Moths use multimodal sensory information to adopt adaptive resting orientations

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Camouflage conceals animals from predators and depends on the interplay between the morphology and behaviour of animals. Behavioural elements of animals, such as the choice of a resting spot or posture, are important for effective camouflage, as well as the animals' cryptic appearance. To date, the type of sensory input that mediates resting site choice remains poorly understood. Previously, we showed that bark-like moths perceive and rely on bark structure to seek out cryptic resting positions and body orientations on tree trunks. In the present study, we investigated the sensory organs through which moths perceive the structure of bark when positioning their bodies in adaptive resting orientations. We amputated (or blocked) each one of the hypothetical sensory organs in moths (antennae, forelegs, wings, and eyes) and tested whether they were still able to perceive bark structure properly and adopt adaptive resting orientations. We found that visual information or stimulation is crucial for adaptively orienting their bodies when resting and tactile information from wings may play an additional role. The present study reveals multimodal information use by moths to achieve visual camouflage and highlights the sensory mechanism that is responsible for the adaptive behaviour of cryptic insects. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, ••, ••–••.

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INTRODUCTION

Camouflage conceals an animal body to avoid detection/recognition by predators (Cott, 1940; Stevens & Merilaita, 2011). Although camouflage has largely been understood as a strategy that results from the colour patterns of animals, it is achieved through the interplay between multiple traits, such as morphological, behavioural or, in some cases, physiological traits (Akino, Nakamura & Wakamura, 2004), which co-evolved to deceive the detector's sensory system (Stevens & Merilaita, 2009). To date, the morphological mechanisms that provide camouflage have been investigated extensively (Stevens & Merilaita, 2011), although behavioural elements remain poorly understood.

Behavioural background matching is a common process in camouflaged animals. For example, many camouflaged animals preferentially stay near a substrate that decreases the chance of detection by predators (Manríquez *et al*., 2008; Cooper & Sherbrooke, 2012; Kjernsmo & Merilaita, 2012). Recent studies revealed that some animals can seek out cryptic positions and postures at a fine scale. Quails know and choose locally the most cryptic egg-laying sites (Lovell *et al*., 2013), probably using visual cues to find cryptic spots for egg laying. Bark-like moths can find better camouflaged spots and body orientations after landing on tree trunks (Kang *et al*., 2012, 2013b). However, it is still not known how these moths seek out cryptic spots and assume body orientations.

A previous study shows that a bark-like moth, *Jankowskia fuscaria* (Leech 1981), perceives and relies on bark structures to find cryptic resting orientations that align the direction of the wing patterns with the direction of bark patterns (Kang *et al*.,

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2013a). It does not use information regarding the direction of light, shadow or gravity when structural cues are present and primarily utilizes the structural cues for orienting their bodies (Kang *et al*., 2013a). In the present study, we tested the follow-up question: through which sensory organs (or, by implication, what type of sensory information) do moths perceive these bark structures. As the potential source of information, we considered sensory inputs from their antennae, forelegs, wings or eyes. Insect antennae play a major role in chemoreception or flight control (Sane *et al*., 2007), although there are mechano-receptors, such as Johnston's organs, that can perceive tactile information by direct contact (Schneider, 1964; Gullan & Cranston, 2009). Insect legs also have mechanoreceptors, such as trichoform or campaniform sensillae, that can perceive tactile stimuli (Gullan & Cranston, 2009). Mechanoreceptors are also present in the wing margin that can detect vibration (Keil, 1997). Insect eyes perceive visual information through photoreceptors (Land & Nilsson, 2012). With respect to the resting posture of moths, we hypothesized that one (or more than one) of these organs may be responsible for the perception of bark structure.

MATERIAL AND METHODS

STUDY SPECIES AND EXPERIMENTAL DESIGN

We used *J. fuscaria* to test our hypothesis. *Jankowskia fuscaria* is a bark-like moth species with wing patterns that resemble those of tree bark. Their natural resting orientations on vertically (naturally) standing tree trunks are towards either the left or right side; this is considered to be adaptive.

To investigate the sensory organs through which moths perceive the structure of tree bark when orienting, we blocked the information input from one of the sensory organs either by amputating the organ or blocking the sensory information input. Then we released the moths on natural/artificial backgrounds and observed whether the moths were still able to perceive background structure properly and assume cryptic orientations. We considered antennae, wings, forelegs, and eyes as the candidate organs. The experimental procedures complied with the 'Guidelines for the Treatment of Animals in Behavioural Research and Teaching' (Animal Behaviour, 2012).

EXPERIMENTAL PROCEDURES

We collected *J. fuscaria* at night near black lights at the field site (Mt Baekwoon, South Korea; 35°,01′,54.30″N; 127°,36′,22.30″E). The moths were kept individually in small plastic containers with sugar moistened tissue. Next morning, we performed experimental manipulation (see below) on the moths and allowed them at least 1 h of resting time after the manipulation. Then we released the moths on natural tree trunks or on a directionally structured background (see below), waited for 1 h to allow them sufficient time to re-position themselves (crucial for the adoption of adaptive resting orientations in *J. fuscaria*; Kang *et al*., 2012), and photographed the moths. When released, individuals with antennae/forelegs amputated were able to fly and land on the nearby tree trunks (usually a tree within a distance of 15 m from the releasing point), although this was impossible for the wing-excised ones. Therefore, for the wing-excised individuals, we allowed the moths to walk from the container boxes to the tree trunks. A previous study shows that moths are still able to perceive background structure and find adaptive resting orientations when released by this method (Kang *et al*., 2013a).

After the experiments, moths were re-collected and released at least 4 km away from the experimental site to avoid re-capturing the same individuals. All of the experiments were conducted during August and September 2013.

ELIMINATION OF INFORMATION INPUTS FROM EACH SENSORY ORGAN

We predicted that, if moths use the information from the sensory organs that we amputated (or blocked), the moths whose sensory information was blocked by our experimental procedure would fail to adopt adaptive resting orientations. We hypothesized that, to perceive bark structure, moths may use tactile information from antennae, forelegs, wings or visual information from eyes.

For antennae treatment, we amputated the basal part of both antennae using surgical scissors. We left less than 1 mm of both antennae to ensure that their antennae do not perceive bark structure by direct contact. For foreleg treatment, we pulled out both forelegs by hand. The forelegs were easily pulled out and no leftovers or additional damage was found on the bodies of moths. For wing treatment, we cut the basal part of both forewings and hindwings. We left less than 5 mm of the wings to ensure that no tactile information is transmitted by direct contact.

For vision treatment, we obviously were unable to block the visual information by amputation of the eyes. Therefore, we decided to eliminate ambient light entirely. We used an experimental box (40 × 33 × 26 cm) into which no light could enter and observed the resting behaviours of moths in the box. A previous study (Kang *et al*., 2013a) shows that moths respond to the directional structure of the

background and adopt adaptive resting orientations (towards either the left or right side on a vertically structured background). We made four backgrounds with vertical directional structures (i.e. the same as those used in our previous study; Kang *et al.*, 2013a) using cardboard (see Supporting information, Fig. S1). Then we attached these backgrounds inside the walls of the experimental box. We covered the opening of the box (the upper part) with transparent acrylic plate and used this plate as a 'door' to introduce the moths. At first, we checked whether the resting orientations of the moths in the experimental box were similar to those in natural situation (control experiment for vision treatment). We released the moths into the box with light present and waited one night and then checked the resting orientations of the moths the next morning. In the experimental box with light present, the orientations of moths were either towards the left or right side (for the results, see the Supporting information, Fig. S2), which assured us that the moths perceived background structure and responded to it in the experimental box when light was present.

For vision treatment, we covered the whole box with three layers of thick black fabric after the moths were released into the box to prevent any light from entering the box. Then we let the moths stay in the box for one night and photographed them the next morning. This overnight procedure was essential because light present during the release could affect the moths' choice of resting orientations for that day. When photographing the moths the next morning, we carefully removed the fabric and photographed the moths through the opening of the box. When a moth flew in response to the removal of the fabric, it was discarded from the sample. This vision treatment was conducted in a warehouse where only dim light could penetrate through small windows. For analysis, we only used moths that rested on the side walls (where the structures were present) and excluded any moths that were found on the floor of the box.

STATISTICAL ANALYSIS

From the photographs, we measured the head orientations of moths (by degrees, 0° if moths oriented upwards and run clockwise) relative to the upright tree trunks or directional structure in the vision treatment. Because our predicted orientations of moths were distributed in bimodal fashion (towards either the left or right side), we transformed the angular data to achieve unimodal distribution (Zar, 1999). Then, for each treatment, we tested whether the head orientations of moths were uniformly distributed or not using Kuiper's test of uniformity (nonparametric uniformity test). We additionally performed Rayleigh's test with a

specified mean angle to test whether moths oriented towards either the left or right side. More details of the methods (mathematical formulae for transformation and null/alternative hypothesis of the test after transformation) are provided in Kang *et al.* (2013a). We used the functions ('kuiper.test', 'rayleigh.test') in the 'circular' package in R (R Foundation for Statistical Computing, available at http://www.R-project.org/). The total number of captured, tested, and analyzed moths is reported in the Supporting infromation (Table S1).

RESULTS

In total, 83% (25/30) of the antennae-amputated moths re-positioned themselves, and the resting orientations of the moths were nonrandomly distributed (Kuiper's test; $V = 2.455$, $N = 30$, $P < 0.001$; Fig. 1A) but towards either the left or right side (Rayleigh's test; test statistic = 0.499, $N = 30$, $P < 0.001$). When forelegs were pulled out, all of the moths re-positioned themselves (27/27). They oriented nonrandomly when resting (Kuiper's test; $V = 3.356$,
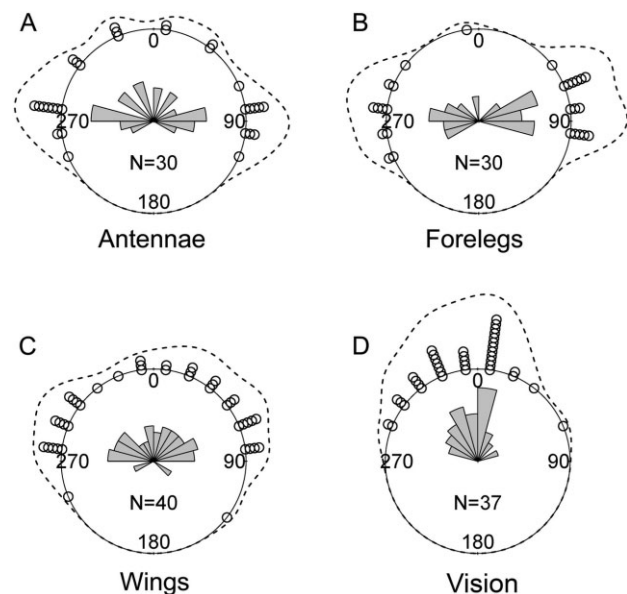

Figure 1. The distribution of the head orientations of *Jankowskia fuscaria* in relation to vertically standing tree trunks (or directional structures for vision treatment). Each subplot shows the distribution of resting orientations of antennae-amputated moths (A), foreleg-amputated moths (B), wing-excised moths (C), and moths when visual information was blocked (D), respectively. Moths were expected to orient towards either the left or right side, if they perceived the background structure properly. We used binned data (24 bins) to draw circular plots and a rose diagram (histogram of circular data), and overlaid circular density plots (dashed lines) to visualize data.

$N = 27$, $P < 0.001$; Fig. 1B) but towards either the left or right side (Rayleigh's test; test statistic = 0.7491, $N = 27$, $P < 0.001$). These results suggest that moths were able to perceive bark structure and adopted adaptive body orientations without the sensory information from antennae or forelegs.

When wings were excised, 79% (30/38; two moths have no data about re-positioning) of the moths repositioned themselves, and the moths oriented in a manner that was not different from random (Kuiper's test; $V = 1.37$, $N = 40$, $P > 0.15$; Fig. 1C). However, the alternative hypothesis of specific resting orientations (towards either the left or right side) was also supported (Rayleigh's test; test statistic = 0.22, $N = 40$, $P = 0.02$). These contrasting results suggest that, although moths require information from wings to accurately orient their bodies, cutting off their wings was not sufficient to entirely remove their preference to orient towards the left or right side.

In darkness, moths could not be observed and we did not determine whether the moths performed re-positioning behaviour. Next morning when the photographs were taken, the moths oriented nonrandomly (Kuiper's test; $V = 3.10$, $N = 37$, $P < 0.001$; Fig. 1D) but upwards rather than towards either of the two sides (Rayleigh's test; test statistic = −0.58, $N = 37$, $P = 1.00$).

DISCUSSION

Because cutting off the antennae or forelegs did not change the body orientations of moths in comparison with natural conditions, we conclude that tactile information from the antennae and forelegs is not crucial for the perception of bark structure needed for adaptive body positioning. However, cutting off the wings resulted in a random distribution of body orientations, suggesting that moths may use some sensory information from wings and the movements of the wings to perceive bark structure during re-positioning behaviour.

Some wing-excised moths were able to orient their bodies towards the sides, and some even re-positioned their bodies and oriented towards the sides (to observe the re-positioning of wing-excised moths, see Supporting information, Video S1). Nevertheless, without wings, the accuracy of adopting cryptic body orientations was reduced considerably, and the overall distribution of the moths' orientations was dispersed (Fig. 1D). These results suggest that wings may play some role in accurately perceiving the direction of bark structures.

We hypothesize that mechanosensory inputs from wings may be used during normal re-positioning behaviour. Typically, when resting, geometrid moths put their wings flat on the bark surface and the frontal edges of both the left and right wings (their frontal edges make an almost straight line; see Supporting information, Fig. S3) align well with the furrows in the bark. If a moth were to rest in a body orientation that results in a lack of alignment between direction of frontal wing edges and bark furrows, the wings would contact the bark in a different manner, which may be detectable by mechanosensory organs on the wings (Keil, 1997). However, there is the possibility, which we cannot fully reject, that changes in body balance (caused by wing excision or the subtle weight difference between the left and right wing remains) might have affected the reduced ability of moths to properly orient their bodies.

In the vision treatment, unlike in the other treatments, we were unable to observe the moths and we therefore cannot determine whether they repositioned their bodies. Thus, this treatment can only provide general information indicating that moths ended up with non-adaptive body orientations in total darkness. If the moths did perform re-positioning behaviour, then our results would mean that moths use visual information to perceive the bark structure. On the other hand, if the moths did not perform re-positioning behaviour, this would indicate that light is important for triggering re-positioning behaviour. In our results, the distribution of body orientations was almost identical to the typical distribution of the body orientations of *J. fuscaria* at landing spots before re-positioning behaviour is performed (upwards; Kang *et al*., 2012). Thus, it is more likely that the moths did not perform the re-positioning behaviour in total darkness, and that the presence of light is needed to trigger re-positioning. Regardless of the two possibilities, blocking the light effectively prevented the moths from assuming adaptive orientations, which suggests that visual information is pivotal for finding cryptic positions on the tree trunk.

In summary, we show that visual information is crucial for assuming adaptive body orientations, although we cannot precisely determine whether this is because vision is used to perceive the bark structure or simply because light is needed to trigger the re-positioning behaviour. We also show that the tactile information from wings may be important for accurately perceiving the direction of bark structure.

REFERENCES

Akino T, Nakamura K-I, Wakamura S. 2004. Diet-induced chemical phytomimesis by twig-like caterpillars of *Biston robustum* Butler (Lepidoptera: Geometridae). *Chemoecology* **14:** 165–174.

Animal Behaviour. 2012. Guidelines for the Treatment of Animals in Behavioural Research and Teaching. *Animal Behaviour* **83:** 301–309.

Cooper WE Jr, Sherbrooke WC. 2012. Choosing between a rock and a hard place: camouflage in the round-tailed horned lizard *Phrynosoma modestum*. *Current Zoology* **58:** 541–548.

Cott H. 1940. *Adaptive coloration in animals*. London: Methuen & Co Ltd.

Gullan PJ, Cranston P. 2009. *The insects: an outline of entomology*. Malden, MA: Blackwell Publishing.

Kang CK, Moon JY, Lee SI, Jablonski P. 2012. Camouflage through an active choice of a resting spot and body orientation in moths. *Journal of Evolutionary Biology* **25:** 1695–1702.

Kang CK, Moon JY, Lee SI, Jablonski PG. 2013a. Cryptically patterned moths perceive bark structure when choosing body orientations that match wing color pattern to the bark pattern. *PLoS One* **8:** e78117.

Kang CK, Moon JY, Lee SI, Jablonski PG. 2013b. Moths on tree trunks seek out more cryptic positions when their current crypticity is low. *Animal Behaviour* **86:** 587–594.

Keil TA. 1997. Functional morphology of insect mechano-receptors. *Microscopy Research and Technique* **39:** 506–531.

Kjernsmo K, Merilaita S. 2012. Background choice as an anti-predator strategy: the roles of background matching and visual complexity in the habitat choice of the least killifish. *Proceedings of the Royal Society of London Series B, Biological Sciences* **279:** 4192–4198.

Land MF, Nilsson DE. 2012. *Animal eyes*. Oxford: Oxford University Press.

Lovell PG, Ruxton GD, Langridge KV, Spencer KA. 2013. Egg-laying substrate selection for optimal camouflage by quail. *Current Biology* **23:** 260–264.

Manríquez KC, Pardo LM, Wells RJD, Palma AT. 2008. Crypsis in *Paraxanthus barbiger* (Decapoda: Brachyura): mechanisms against visual predators. *Journal of Crustacean Biology* **28:** 473–479.

Sane SP, Dieudonné A, Willis MA, Daniel TL. 2007. Antennal mechanosensors mediate flight control in moths. *Science* **315:** 863–866.

Schneider D. 1964. Insect antennae. *Annual Review of Entomology* **9:** 103–122.

Stevens M, Merilaita S. 2009. Animal camouflage: current issues and new perspectives. *Philosophical Transactions of the Royal Society B, Biological Sciences* **364:** 423–427.

Stevens M, Merilaita S. 2011. *Animal camouflage: mechanisms and function*. Cambridge: Cambridge University Press.

Zar J. 1999. *Biostatistical analysis*, 4th edn. Upper Saddle River, NJ: Prentice Hall.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. A photograph of the experimental box that was used for vision treatment and the control experiment for vision treatment.

Figure S2. The distribution of the head orientations of *Jankowskia fuscaria* relative to vertically directional structures in the control experiment for vision treatment. Each circle shows the head direction of a moth. Moths oriented towards either the left or right side (N = 17, test statistic = 0.58, $P < 0.001$), which suggests that moths responded to directional structures when light was present. We used binned data (24 bins) to draw circular plots and a rose diagram (histogram of circular data), and overlaid circular density plots (dashed lines) to visualize data.

Figure S3. Typical resting posture of *Jankowskia fuscaria* on tree trunks.

Table S1. The number of collected, analyzed, and discarded moth individuals. The total number of tested and discarded moth samples for each treatment is shown and the reasons for the discard are presented.

Video S1. Re-positioning behaviour of a wing-excised moth.