moved into a controlled luminance gradient. Three luminance contrasts were used, with the mean value ranging from ~ 2 to 1.5×10^4 lx (Fig. 3A). Newly hatched nymphs (0 days old) exhibited strong phototactic displacement under all luminance conditions, with the frequency of phototactic response (f_p) >0.8 under all light conditions. In older nymphs (i.e. 5 and 10 days old), f_p declined with both age and increasing mean luminance (Fig. 3B). For example, 10 day old nymphs preferred the brighter direction under a mean luminance of 2 lx ($f_p=0.90\pm 0.09$; mean \pm s.e.m.) and tended to move toward the darker direction when mean luminance exceeded 1.5×10^4 lx ($f_p=0.27\pm 0.07$; mean \pm s.e.m.). In control experiments with no luminance gradient, insects showed no directional bias (repeated G -test: $G=0.722$, d.f.=1, $P=0.396$), supporting the hypothesis that nymphal *E. tiaratum* can use luminance gradients for directional reference.

Tactic movement toward visual contrasts

Experimental insects were released into a visual arena surrounded by vertically oriented contrast lines (Fig. 4A). When contrast patterns were formed by equally sized surfaces (black–white, black–

gray and gray–white), insects rapidly moved toward contrast lines (Movie 1), showing the strongest directional preference for a black–gray contrast. Specifically, movements toward a black–gray contrast exhibited the smallest angular deviation from the contrast line (<1 deg), and accordingly the highest concentration ($\kappa>700$) (Fig. 4B,C; Tables S2 and S3). When exposed to black–gray contrasts, they also exhibited the fastest movement speeds (3.51 ± 0.25 cm s $^{-1}$; mean \pm s.e.m.) on the arena floor, and initiated climbing immediately after reaching the wall (<2 s) (Fig. 4D,E). Black–white and gray–white contrasts were less attractive; the insects showed the slowest movements when exposed to the gray–white contrast (2.18 ± 0.3 cm s $^{-1}$; mean \pm s.e.m.). The insects showed a preference for the darker surface paired with a white surface (frequency of moving to the darker surface was 0.57 in BW11 and 0.76 in GW11), but preferred the gray surface when paired with a black surface (frequency of moving to gray surface of 0.62) (Table S1).

Furthermore, when exposed to contrast patterns formed between surfaces of different sizes, insects showed a general preference for the narrower surface independent of its brightness. For such contrast patterns formed between black and white surfaces, the frequency of moving toward the narrower surface (0.76 for BW19 and 0.82 for BW91) was significantly greater than the null expectation (Fig. 4F,G). The tactic response to vertically oriented contrast lines and preference for narrower surfaces may help in localizing vegetational structures with which to initiate ascent (see Discussion).

Ontogenetic decline of ascent endurance

We evaluated ascent behavior of nymphal *E. tiaratum* for six age groups ranging from 2 to 240 h old. Experimental insects were placed on a vertically oriented and speed-controlled treadmill, and were filmed for 60 min (Fig. 5A). Ascents on the treadmill were then digitized to generate speed profiles, consisting of intermittently

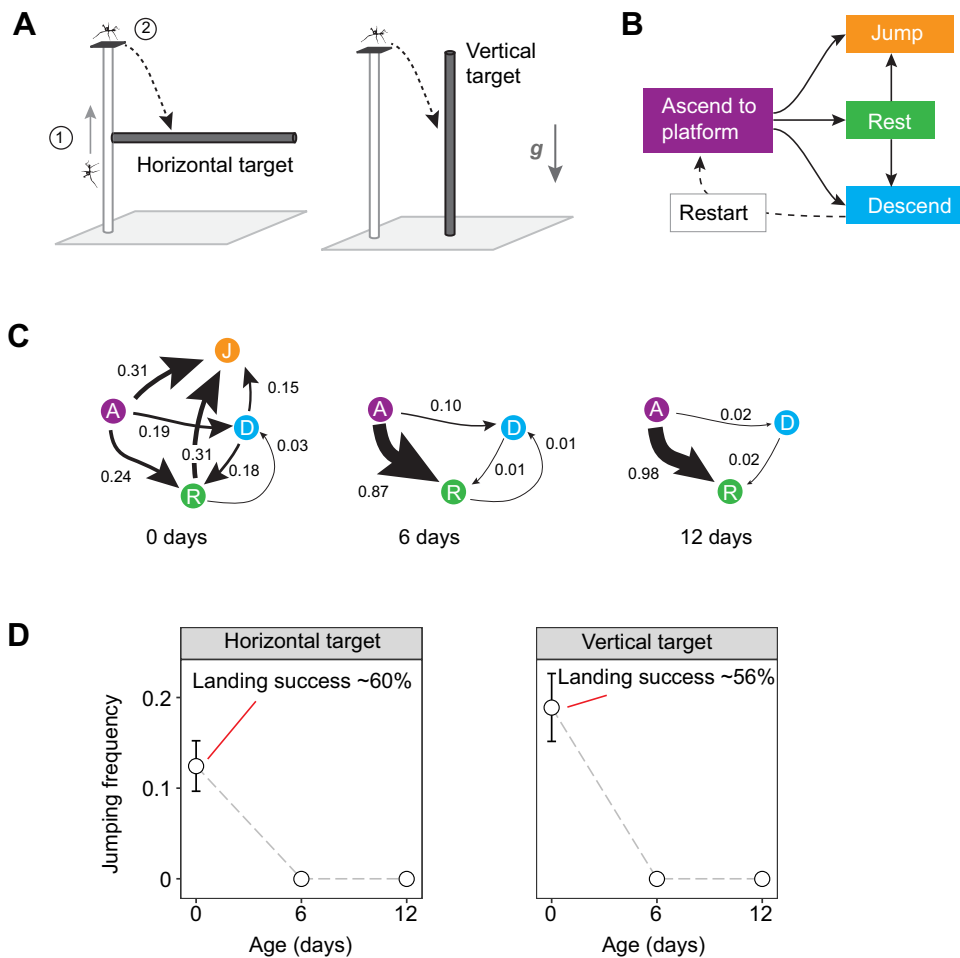


Fig. 7. Newly hatched *E. tiaratum* jump to cross gaps. (A) In each experimental trial, the insect first ascended to the jumping platform through a vertical wooden rod (step 1); its subsequent response to a distant land target (dark rod with a white background) was then recorded (step 2). (B) General behavioral sequences of the insects after ascending to the platform. Having descended, an individual insect was re-introduced to the vertical rod in a head-up orientation. (C) Comparison of behavioral sequences between insects of different ages, combined using data from the two experimental setups. The newly hatched insects exhibit jumping and frequent spatial exploration, whereas the older nymphs mainly rest after ascending to the platform. Arrow width represents behavioral transition probabilities and is annotated with probability values, calculated as the ratio between the number of occurrences and the total number of trials. A, initial ascent; D, descent; J, jump; R, rest. (D) Variation in jumping frequency versus insect age, showing a significant ontogenetic reduction in jumping behavior (frequency: 0.12 ± 0.03 for horizontal target, and 0.19 ± 0.04 for vertical target; mean \pm s.e.m.). See Results for statistical summary. See also Movie 2.

ascending and pausing intervals (Fig. 5B). Ascent speed (range $2.3\text{--}5.3\text{ cm s}^{-1}$) generally tended to decline with increasing age. The 4 and 48 h old nymphs exhibited the highest speed ($5.1\text{--}5.4\text{ cm s}^{-1}$), peaking within the first 3 days of hatching (Fig. 5C). Among insects younger than 3 days, a low ascent speed for 2 h old nymphs derived from frequent pauses ($0.42 \pm 0.07\text{ times min}^{-1}$), which is ~ 2 times greater than that of older ones (Fig. 5D). The 2 h old nymphs exhibited the greatest ascent capacity, including the greatest ascent time ($95 \pm 5\%$ active time; mean \pm s.d.) and the greatest total ascent height (125 m, or ~ 7350 body lengths); older (>5 days old) nymphs were active for <1 min ($<5\%$ active time) and otherwise remained immobile throughout the trial (Fig. 5D,E).

Predator- and perturbation-induced self-dropping

To explore mechanisms of the aerial phase initiation, experimental insects were tested with two tactile perturbations, namely simulated predatory attacks and accidental loss of foothold (see Materials and Methods), of which the latter was a proxy for environmental perturbation and initiated a perturbed descent as in previous gliding experiments (see Zeng et al., 2015). For newly hatched nymphs, simulated attacks led to self-dropping (frequency $0.15\text{--}0.19$; Fig. 6A,B). By contrast, older nymphs generally oriented away from the direction of attack (frequency of dropping $0\text{--}0.07$). High-speed filming revealed that self-dropping was initiated by voluntary withdrawal of the tarsus from the substrate within ~ 50 ms following termination of the simulated attack, followed by mid-air tucking (i.e. flexion of the tibia–femur joint and elevation of the femur) of all leg pairs ($N=30$ trials; Fig. 6A; Movie 2). Preliminary high-speed

recording showed that such leg tucking allows the insect to reduce contact with surrounding structures during descent, and to roll on a 70° incline at an average speed of $40.5 \pm 2.0\text{ cm s}^{-1}$ (mean \pm s.d.; $N=2$ trials; ~ 24 body lengths per second; Fig. 6C; Movie 3). This strategy may allow rapid descent through the clustered space of foliage under natural conditions (see Discussion). Slippery surfaces were used to induce loss of foothold during ascent, such that experimental insects were subjected to an unexpected sudden imbalance and loss of foothold (Fig. 6D). Newly hatched nymphs (0 days old) then exhibited self-dropping behavior at a frequency of 0.56 ± 0.07 (mean \pm s.e.m.; Fig. 6E). High-speed videos revealed that, following loss of foothold by one leg, other leg pairs showed similar flexion and tucking to that described above, leading to complete removal of tarsi from the substrate and self-dropping (Movie 2). Self-dropping after touching the slippery surface was not observed in older nymphs.

Jumping for crossing gaps

To test whether nymphal *E. tiaratum* jump to cross gaps, we placed individual nymphs at the base of an elevated platform with a distant visual target (either a horizontal rod or a vertical rod) which contrasted with a white background (Fig. 7A). After ascending to the platform, the newly hatched insects (0 days old) typically explored the platform, ultimately oriented toward the visual target, and tried to reach out using their forelegs; having failed to cross the gap using the forelegs, these insects would then jump toward the visual target. Sometimes, insects also descended via the vertical rod, in which case the experimenter would re-introduce them to the

vertical rod in an upward orientation. In other cases, the insects rested by the edge of or beneath the platform before either jumping or resting until the end of trial (Fig. 7B). Whereas newly hatched insects exhibited active exploration and jumping on the platform, older insects (i.e. 6 and 12 days old) showed no jumping, and either climbed down the vertical rod of the platform or rested in a still posture after trying to reach out with their forelegs (Fig. 7C).

In newly hatched insects, jumping was observed for 19 out of 24 tested individuals (Movie 2). When exposed to the horizontal target, insects spent 1.8 ± 0.2 min (mean \pm s.e.m., $N=28$ trials from $N=9$ individuals) before jumping and successfully landing on the target in 17 out of 28 trials (success rate $\sim 60\%$); for trials with successful landing, they traveled a mean horizontal distance of 8.2 ± 1.6 cm with a 20 cm descent (and thus with a ratio of horizontal to vertical distance of ~ 0.41) (Fig. 7D). When exposed to the vertical target, the insects spent 2.8 ± 0.3 min ($N=21$ trials from 9 individuals) before jumping, and successfully landed in 13 out of 23 trials (success rate $\sim 56\%$); for trials with successful landings, they descended 14.4 ± 6.1 cm with an 8 cm horizontal translation and a horizontal to vertical distance ratio of ~ 0.55 . No jumping behavior was elicited in control experiments with no visual target ($N=5$ individuals).

DISCUSSION

Behavioral and physiological adaptations to canopy dispersal

The tactic uses of gravity and environmental light gradients by newly hatched *E. tiaratum* support our initial hypotheses on the roles of these two cues for legged movement in canopy space, as found in other ecosystems (e.g. other larval insects, and mites; Perkins et al., 2008; Zhang, 1992). Nymphal *E. tiaratum* lack ocelli and thus depend on compound eyes for visual perception. Previous work on a different phasmid species suggests that eyes are less nocturnally adapted in nymphs than in adults (Meyer-Rochow and Keskinen, 2003), which probably derives from dispersal behavior and a broader temporal niche. In newly hatched *E. tiaratum*, the positive phototaxis persists across a broad luminance range (~ 1 to $\sim 2 \times 10^4$ lx; Fig. 3B), and thus facilitates localization of open canopy

space under a variety of light conditions. This behavior contrasts with negative phototaxis in most phasmid species, except for some winged species attracted by light at night (see Brock and Hasenpusch, 2009). Natural spectrum light was used in our experiments, and *E. tiaratum* may possess wavelength-dependent sensitivity typical of other phototactic insects (Chen et al., 2012, 2013), and probably with the highest sensitivity to blue and green light as in orthopteran insects more generally (Bailey and Harris, 1991). The newly hatched nymphs showed an increased tendency to descend under low light (Fig. 2C), which may represent a detouring strategy for alternative paths or for seeking shelters.

Tactic movement toward vertically oriented contrast lines suggests searching behavior for plant structures such as stems and tree trunks that can be used in ascent; similar behavior has been reported for nymphal crickets (Meille et al., 1994). Black–gray contrasts were more attractive than black–white and gray–white contrasts (Fig. 4A–E), and this preference for dark or shaded surfaces may reduce exposure to visual predators during dispersal. A similar preference was shown in gliding nymphal stick insects (Zeng et al., 2015). Nymphal *E. tiaratum* also showed a preference for narrow strips regardless of the contrast mode (Fig. 4F–H), suggesting a certain level of pattern recognition using spatial frequency characteristics of potential targets (e.g. a stem in the foreground contrasting with a differently shaded background). Overall, these visually mediated behaviors facilitate diurnal dispersal and may serve as a precursor to the evolution of gliding from plants (see below). More systematic investigation is needed to understand the influence of environmental luminance on the insect's visual acuity and how different visual signals are used to enable navigation in a complex canopy space.

Newly hatched nymphs (e.g. 2 h old) showed the longest ascent time, suggesting that they have the lowest fatigue rate; they also exhibited the most frequent pauses, which may be associated with an increased tendency for spatial exploration. A high climbing speed was shown by 4–48 h old nymphs (5.1 – 5.4 cm s^{-1} , or about 3–3.2 body lengths per second), which may be the highest fastest known relative speed for walking phasmids (e.g. mean walking speed in *Carausius morosus* is ~ 2 cm s^{-1} or ~ 1.5 body lengths per

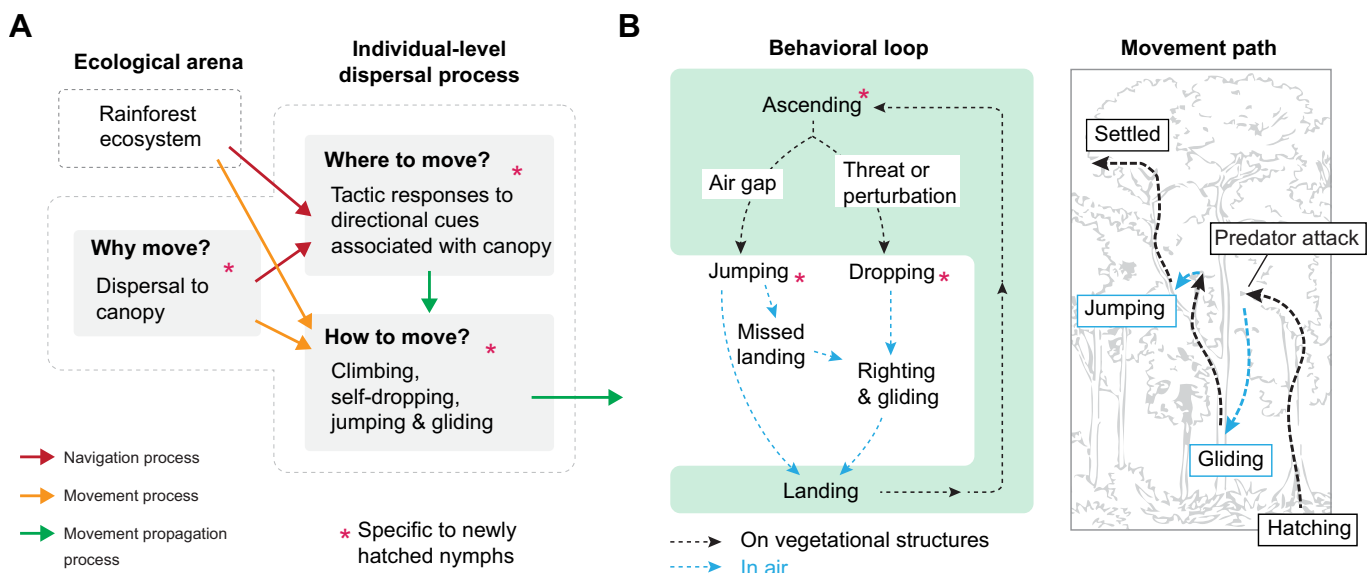


Fig. 8. Ecology of canopy dispersal and gliding in newly hatched *E. tiaratum*. (A) Navigation and movement processes of post-hatch dispersal in *E. tiaratum*, whereby alternation between movements on vegetational structures and in air form a behavioral loop (B), as shown by the schematic diagram (right).

second in the first instar, and $\sim 5 \text{ cm s}^{-1}$ or ~ 0.7 body lengths per second in adults; Graham, 1972). This rapid walking, as fueled by embryonic reserves, likely reflects a strong selective pressure for rapid dispersal in a rainforest ecosystem (see below). Future work may examine the ontogenetic variation of climbing energetics (e.g. aerobic metabolism) to further understand the physiological specializations underlying such rapid movements.

Our experimental setups were repeatedly used with different subjects, and any potential influence of pheromones was assumed to be negligible. Unlike social insects (e.g. ants), phasmids are not known to deposit pheromone trails, which nevertheless does not exclude the possibility that *E. tiaratum* nymphs may use chemical cues for host search during dispersal. The integrative use of various environmental cues for spatial navigation remains to be studied in arboreal arthropods. To further understand cognitive abilities underlying dispersal in nymphal *E. tiaratum*, studies of their movements within structurally and visually more complex environments would be informative. The potential use of other directional cues (e.g. polarized light, chromatic signals, chemical cues, etc.) in the canopy space can be tested using binary choice experiments. Recording movement on natural vegetation (e.g. via a Lagrangian approach; Bagnette et al., 2014) can provide direct evidence for integrative use of various short-range and long-range cues.

Canopy dispersal and the evolution of gliding

A multimodal locomotor strategy, consisting of both aerial and legged phases, can assist small insects moving in the canopy space.

In *E. tiaratum*, mid-air tucking of legs after defensive self-dropping can reduce the body moment of inertia and overall projected size, which also permits rolling along inclines (Fig. 6C). In aggregate, these behaviors may allow for rapid descent through clusters of leaves and branches. Their widespread occurrence in phasmids (e.g. *Phaenoparus khaoyaiensis*; Y.Z., unpublished observation) suggests a general adaptation to arboreal life. Jumping is effective for gap crossing but can be risky given the high chances of a missed landing (Fig. 7D). Our observations suggest that nymphal *E. tiaratum* rely on a variety of mechanisms to assess opportunities for jumping, including leg-search and probing behavior used in gap crossing (Dür, 2001; Blaesing and Cruse, 2004), and active visual scanning for depth perception (e.g. sideways movement of the head or with the legs; Kral, 2009). We predict that, under natural conditions, dispersing *E. tiaratum* may frequently alternate movements on vegetational structures and in air, thus forming a behavioral loop (Fig. 8A,B). An aerial phase may be initiated by missed landings, environmental perturbations and predatory attacks. After dropping, energy required for subsequent ascent can be indirectly reduced by aerial righting and gliding, rather than falling to the ground. The self-dropping reflex that triggers the withdrawal of all tarsi may present a reflex arc similar to avoidance reflexes at the single-leg level (Kittmann et al., 1996). Foothold withdrawal may underlie a variety of voluntary self-dropping mechanisms in phasmids (Bedford, 1978) and some other insects (e.g. ants and aphids; see Haemig, 1997; Humphreys and Ruxton, 2019).

The diurnal niche plays a key role in the gliding behavior of nymphal *E. tiaratum*. Control of mid-air maneuvering and landing

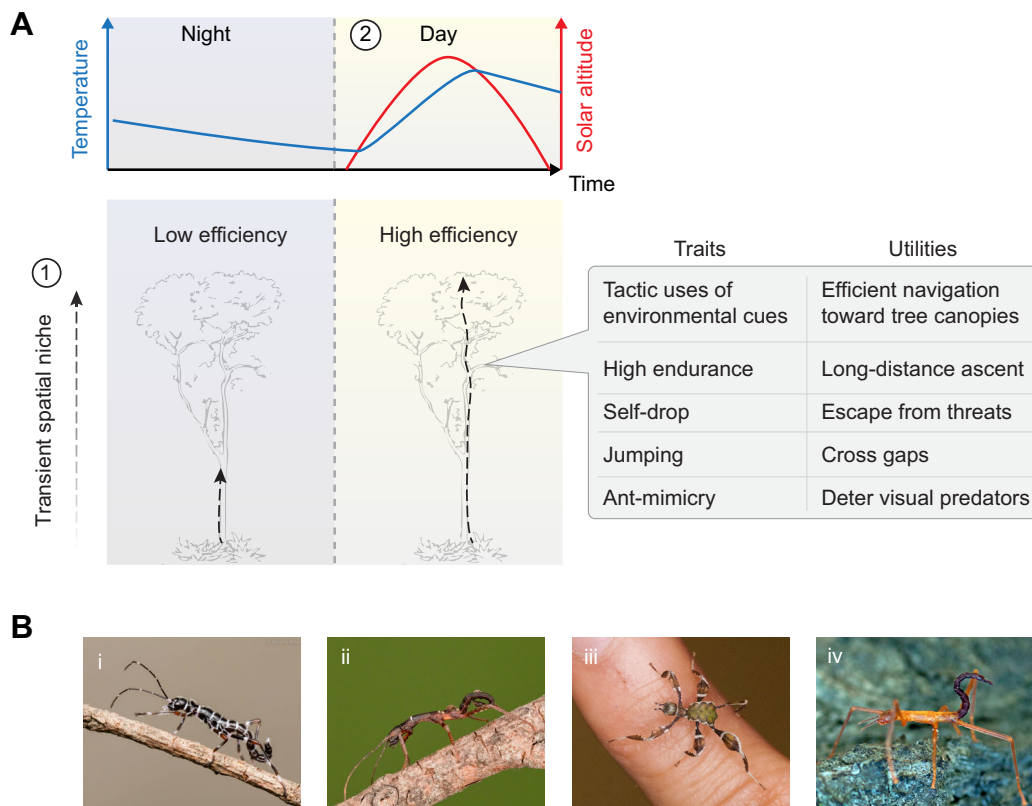


Fig. 9. Evolution of diurnal dispersal in newly hatched stick insects. (A) Diurnal dispersal in nymphal *E. tiaratum* is likely an evolutionary consequence of the interplay between (1) an obligatorily transient spatial niche, and (2) a diurnal temporal niche under selection for greater dispersal efficiency. (B) Examples of newly hatched nymphs that are ground hatched and diurnally active with ant-mimicking coloration and behavior. (i) *Orthomeria* sp. (Aschiphmatidae) from Sarawak, Borneo (photo courtesy of Bruno Kneubühler); (ii) *Paraprisopus* sp. (Prisopodidae) from Panama (photo courtesy of Bruno Kneubühler); (iii) *Phyllium philippinicum* (Phylliidae) from the Philippines; (iv) *Podacanthus* sp. (Phasmatidae) from Queensland, Australia (photo courtesy of Tony Eales).

depends on the strength and continuity of vertically oriented contrast edges (e.g. tree trunks; see Zeng et al., 2015), the qualities of which decrease with and would be less salient at lower light levels. Compound eyes of arthropods generally possess much lower acuity compared with vertebrate eyes (Land, 1997), and will be more dependent in flight on visual contrast associated with tree trunks. Therefore, a diurnal niche may be essential to the evolution of gliding in arthropods, and perhaps less so in vertebrates. This conclusion is indirectly supported by the observation of widespread diurnal gliding in various arthropods (see Dudley and Yanoviak, 2011; Yanoviak et al., 2015), in contrast to crepuscular and nocturnal gliding in various vertebrate gliders (e.g. colugos; Byrnes et al., 2011a,b). Spatiotemporal variation of visual quality of landing targets within vegetational canopies has not yet been examined relative to arthropod gliding, but clearly is relevant to the evolution of this behavior. Similarly, few data exist pertaining to the occurrence and behavioral contexts of aerial descent (e.g. as elicited as a defensive strategy or in response to environmental perturbation) in a range of arboreal invertebrates (see Yanoviak et al., 2011, 2015).

Evolution of diurnal dispersal in nymphal phasmids

Phasmids are generally nocturnal (Bedford, 1978), whereas diurnal hatching in *E. tiaratum* likely evolved under selection for rapid dispersal within a rainforest ecosystem. The ascent of ground-hatched nymphs is a transient behavior that utilizes environmental cues for navigation and movement. As environmental brightness is strongest around midday (Shuttleworth, 1985), hatching around this time maximizes the use of this cue (see Fig. 9A). Even for cloudy days, the luminance-insensitive phototactic response can allow newly hatched *E. tiaratum* to disperse upward toward canopies. During the rainy season of north Queensland, for example, >50% of consecutive wet day intervals last less than 2 days (Cook and Heerdegen, 2001), and the sky is on average only partially cloudy (i.e. 3–5 oktas) with >8 h of daily sunshine (Australian Government Bureau of Meteorology Weather Data Services; www.bom.gov.au/catalogue/data-feeds.shtml; accessed 8 Jun 2020). Furthermore, ant mimicry by newly hatched *E. tiaratum* may help to deter visual predators during movement on vegetational structures. Similar ant-mimicking phenotypes have been described from newly hatched nymphs in several unrelated phasmid species (Fig. 9B; see also Hanibeltz et al., 1995). Nymphs of all such taxa hatch on the ground and are diurnally active. Convergent evolution of this strategy implies a common demand for dispersal efficiency, particularly given the high abundance of nocturnally active predators on vegetation (see Berger and Wirth, 2004). Hatching location, diurnality and vegetational features thus underlie the evolution of diurnal ascent in nymphal phasmids, although a broad phylogenetic survey is now warranted to evaluate possible correlates of other dispersal strategies.

Acknowledgements

We thank Douglas Ewing for inspiration, and David Wake and members of Berkeley Animal Flight Laboratory for comments. We also thank Andrea Lucky for communication about *Leptomymex* ants, and Paul Brock and Jack Hasenpusch for advice on *E. tiaratum* natural history. We further acknowledge research facilities provided by the Center for Interdisciplinary Biological Inspiration in Education and Research (CiBER), UC-Berkeley.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: Y.Z., S.W.C., J.Y.W., L.Y.N., J.T., G.N., R.D.; Methodology: Y.Z., S.W.C., J.Y.W., L.Y.N., J.T., G.N., C.K.; Software: Y.Z.; Validation: Y.Z.; Formal

analysis: Y.Z., S.W.C., J.Y.W.; Investigation: Y.Z., S.W.C., J.Y.W., L.Y.N., J.T., G.N., C.K.; Resources: Y.Z., R.D.; Data curation: Y.Z., S.W.C., J.Y.W., L.Y.N., J.T., G.N., C.K.; Writing - original draft: Y.Z.; Writing - review & editing: Y.Z., S.W.C., J.Y.W., L.Y.N., J.T., R.D.; Visualization: Y.Z., S.W.C.; Supervision: Y.Z., R.D.; Project administration: Y.Z., R.D.; Funding acquisition: Y.Z., R.D.

Funding

This work was partially funded by the Undergraduate Research Apprentice Program (URAP) of University of California, Berkeley.

Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.226266.supplemental>

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Table S1. Detailed frequency data for Figs 2–6.[Click here to Download Table S1](#)

Table S2. Statistical results of movement direction in visual contrast experiments. f_D , the frequency of moving towards the darker surface in each setup, excluding those overlapped with contrast edges. G-values are presented with d.f. and P -values in parentheses (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). See Fig. 4.

setup	N	von Mises distribution		f_D (%)	repeated G-test with William's correction		
		μ (95%CI)	κ (95%CI)		G_{total}	G_{pool}	$G_{heterogeneity}$
BW11	100	-0.06 (6.19, 6.25)	32.38 (20.4, 57.3)	57.1	35.8 (23 d.f., *)	2.0 (1 d.f., 0.158)	33.8 (22 d.f., 0.051)
BG11	188	0.01 (0.01, 0.02)	727.07 (563.47, 1046.45)	37.7	60.4 (38 d.f., *)	7.42 (1 d.f., **)	53.0 (37 d.f., *)
GW11	127	-0.03 (6.25, 6.27)	251.96 (200.78, 340.94)	75.7	72.2 (26 d.f., ***)	30.6 (1 d.f., ***)	41.6 (25 d.f., *)
BW19	100	-0.05 (6.22, 6.24)	265.08 (206.68, 372.66)	85.4	73.8 (20 d.f., ***)	49.1 (1 d.f., ***)	24.7 (19 d.f., 0.170)
BW91	95	0.05 (0.04, 0.07)	243.61 (191.89, 329.1)	19.8	57.5 (19 d.f., ***)	33.5 (1 d.f., ***)	24.0 (18 d.f., 0.155)

Table S3. Statistical results of movement direction in visual contrast experiments. f_D , the frequency of moving towards the darker surface in each setup, excluding those overlapped with contrast edges. G-values are presented with d.f. and P -values in parentheses (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). See Fig. 4.

setup	N	f_{stripe} (%)	repeated G-test with William's correction		
			G_{total}	G_{pool}	$G_{heterogeneity}$
BW91	95	82	43.48 (9 d.f., **)	20.08 (1 d.f., ***)	23.4 (18 d.f., 0.18)
BW19	100	76	86.49 (20 d.f., ***)	61.04 (1 d.f., ***)	25.45 (19 d.f., 0.15)

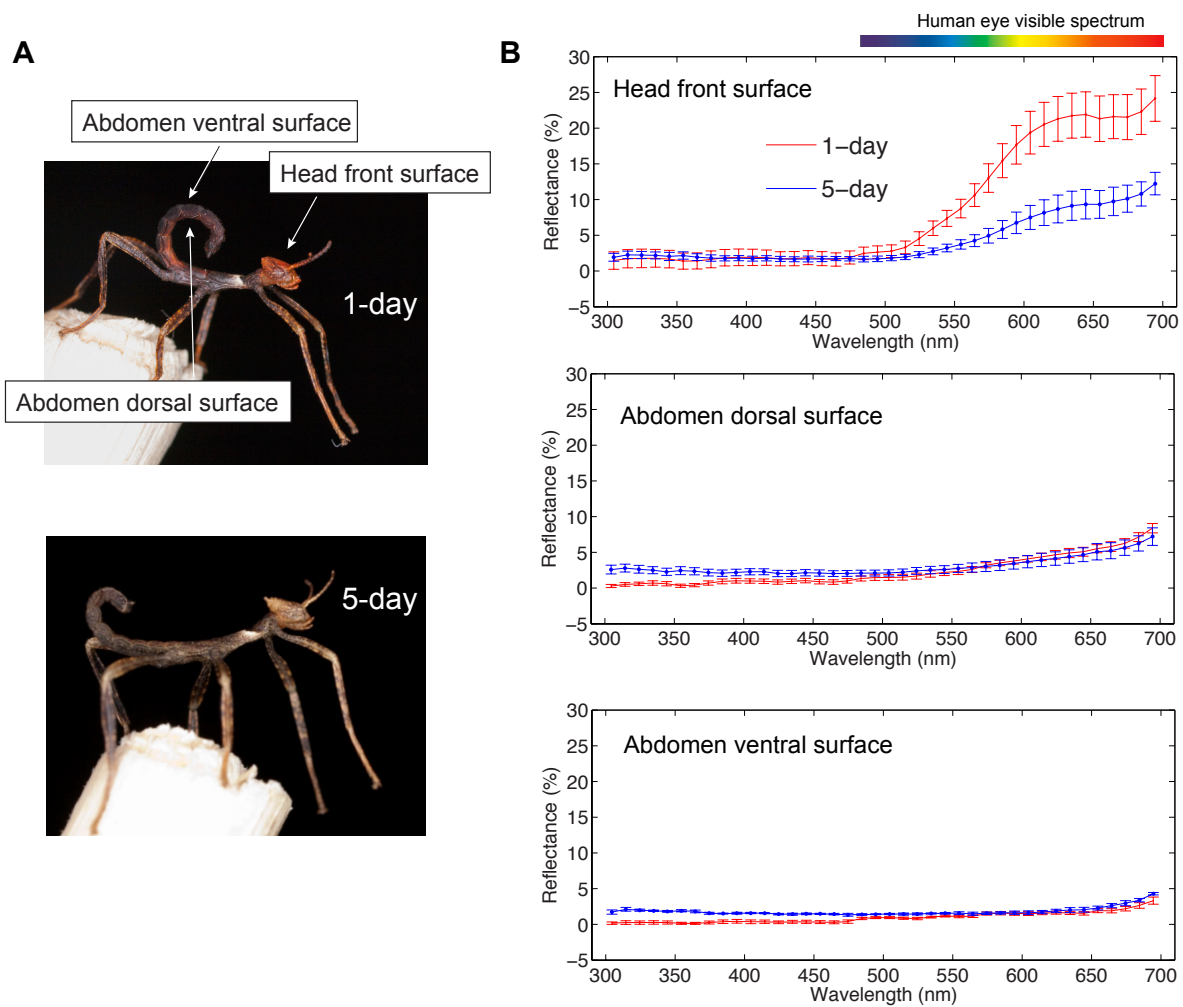


Fig. S1. Ontogenetic loss of ant-mimicry coloration in first instar *E. tiaratum*. (A) Comparison of coloration between a 1-day-old nymph and a 5-day-old nymph. Arrows denote three sampled spots. (B) Comparison of spectral reflectance profiles of three sampled spots between insects of two ages ($N = 5$ individuals per age group), showing the ontogenetic loss of red coloration on head. Spectral reflectance was measured using a spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA) with a deuterium-halogen light source (AIS Model MIMI DTA, Analytical Instrument Systems, Flemington, NJ, USA) calibrated with a diffuse white standard (Labsphere Inc., NH, USA). Values are means \pm S.D.

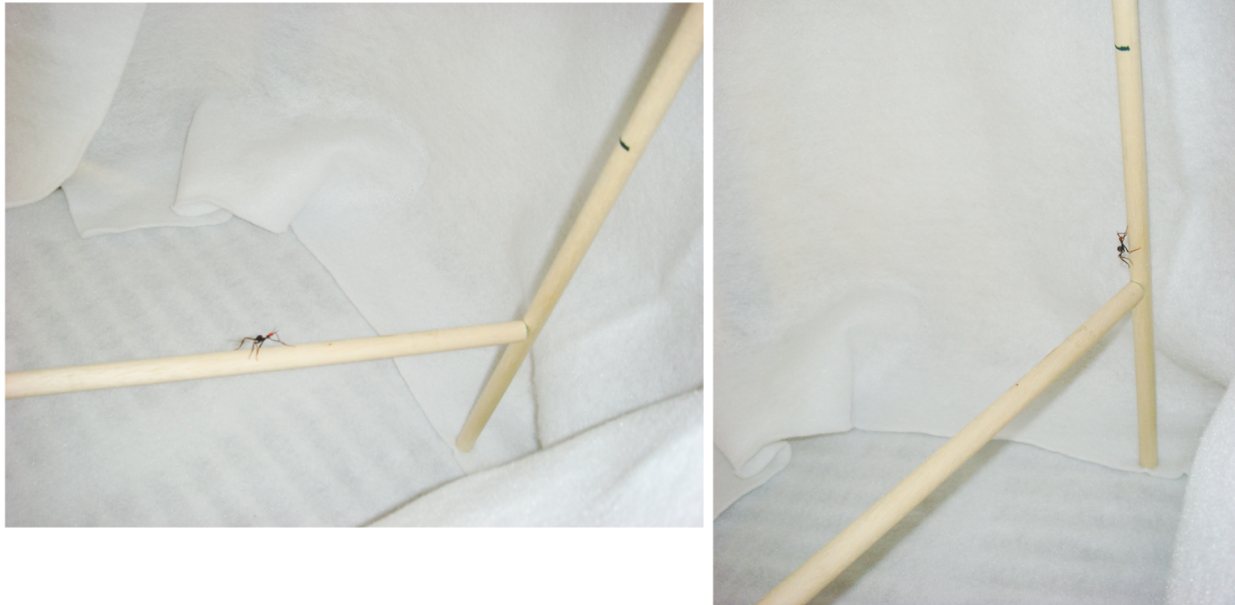


Fig. S2. Inner view of the experimental setup for recording gravitactic behaviors.



Fig. S3. Inner view of the experimental arena for recording tactic response to visual contrasts.