Spatial orientation of social caterpillars is influenced by polarised light

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Methods continued from Uemura *et al.*

Morphological analyses

Preparations for the following methods were all done under the biosafety cabinet/fume hood because *T. pityocampa* and *O. lunifer* have urticating hairs. For scanning electron microscopy (SEM), five *O. lunifer* final instar larvae were used. Live larvae were decapitated instantly using a razor blade. Hairs on the head capsule were cut as short as possible using micro-scissors to reduce the amount of air bubbles that could be trapped during the fixation period. The fixative was a combination of 3.5% glutaraldehyde and 4% formaldehyde. After 24 h of fixation, the head capsules were rinsed three times and stored in sodium cacodylate buffer. Head capsules were cleaned by gently rinsing in 70% ethanol and a fine paintbrush was used to remove debris. Specimens were then sonicated in 70% ethanol for 5 min and dehydrated in ascending concentrations of ethanol (70-100% in 10% steps). Samples were dried at critical point using an Autosamdri-815 series A critical point dryer (Tousimis, Rockville). Specimens were mounted on aluminium stubs using carbon tabs and coated with gold with a SPI module sputter coater prior to viewing with a Neoscope JCM-5000 SEM (JEOL, Japan) or TM4000plus (Hitachi, Japan). Similar methods were used for *T. pityocampa* final instar larvae, following Belušič et al. (2017).

For light microscopy (LM), ten live *O. lunifer* larvae were decapitated instantly using a razor blade. Methods for fixation and buffer storage were the same as described for SEM. However, prior to fixation, the head capsules were cut in half through the middle and the muscles were removed to allow the fixative to efficiently penetrate through the soft tissue. After 3 h of fixation, the head capsules were rinsed in 0.1 M cacodylate buffer. Stemma I – IV and V – VI

were grouped together and dissected from the head capsule using surgical forceps attached with a razor blade, viewed under a stereomicroscope. The dissected groups of stemmata were fixed in 1% osmium tetroxide followed by dehydration in ascending concentrations of ethanol (50-100% in 10% steps). Finally, resin infiltration of dissected stemmata was done using Epon resin and propylene oxide. Semi-thin cross sections were cut and mounted on glass slides, stained with Azure II (Sigma-Aldrich) and observed with an AxioImager Z1 microscope (Zeiss, Oberkochen, Germany). Similar methods were used for *T. pityocampa* final instar larvae.

For transmission electron microscopy (TEM), same methods were applied as described for SEM and LM. Silver ultra-thin cross sections were cut with a diamond knife (Histo 45, Diatome, Nidau, Switzerland), contrasted with 0.7% uranyl acetate in water for 20 min and with 2.5% lead citrate in water for 10 min, and observed with CM 100 (Philips, The Netherlands) and Talos L120C (Thermo Fisher Scientific, USA).

References

Belušič G, Šporar K, Meglič A. 2017 Extreme polarisation sensitivity in the retina of the corn borer moth Ostrinia. *J. Exp. Biol.* **220**, 2047–2056. (doi:10.1242/jeb.153718)