Diurnal colour change in the Andaman Island stick insect *Sceptrophasma hispidulum*

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Abstract

Here, we report the previously undescribed diurnal (i.e. day-night) colour change of the Andaman Island stick insect *Sceptrophasma hispidulum*. We show that *S. hispidulum* has a tan-yellow body colour during the day, but undergoes a relatively rapid change to dark red-orange body colour at night. This change takes place in both males and females of the species, and appears to be induced by changes in environmental light. When considered alongside previous reports of diurnal colour change in the Indian stick insect *Carausius morosus*, these findings could point to diurnal colour change as a general and underappreciated feature of stick insects.

Introduction

Many animals undergo reversible colour changes to enhance crypsis (i.e. camouflage) as warning colouration to predators or for intraspecific communication. Some of the best-known examples of reversible colour change are seen in vertebrates and cephalopod molluscs: for example, chameleons can rapidly change their body colour through a combination of pigment dispersion and nanocrystal-mediated light reflection within their skin (Teyssier *et al.*, 2015), and squid can display a striking and dynamic array of skin patterns through the rapid expansion and retraction of pigmented chromatophore cells over an adjacent layer of iridescent iridophore cells (Hanlon, 1982; Mäthger and Hanlon, 2007). However, many arthropods are also capable of reversibly changing their colour (reviewed by Umbers *et al.*, 2014). In arthropods, reversible colour change can occur on a scale of seconds (e.g. in the spider *Cyrtophora cicatrosa* – Blanke, 1975, as cited by Umbers *et al.*, 2014; Oxford and Gillespie, 1998), minutes (e.g. the Antarctic krill *Euphausia superba,* or the Golden Tortoise Beetle *Charidotella egregia* – Vigneron *et al.*, 2007; Auerswald *et al.*, 2008), hours (e.g. damselflies – May, 1976) or days (e.g. in the beetle *Cryptoglossa verrucosa* – Hadley, 1979). In some cases, body colour may also change on a daily (diurnal) cycle.

Diurnal colour change in arthropods has been most extensively studied in decapod crustaceans (reviewed in Stevens, 2016). Many species of fiddler crabs (*Uca* spp.) shift between a lighter body colour at night and a darker body colour in the day, likely as a means of thermoregulation and protection against UV radiation (Atkins, 1926; Abramowitz, 1937; Brown and Webb, 1948; Darnell, 2012). Conversely, the Horned Ghost crab *Ocypode ceratophthalmus* undergoes diurnal change from lighter during the day to darker at night to enhance camouflage under differing day and night light conditions (Stevens *et al.*, 2013). In many animals, processes that exhibit recurring daily cycles may be regulated by an intrinsic circadian "clock": a complex network of endogenous molecular feedback loops that oscillate with a period of 24 hours, and that regulate a variety of physiological processes (Harmer *et al.*, 2001). A hallmark of processes under circadian clock control is continued rhythmicity, even under constant environmental conditions. The Chameleon prawn *Hippolyte varians* exhibits a striking diurnal colour change, from green, red or brown during the day to transparent blue at night, and the reported persistence of this colour change, even when animals were maintained in constant light or dark, was taken as evidence of its regulation by an endogenous circadian clock (Gamble and Keeble, 1900). However, Kleinholz and Welsh (1937) reported that *H. varians* will, in fact, change to their night colouration when moved into darkness during the day, rather suggesting that this colour change may be directly regulated by sensory input (e.g. changing light conditions). Another example of diurnal colour change under circadian control comes from the Indian stick insect *Carausius morosus*. Indian stick insects change from pale during the day to darker at night, and it has been reported that this change continues for several weeks, even when animals are maintained in complete darkness (Schliep, 1910, 1915 as cited by Wigglesworth, 1972).

Diurnal colour changes have not been widely studied in insects. Here, we report a novel and striking instance of diurnal colour change in the Andaman Island stick insect *Sceptrophasma hispidulum*. We show that this insect undergoes a relatively rapid and dramatic change in body colour, from tan-yellow in the day to dark red-orange at night, and we provide preliminary evidence that this colour change is not under circadian control, but rather occurs in response to changing light conditions. The amenability of *S. hispidulum* to maintenance in captivity and experimental manipulation makes it an exciting new model with which to investigate mechanisms of reversible colour change in insects.

Materials and Methods

Animals

Andaman Island stick insects *Sceptrophasma hispidulum* were maintained in Cambridge, UK at room temperature under ambient light (~16:8 hours light: dark at the time of study – April-May 2020). Animals were kept in an ELC cage from Small-Life Supplies (Peterborough, UK), fed on bramble, and misted daily with fresh water.

Qualitative body colour assessment

To measure colour changes between day and night, three males and three females were scored at 11:00 (day) or 23:00 (night) as belonging to one of eight numbered sections evenly distributed across the colour gradient shown on the Y-axis in figure 2. A score of “1” corresponded with the extreme tan end of the spectrum, while a score of “8” corresponded with the darkest red end of the spectrum. Animals were scored for seven consecutive days, and all day and night colour scores were then used to generate a box and whisker plot in Microsoft Excel.

Dark/light colour change experiments

To test effects of light on colour change, animals were reared **1)** under constant darkness (inside a large cardboard box, in a dark closet), **2)** under constant light (cage illuminated on all sides by LED light) or **3)** on a reverse light cycle (illuminated as in constant light experiment until 00:00, with lights then switched off, leaving animals in complete darkness). Due to a limited number of animals, all experiments were performed with two animals, and compared against one control animal (left under ambient light conditions). Animals were very briefly removed from their cages throughout the experiments for imaging (see below), and then quickly returned.

Imaging

For imaging of live animals at various stages of colour change, the insects were gently removed from their cages, and held under a stereomicroscope with gentle pressure while the head or abdomen were photographed. Animals were then promptly returned to their cages. Figure 2 imaging was performed with a Leica M165FC stereomicroscope and DFC7000 camera, and figures 4-5 imaging was performed with a Leica S9E stereomicroscope and LabCam Pro for iPhone 8 (iDu Optics, New York, NY, USA). All imaging within a figure was performed under constant light and exposure conditions, to ensure comparability of images.

Results

Rapid diurnal colour change in S. hispidulum

From having kept Andaman Island stick insects *Sceptrophasma hispidulum* (figure 1) for many years, we noticed that this species exhibits a striking colour change from day to night. During the day, the head, thorax and abdomen is uniformly tan-yellow (figure 2a, b), while at night, the body colour changes to

Figure 1: A female Andaman Island stick insect *Sceptrophasma hispidulum*.

a dark red-orange (figure 2c, d). To qualitatively assess this colour change, we scored the body colour of three male and three female insects against a colour spectrum (from tan-yellow to red-orange), during the day (11:00) and at night (23:00), for seven consecutive days. We found that both males and females were invariably tan-yellow at the point of daytime observation, and exhibited a striking shift in body colour to the red-orange end of the spectrum at the point of night time observation (though with some variation in the final night time colouration – figure 3). This suggests that diurnal change in body colour is a general feature of this species, and that it occurs in both males and females.

To assess how quickly the transition from day to night colouration occurred, we observed three *S. hispidulum* females throughout the evening, at ten-minute intervals, over the course of several days. We found that the onset of colour change was generally at about 21:00, and was usually completed in an hour or less (see figure 4 for a typical colour change sequence, with timing). From the starting point of daytime tan-yellow (figure 4a), colour change was first evident with the appearance of red pigmentation at the posterior boundary of each body segment and along the dorsal midline (figure 4b). Zones of red pigmentation at posterior segment boundaries expanded, and merged with islands of red pigmentation that grew out from the dorsal midline (figure 4c, d). Within an hour or less, the change to a relatively uniform red-orange body colour was complete, with a few small patches of tan-yellow remaining (figure 4e, f).

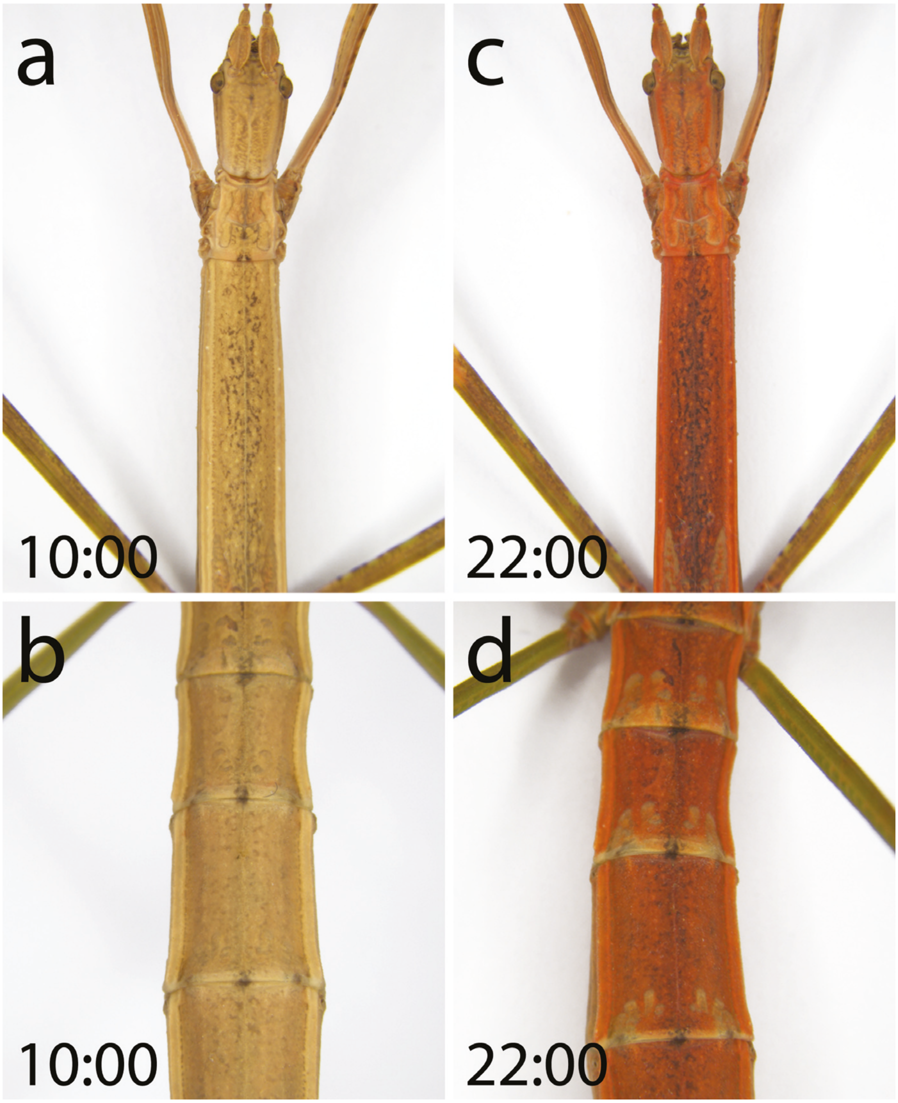

Figure 2: Diurnal colour change in *S. hispidulum*. **a)** Head, thorax and **b)** abdomen of a female *S. hispidulum* at 10:00, showing typical tan-yellow daytime body colour. **c)** Head, thorax and **d)** abdomen of the same insect at 22:00, showing typical red-orange night-time body colour.

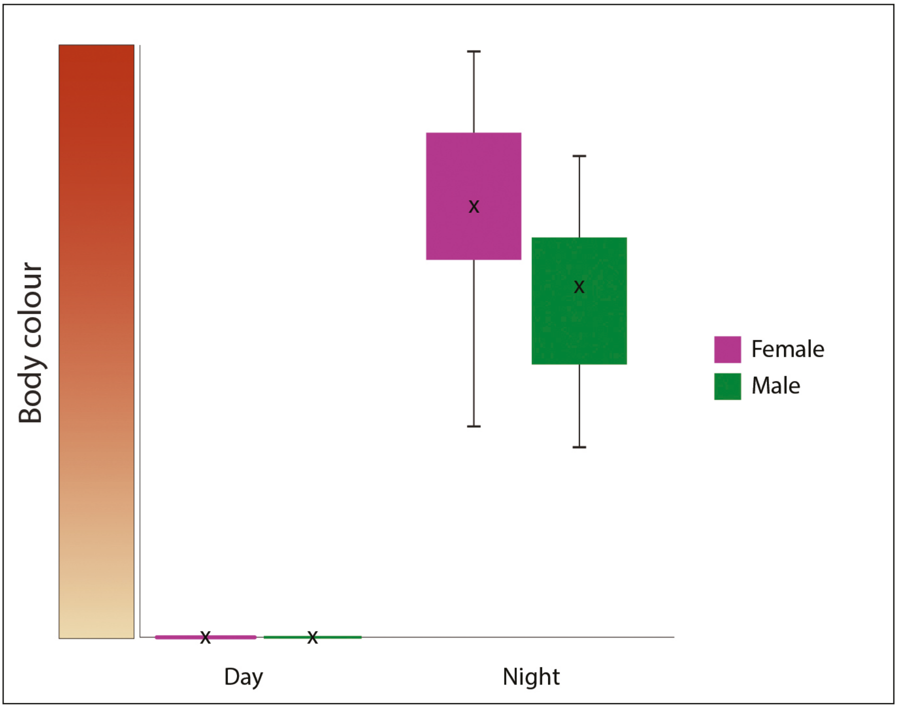

Figure 3: Diurnal colour change in *S. hispidulum* occurs in both males and females. Scoring of *S. hispidulum* body colour in the morning (11:00) and evening (23:00) over seven consecutive days reveals that diurnal colour change occurs in both males and females.

S. hispidulum body colour changes in response to light

Colour change in *S. hispidulum* correlates with the transition from daytime to night-time, with onset of change from tan-yellow to red-orange occurring roughly at sunset. To test whether *S. hispidulum* colour change is under circadian regulation or a response to changing light conditions, we reared females under constant darkness or light and assessed colour change. We predicted that, if colour change is under circadian control, we would continue to see a normal timing of diurnal colour change even when animals were held in constant darkness or light. Conversely, if colour change is a response to changing light conditions, we would expect that constant darkness or light would immediately induce or prolong night-time or daytime colouration, respectively.

We first tested the effect of rearing animals in darkness during the day. At 11:00, animals were moved to complete darkness, and were maintained in darkness and monitored hourly alongside a control animal (left at ambient

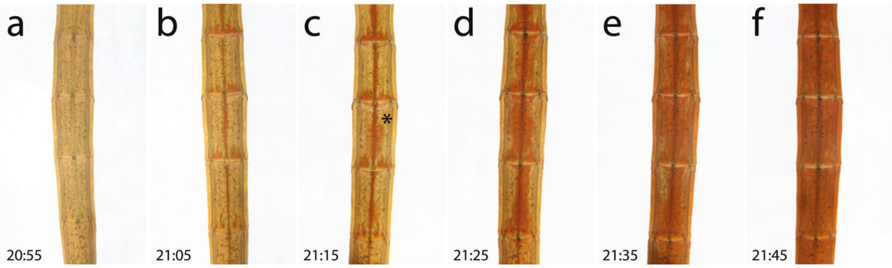

Figure 4: Progression of change from daytime to night-time colouration in *S. hispidulum*. The change from daytime tan-yellow to night-time red-orange in *S. hispidulum* is relatively rapid, and is typically complete in ≤1 hour. **a)** From daytime colour, **b)** the onset of colour change is marked by the appearance of red patches immediately anterior to segment boundaries, and along the dorsal midline. **c)** These patches of red-orange expand, with midline patches giving rise to islands of red-orange pigmentation (*). **d) and e)** Patches of red-orange pigmentation expand until **f)** the body is a uniform red-orange, with few patches of tan-yellow tissue remaining. All images are of the same individual, and times shown are representative of the typical sequence of colour change.

light). While the control animal maintained its normal tan-yellow daytime colouration (figure 5a), animals that had been moved into complete darkness transitioned to their night-time red-orange colouration within an hour (figure 5b). This suggests that a decrease in environmental light is sufficient to induce the transition from daytime-to-night-time colouration during the day in *S. hispidulum*.

We next tested the effect of rearing animals in constant light. From 11:00, animals were placed in a cage that was illuminated on all sides by three LED lights, and were again monitored hourly alongside a control animal at ambient light. Strikingly, the animals maintained under constant light did not undergo a transition from daytime-to-night-time colouration, but rather maintained their daytime tan-yellow colouration into the night (figure 5c). This suggests that darkness is required for the transition from daytime-to-night-time colouration in *S. hispidulum*.

Finally, we tested whether animals reared under constant light could be induced to change colour during the night with a sudden change to darkness. Animals reared under constant light until 00:00 maintained their daytime colouration, as described previously. We then switched off the lights, and noted that animals underwent a rapid change from daytime to night-time colouration, with the change complete within 30 minutes (figure 5d). Taken together, these experiments show darkness is necessary for the transition from daytime tan-yellow to night-time red-orange in *S. hispidulum*, and that darkness is sufficient to induce this change (during the day or during the night). These observations suggest that diurnal colour change in *S. hispidulum* is not under circadian control, but rather is a response to changing light conditions.

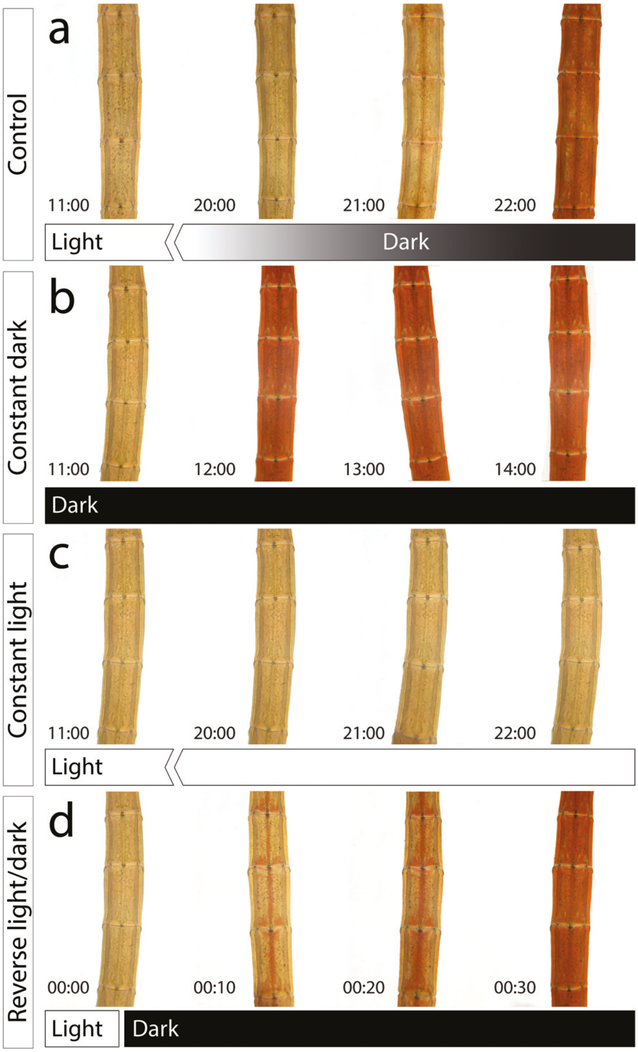

Figure 5: *S. hispidulum* body colour changes in response to light. **a)** A control animal in ambient light. Onset of change from daytime to night-time colouration is noted at ~21:00, and is complete by 22:00. **b)** An animal moved to complete darkness at 11:00. By 12:00, the animal has undergone a complete change from daytime to night-time colouration. **c)** An animal maintained under constant illumination from 11:00. The animal maintains its daytime colouration into the night. **d)** An animal maintained under constant illumination until 00:00 maintains its daytime colouration. When the illumination is switched off and the animals are then in complete darkness, we begin to see a transition to night-time colouration after ten minutes. After thirty minutes in darkness, the change to night-time colouration is complete. For each experiment, images are of the same animal photographed sequentially at each time point.

Discussion

We have shown that the Andaman Island stick insect *Sceptrophasma hispidulum* undergoes a diurnal colour change, from tan-yellow in the day to red-orange at night. This colour change is seen in both males and females of the species, and appears to happen largely in response to changing light conditions: moving animals into the dark during the day immediately led to a change from daytime to night-time colouration, while maintaining animals in constant light at night prevented the change from daytime to night-time body colour. Relatively little is known about the ecology of *Sceptrophasma hispidulum*, and so it is difficult to speculate on the function of their diurnal colour change – though it seems likely that their transition to a darker body colour at night could enhance crypsis and aid in the avoidance of predation.

Most instances of colour change in animals fall into one of two broad categories: physiological or morphological (Figon and Casas, 2018). Physiological colour change is relatively rapid (i.e. it occurs on a scale of seconds to hours), and is often a result of the movement of nanostructures (such as pigment granules or light-reflecting nanocrystals) within cells of the epidermis. Physiological colour changes may be controlled by the direct innervation of pigment-containing chromatophore cells from the brain (Young, 1974), by the intrinsic sensitivity of the skin to light (Ramirez and Oakley, 2015), or through the action of hormones released from the nervous system (Fingerman, 1966; Fingerman and Yamamoto, 1967). Morphological colour change, on the other hand, is generally slower (i.e. occurring on the scale of hours to days), and occurs by synthesis, degradation or chemical modification of pigment. Given the speed of colour change observed in *S. hispidulum*, we speculate that this represents a case of physiological colour change. However, additional studies of neuroanatomy, epidermal microstructure in animals with day- and night-time colouration, and colour change-inducing ability of haemolymph are needed to resolve the detailed mechanisms of colour change in this species.

Diurnal colour change in insects has been studied most extensively in another species of stick insect, the Indian stick insect *Carausius morosus*. Indian stick insects can show a range of body colours, including beige, brown and green, depending on the relative amounts of orange, red, yellow, green and brown pigment in their epidermis (Hoffman, 1985). Additionally, Indian stick insects also show a pronounced darkening at night and lightening during the day, and this is caused by the dispersal and clumping of orange-red and brown pigment granules with the epidermis during the night and day, respectively (Giersberg, 1928, as cited by Wigglesworth, 1972). The observation that diurnal darkening and lightening will continue for several weeks when animals are moved to complete darkness suggests that this physiological colour change may be under circadian control. However, there is also evidence that diurnal darkening and lightening of Indian stick insect may be regulated by light changes as detected by the visual system: severing the eye stem or covering the eyes reportedly causes all colour change to cease (Atzler, 1930, as cited by Wigglesworth, 1972). Control of colour change in Indian stick insects is probably hormonal, as pigmented epidermal cells are not innervated, and pieces of cuticle and epidermis that are excised and transplanted into a new animal will change colour in line with their new host tissue (Janda, 1936, as cited by Wigglesworth, 1972).

Parallels in diurnal darkening and lightening between the Indian and Andaman Island stick insects could indicate shared mechanisms of colour change in these species. Indeed, diurnal, light-induced colour change could be a general feature of stick insects that has, so far, remained largely undocumented. Stick insects are generally easy to rear, and a great number of species are routinely kept by professional scientists and hobbyists alike. We hope that this report illustrates how relatively simple observations, experiments and photography by stick insect enthusiasts could further shed light on this fascinating aspect of insect biology.

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