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UNCOVERING FUNCTIONAL MORPHOLOGY OF BUFF-TAILED BUMBLEBEE OCELLI USING X-RAY MICROTOMOGRAPHY

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Summary: Using a combination of x-ray microtomography, optical measures, and virtual ray-tracing, the functional morphology of the ocelli of *Bombus terrestris* was characterised. Lateral and median ocelli of both workers and drones show an irregularly shaped, biconvex lens, separated by a small vitreous body from a horizontally stretched, ventrally bipartite retina. The field of view of the ocelli seems horizontally stretched, with a focussed view in the median portion.

1. INTRODUCTION

The visual system of many insect species is based primarily on two different types of eyes, a pair of **compound eyes**, thought to provide detailed visual information about the environment, and a set of camera-type eyes called **ocelli**, which are thought to provide information necessary for quick flight stabilisation [1]. Until now, the structure and function of ocelli have mostly been studied using electrophysiological measurements and standard microscopy techniques (e.g. [2,3]). However, neither of these techniques allow for complete and exact reconstructions of the visual field through analysis of three-dimensional optic properties of these eyes. Using X-ray microtomography (micro-CT), 3D structure of different parts of the ocelli can reliably be reconstructed, without the adverse effects of reconstruction based on destructive and highly laborious standard sectioning and microscopy techniques [4]. By combining the three-dimensional morphology of the different parts of the ocelli acquired through micro-CT with measurements of the optical properties for the materials making up these parts acquired through methods such as “hanging drop”, visual fields can be reconstructed with ray-tracing techniques [5]. This has already led to an expanded understanding of ocellar functioning in the tropical orchid bee *Euglossa imperialis*, which indicated a greater and more intricate role for ocelli in visual processing than initially thought. The aim of this study is to apply these same techniques to reconstruct the visual fields of the ocelli the buff-tailed bumblebee *Bombus terrestris* and to expand on these techniques to gain more detailed insight into the visual fields.

2. EXPERIMENTAL METHOD

Three *B. terrestris* reproductive males (drones) and three non-reproductive females (workers) were taken from a commercial hive (Natupol, Koppert Biological Systems). They were cold anesthetised and the lower portions of the head were removed. Samples were fixed and prepared for micro-CT as described in [5]. Synchrotron micro-CT was then conducted at beamline I13-2 of Diamond Light Source in Manchester [6] and the radiographic projections were reconstructed into 3D volumes using DAWN v1.7. Of two drones and two workers, the ocelli were imaged in detail, while of the other drone and worker, the whole head was imaged. Visible ocellar structures (lens, retina, and iris) in the 3D volumes were segmented, annotated, registered onto the scanned heads in Amira (FEI). Median and lateral ocelli from individuals of a different hive were isolated and used to perform the “hanging-drop technique” as described in [5], in order to determine the back focal distance (the distance at which a focussed image is formed behind the lens). The annotated volumes of the ocelli were imported into MATLAB (Mathworks). The annotated volumes together with their placement in the head as well as the results from the hanging-drop experiments were used to determine the path of virtual light rays through the lens onto the retina.

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3. RESULTS

Reconstructions based on the scans of the ocelli show that both lateral and median ocelli show an asymmetrically shaped biconvex lens, as well as a large iris, a "banana-shaped" cupped retina, and a small vitreous body (Figure. 1). The retina is stretched along the horizontal axis and is distinctly bipartite in its ventral portion (which receives light from the dorsal part of the world). This partition is further enforced by the large iris, which covers the median part of the ventral portion of the retina. Preliminary results from ray tracing indicate a horizontally elongated field of view. Light rays seem to converge on the back of the retina in the median portion, indicating that portions of the world projecting onto these parts of the retina are viewed in detail by the ocellar system.

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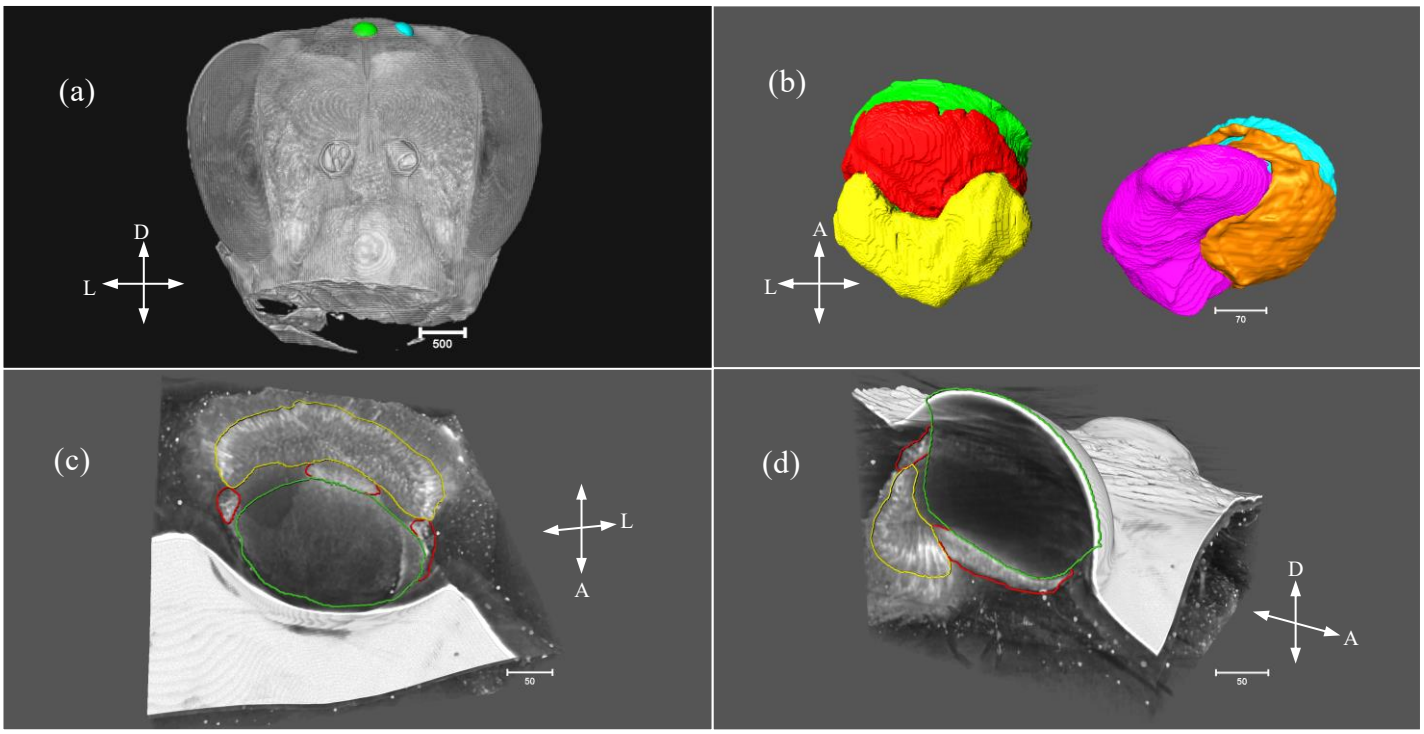

Figure 1: (a) Location of the median (m) and left lateral (l) ocelli (lenses coloured green and cyan respectively) on the *B. terrestris* head. (b) A ventral, 3D view of the ocelli (m left, l right), clearly showing the curved, elongated shape of the retina (m yellow, l pink), as well as the large iris (m red, l orange). (c) Virtual horizontal section of the ventral part of the median ocellus, showing the curved, elongated shape of the retina (yellow) and the covering of the median portion of the retina by the iris (red). A clear zone (space between the lens [green] and the retina) is also distinguishable. (d) Virtual sagittal section through the median part of the median ocellus (lens: green, retina: yellow, iris: red), showing the asymmetry of the lens. Scale bars indicate 500 (a), 70 (b) or 50 (c,d) µm. D=dorsal, L=lateral, A=anterior.